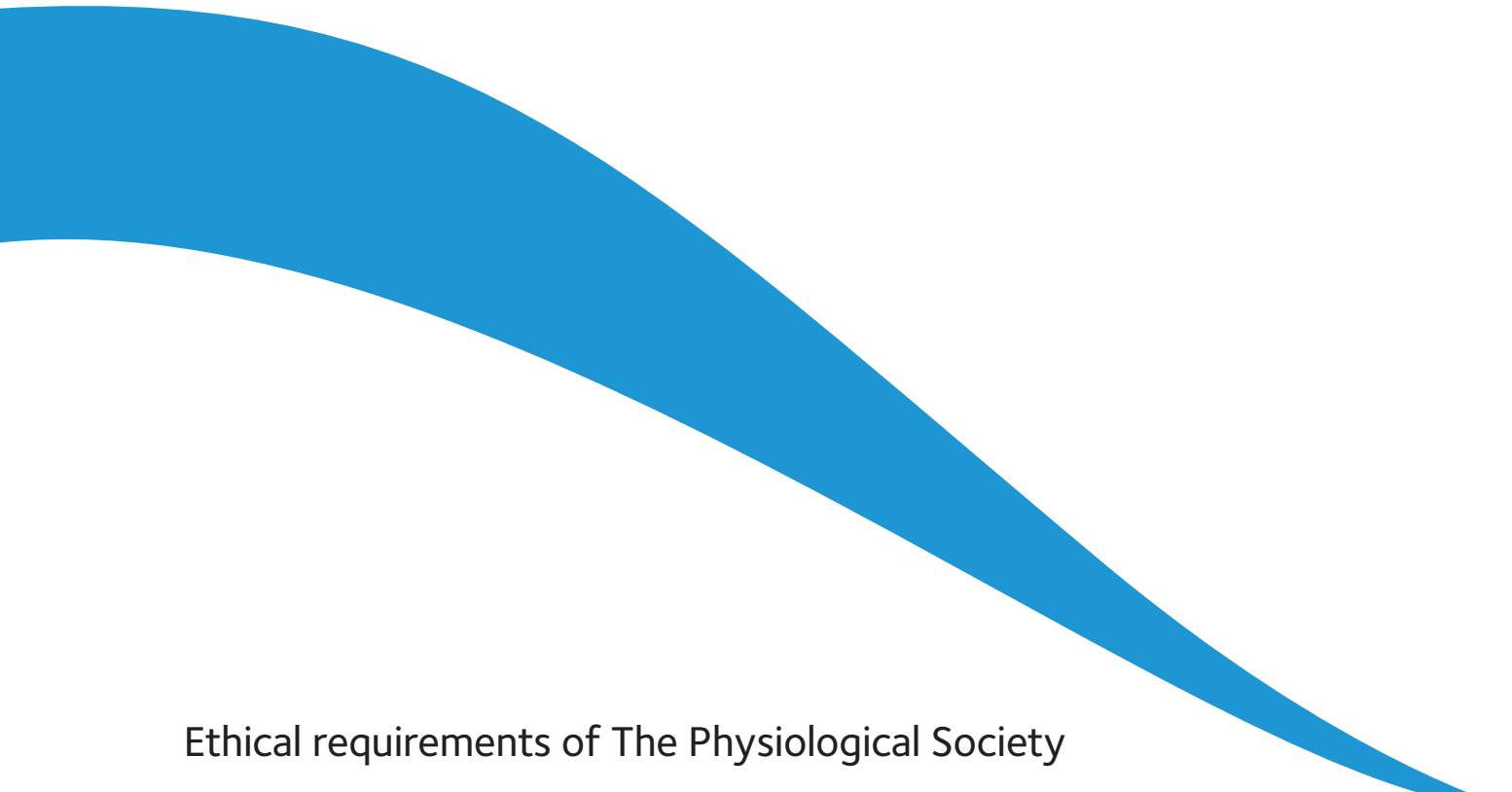




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Ethical requirements of The Physiological Society

Experiments on animals or animal tissue

For work conducted in the UK all procedures must conform with current UK legislation. For work conducted elsewhere all procedures must accord with current national guidelines or, in their absence, with current local guidelines.

Experiments on humans or human tissue

All procedures must accord with the ethical standards of the relevant national, institutional or other body responsible for human research and experimentation, and with the principles of the World Medical Association's Declaration of Helsinki.

PL1

How cool are ectotherm hearts

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Changes in temperature, pH, or respiratory gases can dramatically affect heart function. Yet many ectothermic animals experience these environmental changes routinely as part of their day to day life. This talk will explore strategies of cardiac adaptation that permit maintenance of heart function in animals living in fluctuating environments. Interrelated aspects of ectotherm cardiac physiology will be discussed across levels of biological organization and across a variety of fish and reptile species. Excitation-contraction coupling during temperature change will be explored, in particular, the 'mysterious' role of the ectotherm sarcoplasmic reticulum. The talk will also consider mechanical regulation of ectotherm heart function and sarcomeric adaptations that allow extremes of stroke volume. The overall aim is to share my excitement for ectotherm cardiac physiology and highlight the growing importance of understanding the tolerances and adaptability of ectotherm hearts as our climate changes.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PL2

Neural circulatory control during exercise: Early insights

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During exercise, the cardiovascular response is rapidly and appropriately matched to the intensity of the physical activity. The autonomic nervous system plays an important role in achieving this closely matched circulatory response by an increase in the sympathetic nerve activity to the heart, blood vessels, and adrenal medulla and a decrease in the parasympathetic nerve activity to the heart. Early insights into the mechanisms that controlled these cardiovascular changes during exercise were reported in the 19th century. At that time, two mechanisms were hypothesized to be responsible for these changes. In one mechanism, a signal arising in a central area of the brain causes a parallel activation of skeletal muscle contraction and of autonomic nervous system changes (now termed, Central Command). In the other mechanism, a signal arising in the contracting skeletal muscle causes a reflex activation of the autonomic nervous system changes (now termed, Exercise Pressor Reflex). Some important investigators involved in early studies include Johan Johansson, August Krogh, Johannes Lindhard, and Horace Smirk. Also, Frances Buchanan and Louis Fridericia should be recognized for their contributions. Since those early insights, experiments in both animals and humans have confirmed these early hypotheses (Exp.Physiol. 97:10-69, 2012). In more recent years, the important involvement of a third mechanism, the Arterial Baroreflex, has been elucidated. Thus, all three of these mechanisms (Central Command, the Exercise Pressor Reflex, and the Arterial Baroreflex) contribute to the closely matched and coordinated response of the circulatory system to the intensity of the exercise being performed.

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PL3

The Loving Brain: Monogamy to maternity

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A baby sucks at a mother's breast for comfort, and of course for milk. Milk is made within specialised cells of the mammary gland, and is normally stored inside those cells. For a baby to feed, the milk must be released from those cells; when it is released it gathers in a collecting chamber from where it can be extracted by sucking. Milk is released or "let down" as a reflex response to the sucking and kneading of the baby at the breast, and sometimes also in response to the sight and smell and sound of the baby. The reflex originates from stimuli that are sensed by nerve endings, and which are communicated to the brain through a chain of nerve cells, and the reflex is effected by the release into the circulation of a hormone, oxytocin, from the pituitary gland.

The reflex, first described in the rat by Wakerley and Lincoln in 1973 (1), is a spectacle of power and economy. When hungry pups suckle at the nipples of their mother, oxytocin cells in the hypothalamus all discharge an intense burst of spikes every 5-10 min, resulting in an abrupt milk let-down. Each burst lasts for just 1-2 s, and every oxytocin cell will burst within about half a second of one another. These bursts cause oxytocin to be released not only into the blood, but also into the brain - from the dendrites of oxytocin cells, and this stimulates yet more oxytocin release (see 2-4). So much oxytocin is released from these dendrites (5) that it can reach sites throughout the forebrain, where it causes striking changes in behaviour, reinforcing the bond between the mother and her offspring (6).

But oxytocin is also released during sexual activity, in both males and females, and experiments in the prairie vole showed that this release is instrumental in forming bonds between partners (7). The prairie vole is one of those unusual mammalian species, which, like humans, forms monogamous bonds, and oxytocin release in the female brain, stimulated by sexual activity, is essential for the formation of these bonds. Oxytocin is also released in the male brain, as is a closely related hormone-vasopressin, which in the male brain triggers the aggressive, territorial behaviour, akin to jealousy, that is a signature that a bond has been formed by the male.

In humans, some studies indicate that intranasal application of oxytocin can induce trust, and there is evidence that links deficiencies in oxytocin with autism and related deficits in social behaviour (8). These have attracted intense interest in the search for novel therapies - and have also raised some difficult ethical questions.

Wakerley JB, Lincoln DW (1973) The milk-ejection reflex of the rat: a 20- to 40-fold acceleration in the firing of paraventricular neurones during oxytocin release. *J Endocrinol.* 57:477-93.

Ludwig M, Sabatier N, Bull PM, Landgraf R, Dayanithi G, Leng G. (2002) Intracellular calcium stores regulate activity-dependent neuropeptide release from dendrites. *Nature* 418:85-89

Ludwig M, Leng G (2006) Dendritic peptide release and peptide-dependent behaviours. *Nature Neurosci Rev* 7:126-36

Leng G, Ludwig M (2008) Neurotransmitters and peptides: whispered secrets and public announcements. *J Physiol* 586:5625-32

Rossoni E, Feng J, Tirozzi B, Brown D, Leng G, Moos F (2008) Emergent synchronous bursting of oxytocin neuronal network. *PLoS Comput Biol* Jul 18;4(7):e1000123.

Leng G, Meddle SL, Douglas AJ (2008) Oxytocin and the maternal brain. *Curr Opin Pharmacol* 8:731-4

Young KA, Gobrogge KL, Liu Y, Wang Z. (2011) The neurobiology of pair bonding: insights from a socially monogamous rodent. *Front Neuroendocrinol.* 32:53-69.

Reference 8. Baumgartner T, Heinrichs M, Vonlanthen A, Fischbacher U, Fehr E. 2008 Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. *Neuron.* 58:639-50.

Reference 9. Hammock EA, Young LJ (2006) Oxytocin, vasopressin and pair bonding: implications for autism. *Philos Trans R Soc Lond B Biol Sci.* 361:2187-98.

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PL4

Decoding the olfactory world in time and space

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How do genes and the environment interact to generate behaviors? How are innate genetic templates for behavior modified by context and experience? To answer these questions we need to understand the relationships among genes, neurons, and circuits that generate behaviors.

The nematode worm *Caenorhabditis elegans* provides an opportunity to understand behaviors at levels of resolution ranging from the molecule to the organism. Furthermore, an anatomical wiring diagram of *C. elegans* has been known for over twenty years. Still, we cannot predict the animal's behavior from its connectivity, with the exception of deterministic escape behaviors. To gain a deeper insight into the key functions of the neurons and circuits, we are mapping a probabilistic goal-directed behavior, chemotaxis to attractive odors. Our approaches include genetic manipulation of neurons and circuits, high-resolution behavioral analysis, and quantitative analysis of neuronal responses.

The transformation of olfactory information into goal-directed behavior begins with the activation of odorant receptors by their ligands. The neuronal responses to these inputs are shaped on at least two different timescales by intrinsic neuronal dynamics: a fast linear response permits rapid odor tracking, and a slower nonlinear response follows mean odor levels. In some respects these two computations resemble the fast detection and slow adaptation in bacterial chemotaxis that were defined by Howard Berg and his colleagues. Interestingly, different sensory neurons operate in distinct temporal regimes that are suited to their behavioral functions. These sensory signals are interpreted by interneurons with different dynamic properties.

By exposing animals to defined temporal and spatial odor patterns and measuring specific parameters of the resulting behaviors, we can preferentially reveal turning dynamics in a biased random walk, directed orientation into an odor stripe, and speed regulation by odor. In addition, we have uncovered information that shapes the connectivity map and resulting behaviors: neuromodulators that are not apparent in the anatomical circuits, flexible information flow through alternative synaptic pathways, and neuronal dynamics that shape responses over time.

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PL5

Dangerous assumptions and misconceptions: Can physiology teaching help to improve patient safety?

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There is nothing more certain and unchanging than uncertainty and change. John Fitzgerald Kennedy

I would like to use this opportunity to share with you my reflections upon the privileges and the challenges of a decade of teaching of teaching medical students. I'll describe how my clinical practice in the field of emergency medicine informs my teaching sessions and why I believe a good understanding of physiological systems is vital for safe and effective patient care. I'll also tell you about the fatal consequences of a doctor that didn't appreciate that the QRS complex of the electrocardiogram is due to ventricular depolarization and the misconceptions of a diabetes specialist who thought that osmotic diuresis caused glycosuria.

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PL6

Oxygen sensing in animals

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Maintenance of oxygen homeostasis is a fundamental physiological challenge. However the general operation of animal systems responding directly to oxygen availability was not appreciated until work on regulation the regulation of the erythropoietin gene led to the discovery of a system of direct oxygen sensing that operates in all animals. Post translational hydroxylation of specific prolyl and asparaginyl residues by a group of Fe(II) and 2-oxoglutarate (2-OG) dioxygenases directly couples the availability of oxygen to the regulation of HIF, the key transcription factor directing a very wide range of cellular and systemic responses to hypoxia. Co-factor requirements for Fe(II), ascorbate and 2-OG, as well as oxygen, potentially enable these enzymes to link biological responses to hypoxic, metabolic and redox stresses. The lecture will review the biochemistry, evolution and pan-genomic organization of the HIF hydroxylase pathways, and will address the wider role of intracellular protein hydroxylation in biological systems. It will also consider the implications of dysregulating large interconnected physiological pathways for cancer.

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PL7

There and back again: G proteins inhibit neuronal calcium ion channels

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Voltage-gated calcium ion channels (Ca_v) link neuronal activity to a direct and rapid change in intracellular calcium. The resulting calcium signal, at the inner face of the plasma membrane, triggers a myriad of cellular responses depending on its location, magnitude, and duration. Presynaptic calcium entry through Ca_v2 (P, N, R -types) channels and, at some synapses Ca_v1 (L-type) channels triggers transmitter release, whereas calcium that enters through dendritic postsynaptic Ca_v1 channels regulates gene expression. Their strategic location at both sides of the synapse places voltage-gated calcium ion channels in a privileged position to integrate electrical and chemical signals, and to influence synaptic efficacy. Presynaptic $\text{Ca}_v2.2$ (N-type) channels are major targets of a large number of neurotransmitters and drugs, including morphine, that act through their respective G protein coupled receptors (GPCR) to modulate neuronal activity. This signaling pathway - GPCR to $\text{Ca}_v2.2$ channel – underlies presynaptic inhibition of transmitter release at many synapses. The actions of GPCRs on $\text{Ca}_v2.2$ channels are cell-type and synapse specific. Our studies show that cell-type specific alternative pre-mRNA splicing sets the sensitivity of $\text{Ca}_v2.2$ channels to GPCR inhibition. Cell-specific splicing factors therefore influence the efficacy of G protein signaling to $\text{Ca}_v2.2$ channels and ultimately synaptic function.

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C1

Angiotensin-(1-9) antagonises Angiotensin II-induced cardiac remodeling via the angiotensin type 2 receptor

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The renin-angiotensin system (RAS) regulates cardiovascular physiology mainly via angiotensin II (AngII) engaging the angiotensin type 1 or type 2 receptors (AT1R and AT2R). Classical AngII actions are AT1R-mediated, while the AT2R may counteract AT1R signalling. However recent identification of the ACE2/Ang-(1-7)/Mas has led to the definition of a counter-regulatory axis of the RAS. Novel peptides of the counter-regulatory axis of the RAS, angiotensin 1-7 [Ang-(1-7)] and angiotensin 1-9 [Ang-(1-9)], have been identified as potential therapeutic molecules. Ang-(1-7) has been shown to antagonise the pathological actions of AngII through the receptor Mas. Recently, we showed that Ang-(1-9) has an anti-hypertrophic effect on AngII-induced rat neonatal cardiomyocytes, signalling through the angiotensin type 2 receptor (AT2R)1. Furthermore, infusion of Ang-(1-9) via osmotic minipumps to the stroke-prone spontaneously hypertensive rat reduced cardiac fibrosis via the AT2R2. To further investigate the actions of Ang-(1-9) in an acute model of hypertension we infused AngII (24µg/kg/hr) in mice to induce hypertension. Ang-(1-9) (48µg/kg/hr), Ang-(1-9)+PD123,319 (AT2R blocker) (20mg/kg/day), or water (control) were co-infused with AngII for 2 weeks via osmotic minipumps. Blood pressure was monitored via radiotelemetry (for radiotelemetry probe and osmotic minipump implantation mice were anesthetized with 1.5% isoflurane in 1 L/min O₂ for the duration of the surgical implantation procedure). Significant increases in mean arterial pressure (MAP) were observed in AngII infused mice compared to control (control 108.8 ± 5.7 mmHg; AngII 125.1 ± 8.4 mmHg; p>0.05; n= 6 mice; two way ANOVA) however co-infusion of Ang-(1-9) or Ang-(1-9) and PD123,319 did not affect AngII-induced hypertension (AngII + Ang-(1-9) 122.4 ± 10.3 mmHg; AngII + Ang-(1-9) + PD123,319 118.3 ± 6.8mmHg; n=6 mice; two way ANOVA). However, Ang-(1-9) co-infusion was able to reduce AngII-induced cardiac hypertrophy (ratio heart weight/tibia length: Control 9.43 ± 1.2 mg; AngII 11.69 ± 0.6 mg; Ang-(1-9) 8.22 ± 0.7 mg; p<0.01; n=6 mice; one way ANOVA). Staining of cardiac sections with wheat germ agglutinin and measurement of cardiomyocyte size confirmed these results (Control 25.73 ± 3.8 µm; AngII 28.78 ± 3.8 µm; Ang-(1-9) 24.6 ± 4.8 µm; p<0.05; one way ANOVA). Cardiac fibrosis was assessed by picrosirius red staining and quantified by image analysis software. Mice co-infused with Ang-(1-9) showed a reduction in cardiac fibrosis when compared to AngII-infused animals (control 0.12 ± 0.16%; AngII 1.28 ± 2.2%; Ang-(1-9) 0.52 ± 0.75%; p<0.05; one way ANOVA). The effects of Ang-(1-9) were reversed by PD123,319 co-infusion. In summary Ang-(1-9) was able to reduce cardiac hypertrophy and fibrosis in an acute animal model of hypertension without effecting blood pressure. These data support a direct biological role for Ang-(1-9) in cardiac remodeling and highlights Ang-(1-9) as a potential new therapeutic target in cardiovascular disease.

FLORES-MUNOZ, M., SMITH, N. J., HAGGERTY, C., MILLIGAN, G. & NICKLIN, S. A. (2011) Angiotensin1-9 antagonises pro-hypertrophic signalling in cardiomyocytes via the angiotensin type 2 receptor. *J Physiol*, 589, 939-51.

FLORES-MUNOZ, M., WORK, L. M., DOUGLAS, K., DENBY, L., DOMINICZAK, A. F., GRAHAM, D. & NICKLIN, S. A. (2012) Angiotensin-(1-9) attenuates cardiac fibrosis in the stroke-prone spontaneously hypertensive rat via the angiotensin type 2 receptor. *Hypertension*, 59, 300-7.

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C2

Direct, simultaneous measurements of free intra-SR and cytosolic Ca²⁺ during Ca²⁺ waves

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When ventricular myocytes are overloaded with Ca²⁺, spontaneous waves of Ca²⁺-induced Ca²⁺-release are triggered from the sarcoplasmic reticulum (SR). These waves can result in arrhythmias. Previous work has suggested waves occur when the SR Ca²⁺ content reaches a threshold level, with the time between waves depending on the rate that the SR refills with Ca²⁺. It is, however, also possible that the recovery of the SR Ca²⁺ release channel (Ryanodine Receptor, RyR) from inactivation also plays a role in wave initiation. Most previous work has used indirect methods to measure SR Ca²⁺ content, and therefore, the mechanisms underlying wave initiation have not been clarified. In order to investigate this issue, we measured free intra-SR and cytosolic Ca²⁺ simultaneously in canine ventricular myocytes displaying spontaneous Ca²⁺ waves. Canine myocytes were isolated and dual loaded with Mag-Fura-2AM/Fluo-3AM. Waves were induced via two methods; in some experiments, external Ca²⁺ concentration was increased up to 15 mM, and in other experiments the cells were voltage clamped in the perforated patch configuration with an elevated intracellular Na⁺ (15mM). In both cases, wave frequency was altered by changing external Ca²⁺ concentration. We find that the cytoplasmic Ca²⁺ wave is associated with a depletion of SR free Ca²⁺, and recovery of cytoplasmic Ca²⁺ is associated with a rapid partial recovery of SR free Ca²⁺. The remainder of the recovery of SR free Ca²⁺ occurs more slowly. Elevation of external Ca²⁺ concentration accelerates this slow phase of recovery. When SR free Ca²⁺ has recovered to the pre-wave level, another wave is initiated, suggesting that a threshold level has been attained. The slow phase of restoration of SR free Ca²⁺ is associated with either a small decrease of cytoplasmic Ca²⁺ or no measurable change. Our results confirm a threshold-mediated mechanism for induction of spontaneous waves, with little evidence of a role for RyR refractoriness.

This study was funded by The British Heart Foundation.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C3

Use of echocardiography for the phenotypic assessment of a mouse model of coronary heart disease

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Background: Transthoracic echocardiography provides a non-invasive approach for in-vivo evaluation of the mouse heart. The present study examines its use in assessing the functional phenotype of the left ventricle (LV) in a model of chronic coronary heart disease (CHD).

Methods: 26 male apolipoprotein E knockout mice (ApoE^{-/-}) were fed either a western-type high-fat diet (n=13; 21% lard and 0.15% cholesterol) or chow diet (n=13) for 12 weeks from weaning. High-fat diet ApoE^{-/-} mice develop progressive atherosclerosis including in their coronary arteries (CHD). In contrast, littermates fed a chow diet exhibit no coronary disease (control).

Animals underwent echocardiography with a Vevo 770 (Visualsonics, Canada) at 12 weeks of age. Heart rate and core temperature were continuously monitored. Normothermic mouse core temperatures were maintained using a heated platform. Mice were anaesthetised with isoflurane at a concentration of 2% (induction) and 1-1.5% (maintenance) in 100% Oxygen. To minimize the confounding effects of anaesthesia between experiments, recordings were taken when the heart rate was in the range 400-500 beats/min. LV systolic function was evaluated with the fractional area shortening (FAS%) in a parasternal long axis. Pulsed wave and tissue doppler modes were used to assess the diastolic function. Data were analysed using an unpaired Student's t-test.

Results: 12-wk old apoE^{-/-} mice when fed an atherogenic diet had comparable body mass (31.1 ± 1.46 g; CHD vs. 31.7 ± 2.6 g control), corrected cardiac mass (96 ± 23 mg; CHD vs. 100 ± 21 mg control) and cardiac output (16.7 ± 4 ml/min; CHD vs. 16.2 ± 5.6 ml/min control). There were no significant differences in short-axis B- and M-mode measurements: Aortic root (1.56 ± 0.028 mm; CHD vs. 1.542 ± 0.035 mm control); LV internal systolic diameter (2.24 ± 0.39 mm; CHD vs. 2.3 ± 0.3 mm control); LV internal diastolic diameter (3.59 ± 0.31 mm; CHD vs. 3.46 ± 0.49 mm control), fractional shortening (38.4 ± 4 %; CHD vs. 34.4 ± 6 %), and ejection fraction (68.5 ± 6 %; CHD vs. 62.1 ± 8 % control).

There was no change in long axis FAS% (65 ± 5 %; CHD vs. 68 ± 9 %), peak aortic velocity (905 ± 144 mm/s; CHD vs. 890 ± 88 mm/s) and mitral valve early and late diastolic filling (E/A) ratio (1.8 ± 0.5 ; CHD vs. 2 ± 0.6) between the two groups.

Conclusions: We have performed a complete characterisation of the left ventricular function in a mouse model of coronary disease in its early stage of atheroma deposition. These reference values will enable a serial study of the heart as the disease progresses.

Heart Research UK (HRUK)

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C4

Dysfunction of the Purkinje fibres underlies delayed interventricular conduction in heart failure

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About one-third of heart failure (HF) patients have left bundle branch block and resynchronization therapy is beneficial. We have investigated possible mechanisms in a rabbit model of left-sided volume and pressure overload. Male New Zealand rabbits (2.5-3 Kg) were anaesthetised with ketamine and isoflurane. Aortic incompetence was induced by inserting a catheter through the valve leaflets to cause LV volume overload. Then pressure overload was introduced by placing a silver clip (internal diameter 2.44 mm) just above right renal artery, 3 weeks later. The control group underwent sham procedures. The study was conducted in accordance with the Guide for the Care and Use for Laboratory Animals and approved by the local animal research ethics committee. By the end of 8 weeks, the rabbits showed significant LV hypertrophy, diminished LV fractional shortening and increased LV internal dimension. Echocardiography revealed an increase in the left ventricle (LV) internal diameter (in diastole) from 1.49 ± 0.03 to 2.16 ± 0.07 cm (n=6/8; $P < 0.001$) and a decrease in LV fractional shortening from 41.3 ± 1.4 to 25.5 ± 3.2 % (n=6/8; $P = 0.002$). The study was conducted in accordance with the Guide for the Care and Use for Laboratory Animals (US NIH Publication No 85-23, revised 1985). Micro-CT imaging as well as visual inspection showed hypertrophy of the Purkinje fibres. The ECG was recorded from anaesthetized sham-operated and HF rabbits before and after autonomic blockade. In the HF rabbits, there was an increase in the PR interval and duration of the QRS complex: e.g. following autonomic blockade, there was an increase in the PR interval from 68.5 ± 0.9 to 83.9 ± 2.9 ms (n=7/9; $P = 0.001$) and the QRS duration from 38.4 ± 2.0 to 48.2 ± 2.9 ms (n=7/10; $P < 0.05$). This suggests that there is delayed His-Purkinje conduction in the HF model. Free-running Purkinje fibres in the two ventricles were collected and RNA was extracted and reverse transcribed to produce cDNA. Quantitative PCR was carried out to measure the abundance of different ion channel and Ca^{2+} -handling transcripts. In the LV Purkinje fibres from the HF group, we found significant downregulation of many ion channel mRNAs, including mRNAs for funny channels (HCN1 by 66%, HCN4 by 82%), Na^+ channels ($\text{Na}_v1.5$ by 51%), Ca^{2+} channels ($\text{Ca}_v1.2$ by 63%, $\text{Ca}_v1.3$ by 90%), K^+ channels ($\text{K}_v1.5$, ERG, $\text{K}_v\text{LQT1}$, $\text{K}_{ir}2.1$, $\text{K}_{ir}3.1$, $\text{K}_{ir}6.2$ and SUR2a by 48-93%), Ca^{2+} -handling proteins (RYR2, SERCA2 and NCX1 by 55-62%), a Cl^- channel (ClC-2 by 46%) and connexins (Cx40 by 50%, Cx43 by 49%). The change in HCN4 appears to have been driven by a significant downregulation in the transcription factor, Tbx3, by 70%. In the RV Purkinje fibres, fewer differences were observed. At the protein level (measured using immunohistochemistry), there was a significant decrease in Cx43 in the LV Purkinje fibres from the HF group. We propose that remodelling of ion channels etc. in the LV His-Purkinje network may occur in response to LV dilatation and consequent stretching of the His-Purkinje tissue, particularly given that stress/stretch is thought to play a role in Purkinje fibre differentiation. Strategies to limit acute ventricular dilatation may be worthwhile in preventing long-term LV dysfunction in HF.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C5

Exercise rectifies contractile dysfunction in heart failure by activating CaMKII

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Ca²⁺ calmodulin dependent protein kinase II (CaMKII) is an important regulator of cardiac excitation-contraction coupling (ECC). Abnormal ECC leads to cardiac dysfunctions in heart failure (HF) that are partly due to changes in CaMKII activity. Exercise training improves ECC parameters in HF, but the role of CaMKII in this change is unknown, however exercise is seen to positively affect CaMKII and thus ECC in healthy individuals. The purpose of this study was to test whether exercise reverses the contractile dysfunction in myocardial infarction (MI)-HF, and whether this is altered by CaMKII. Young adult Wistar rats (male, 250-350g, n=42) were anaesthetised (1.5% isoflurane), MI-HF induced via permanent coronary artery ligation, and treadmill running (5 d/week, 6-8 weeks, 90-95% maximal capacity) initiated four weeks post-surgery (MI-TRN, n=14). Sham-operated (SHAM, n=12) and sedentary MI-HF (MI-SED, n=16) rats served as controls. Values are means±SEM, compared by One-Way ANOVA. MI-HF reduced aerobic capacity by 16% (p<0.01); exercise normalised this. Contractile function of twitch-stimulated (1-4Hz at 37°C) cardiomyocytes was assessed with edge detection video and Fura-2 fluorescence microscopy with and without the CaMK inhibitor autocamtide-2 related inhibitory peptide (AIP 10uM). MI reduced contractility by 40% (fractional shortening (FS) 13.5±0.5% vs 8.1±0.6% (p<0.01) in SHAM and MI-SED) and increased contraction and relaxation times (SHAM 77±4ms and 195±12ms vs MI-SED 106±4ms and 289±10ms; p<0.01). These effects were explained by reduced Ca²⁺ transient amplitude (SHAM 0.69±0.04au vs MI-SED 0.33±0.03au; p<0.01) and increased Ca²⁺ peak and decay times (SHAM 31±4ms and 263±13ms vs MI-SED 44±4ms and 363±9ms; p<0.01). Exercise corrected FS (MI-TRN 11.5±0.5%; p<0.01), tended to correct contraction and relaxation times (100±4ms and 243±10ms; both p<0.1), and improved Ca²⁺ handling (Ca²⁺ transient amplitude 0.49±0.04au, time to Ca²⁺ peak 39±3ms and decay 330±14ms; all p<0.01). CaMKII was then inhibited and experiments repeated, this abolished exercise-induced improvement in FS and Ca²⁺ transient amplitude (both p<0.01) and impaired relaxation and Ca²⁺ transient decay times (p<0.01) more in MI-TRN than MI-SED, but had no effect on contraction and systolic Ca²⁺ release time. MI-HF was associated with increased diastolic Ca²⁺ (p<0.01), while exercise reduced, but did not normalise this (p<0.01); however, CaMK-inhibition only partly explained this effect. In conclusion, this study confirmed that CaMKII plays an important role in modulating exercise-induced improvements in cardiomyocyte function following MI-HF. In particular, exercise improved sarcoplasmic reticulum (SR) Ca²⁺-uptake and SR loading parameters by activating CaMKII, whereas Ca²⁺ release parameters were less affected. This suggests that exercise activated cytoplasmic, but not dyadic CaMKII.

British Heart Foundation

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C6

Oral simvastatin dosing in rat enhances myocardial nitric oxide synthase expression, troponin I phosphorylation and cardiomyocyte lusitropy

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Statins inhibit the biosynthesis of L-mevalonate, a precursor of cholesterol. They improve cardiovascular morbidity and mortality, in part, through regression of atherosclerosis secondary to reduced serum cholesterol. However, a body of research has shown that statins also have direct effects on endothelial cells, vascular smooth muscle cells and cardiac myocytes through cholesterol dependent and independent mechanisms, which may contribute to their efficacy. Cholesterol regulates the expression of proteins like caveolins, structural and regulatory components of caveolae, through sterol regulatory elements in promoter DNA sequences. Caveolae are important signalling platforms [1]. Depletion of cellular mevalonate by statins also attenuates production of isoprenoid intermediates and therefore inhibits prenylation and activation of certain signalling molecules, including the small and heterotrimeric G-proteins. Here we investigate for the first time the impact of *in vivo* statin treatment on contractile function of the adult ventricle. Adult male Wistar rats were given 40 mg/kg simvastatin or saline daily by oral gavage for 2 weeks. Simvastatin treatment decreased time to half (t_{0.5}) relaxation by 25% in field-stimulated single ventricular myocytes (P<0.001; n=34-48 cells; Student's t-test), without affecting shortening, [Ca²⁺]_i transient amplitude or t_{0.5} transient decay. This was accompanied by a 173% increase in phosphorylation of troponin I (TnI) at Ser^{23/24} in ventricular homogenates (P<0.01; n=6). Levels of endothelial nitric oxide synthase (eNOS) and Akt were 265% and 100% higher, respectively, in ventricular homogenates from statin-treated animals (P<0.01; n=6). Additionally, caveolin 1 (Cav-1) and caveolin 3 (Cav-3) expression were attenuated by 34% and 42%, respectively, with statin treatment (P<0.05; n=6). Despite this, myocardial cholesterol was not significantly different between saline and statin groups (7.8 ± 0.4 vs. 7.6 ± 0.2 µg cholesterol/mg total protein; mean ± S.E.M., n=6). Our results show significant positive lusitropy in cardiomyocytes after *in vivo* statin administration. This is consistent with the observed increase in TnI phosphorylation. Protein kinases A and G (PKA and PKG) both phosphorylate TnI at Ser^{23/24}; this modification reduces Ca²⁺ sensitivity of the myofilaments and hastens relaxation. PKG could be more relevant since we also observe an upregulation of Akt and eNOS which, through NO, activates guanylyl cyclase, and produces cGMP. Reduced Cav-1 and Cav-3 might further promote NO production since both interact with and inhibit eNOS. Overall, we show a novel effect of statin treatment which could improve diastolic filling of the heart *in situ*.

[1] Harvey R. & Calaghan S. (2012) J Mol Cell Cardiol 52:366-75

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C7

Blood oxygenation and cardiorespiratory function in steelhead trout (*Oncorhynchus mykiss*) challenged with an acute temperature increase and zatebradine-induced bradycardia

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In this study, steelhead trout were fitted with a Doppler blood flow probe around their ventral aorta, a dorsal aortic cannula (for drug injections and blood sampling), and an opercular cavity cannula for ventilatory measurements. Then we injected these fish with saline (control) or zatebradine hydrochloride (at a concentration of 1.0 mg kg⁻¹), and measured blood oxygen status, cardiorespiratory variables and cardiorespiratory synchrony during a critical thermal maximum (CT_{Max}) test to examine whether temperature-dependent increases in cardiac output (*Q*) are mediated solely through heart rate (*f_H*) in fish to ensure adequate/efficient blood oxygenation. CT_{Max} was determined as the temperature at which the fish lost equilibrium, at which point, water temperature was rapidly decreased to 10°C to allow the fish to recover before heart sampling. The increasing temperature regimen itself (from 12 °C to CT_{Max}) resulted in large decreases in arterial oxygen partial pressure (P_aO₂) and content (C_aO₂) (by ~35% and 25%, respectively). Further, there was little evidence of cardiorespiratory synchrony at 12 °C, and the number of fish that showed synchrony at high temperatures only increased marginally (to 3 out of 7) despite the large decrease in P_aO₂. These results: (1) indicate that in some situations (e.g. when ventilation is exclusively/predominantly dependent on buccal–opercular pumping) the upper thermal tolerance of fish may be constrained by both cardiovascular and ventilatory performance; and (2) question the importance of cardiorespiratory synchrony (ventilation–perfusion matching) for gas exchange in salmonids, and fishes, in general.

Zatebradine injection decreased heart rate (*f_H*) at 12 °C by 11% and limited maximum *f_H* to 78.7 vs. 116.6 beats min⁻¹ in controls. However, it did not affect maximum cardiac output (due to a compensatory increase in stroke volume), ventilation, cardiorespiratory synchrony or PaO₂. In contrast, metabolic scope and CT_{Max} were lower in the zatebradine vs. control group [184.5±17.4 vs. 135.7±21.5 mL kg⁻¹ h⁻¹ (p<0.05) and 23.7±0.2 vs. 22.6±0.4 °C (p<0.08), respectively]. These effects were unrelated to maximum *f_H* or scope for *f_H*, and occurred despite higher values for blood oxygen content and haematocrit at >18 °C in the zatebradine-treated fish. These latter findings suggest that zatebradine has non-pacemaker effects that limit tissue oxygen utilization and its usefulness for *in vivo* studies. Gamperl, A.K., Swafford, B.L., Rodnick, K.J., 2011. Elevated temperature, per se, does not limit the ability of rainbow trout to increase stroke volume. *J. Therm. Biol.* 36 (1), 7–14.

Clark, T.D., Sandblom, E., Cox, G.K., Hinch, S.G., Farrell, A.P., 2008. Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*). *Am. J. Physiol.* 295 (5), R1631–R1639.

Steinhausen, M.F., Sandblom, E., Eliason, E.J., Verhille, C., Farrell, A.P., 2008. The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). *J. Exp. Biol.* 211

(24), 3915–3926.

Heath, A.G., Hughes, G.M., 1973. Cardiovascular and respiratory changes during heat stress in rainbow-trout (*Salmo gairdneri*). *J. Exp. Biol.* 59 (2), 323–338.

Portner, H.O., 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* 213 (6), 881–893.

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C8

Latency of CICR during rat cardiac action potentials

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Ca²⁺-induced Ca²⁺ release (CICR) is fundamental to cardiac excitation-contraction (EC) coupling. In this process, ryanodine receptors (RyRs) in the sarcoplasmic reticulum (SR) are activated by a small Ca²⁺ influx via L-type Ca²⁺ channels (LCCs) in the surface membrane. It is thought that a group of RyRs within a 'couplon' (Franzini-Armstrong, et. al., 1999) are responsible for Ca²⁺ sparks, whose spatio-temporal summation produces the Ca²⁺ transient and contraction. It has been shown that Ca²⁺ transients occur with no detectable delay from the appearance of 'trigger' Ca²⁺ via LCCs and Ca²⁺ spark activation is relatively synchronous under physiological conditions (Cannell, et. al., 1994). Nevertheless, it is unclear how many LCCs contribute to the trigger Ca²⁺ during APs (Altamirano and Bers, 2007) and what process determines the variability in the timing of Ca²⁺ spark activation during EC coupling.

Cardiac ventricular myocytes were obtained by enzymatic dissociation of Langendorff-perfused rat hearts. Animals were anaesthetised with a lethal dose of pentobarbital (140 mg/kg; I.P.) before any procedures were carried out (in accordance with the University of Auckland Animal Ethics Committee guidelines). Latencies of Ca²⁺ sparks and transients evoked by APs and voltage-clamp steps were measured. To determine the latency arising from LCC gating, the difference in Ca²⁺ spark timing between depolarising and repolarising steps was compared. During a repolarising step, Ca²⁺ influx is started by repolarisation from a very positive membrane potential (+100 mV) where LCCs are already open (e.g. Poláková, et. al., 2008). In the presence of 15 µM nifedipine, the mean Ca²⁺ spark latency was 5.6 ± 0.5 and 15 ± 2 ms (S.E.M., n = 11) for repolarising (no LCC delay) and depolarising steps to -15 mV, respectively. The difference between these values (~8.8 ms) is similar to the expected latency for first LCC opening (Josephson, et. al., 2010). At +5 mV, the difference in latencies decreased to ~4.5 ms, which suggests that CICR latency is dominated by the waiting time for LCC opening, rather than the increase in [Ca²⁺] produced by LCC openings (within this voltage range). Although it was only possible to examine a relatively narrow voltage range (due to the requirement to activate sufficient Ca²⁺ sparks from both depolarising and repolarising steps), the results were consistent with a simple model for CICR, where ~4 LTCCs were available in a couplon and each LCC could activate ~4 RyRs. The model can explain the observed latency of CICR during APs and suggests that CICR operates with a much

higher coupling fidelity than would be expected from previous studies (e.g. Poláková, et. al., 2008).

Altamirano, J and Bers, DM. Voltage dependence of cardiac excitation-contraction coupling: unitary Ca^{2+} current amplitude and open channel probability. *Circ Res.* 2007;101(6):590-7.

Cannell, MB, Cheng, H, Lederer, WJ. Spatial non-uniformities in $[\text{Ca}^{2+}]_i$ during excitation-contraction coupling in cardiac myocytes. *Biophys J.* 1994;67(5):1942-56.

Franzini-Armstrong, C, Protasi, F, Ramesh, V. Shape, size, and distribution of Ca^{2+} release units and couplons in skeletal and cardiac muscles. *Biophys J.* 1999;77(3):1528-39.

Josephson, I, Guia, A, Sobie, E, Lederer, J, Lakatta, E, Stern, M. Physiologic gating properties of unitary cardiac L-type Ca^{2+} channels. *Biochem Biophys Res Commun.* 2010;396(3):763-6.

Poláková, E, Zahradníková, Pavelková, J, Zahradník, I, Zahradníková, A. Local calcium release activation by DHPR calcium channel openings in rat cardiac myocytes. *J Physiol.* 2008;586(16):3839-54.

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C9

NMDA receptor stimulation triggers PFKFB3 stabilization causing pentose-phosphate pathway to glycolysis shift in primary cortical neurons

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Persistent activation of N-methyl-D-aspartate (NMDA) subtype of glutamate receptors (NMDAR) is behind the pathogenesis of several neurological disorders, such as Alzheimer's disease, amyotrophic lateral sclerosis or stroke (1). 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) is a master activator of glycolysis by its ability to synthesize fructose-2,6-bisphosphate, a potent positive allosteric effector of 6-phosphofructo-1-kinase. Previous results from our laboratory have shown that, in neurons, PFKFB3 is constantly destabilized through the ubiquitin-proteasome pathway by the E3 ubiquitin ligase anaphase promoting complex/cyclosome-Cdh1 (APC/C-Cdh1), which acts on a PFKFB3-destabilizing KEN motif (2). Upon NMDAR stimulation, Cdh1 is phosphorylated by the cyclin-dependent kinase-5 (Cdk5) and accumulates in the cytosol, thus inhibiting APC/C activity (3). To investigate whether glutamate receptor activation regulates PFKFB3 stability, here we have incubated rat cortical neurons in primary culture with glutamate or NMDA (100 μM /15 minutes each), and the PFKFB3 protein abundance was assessed several hours later. We found that NMDAR activation promoted a progressive accumulation of PFKFB3 protein through a mechanism that involved the inhibition of APC/C-Cdh1. Furthermore, NMDAR-mediated increase in PFKFB3 stability yielded neurons having a higher glycolytic, and lower pentose-phosphate pathway (PPP) rates. By regenerating NADPH cofactor, PPP activity is known to be essential at maintaining the glutathione redox status; in good agreement with this notion, we found that stimulation of this NMDAR-PFKFB3 pathway led to oxidative stress and apoptotic neuronal death. Furthermore, these effects were counteracted by overexpression of the rate-limiting enzyme of the PPP, glucose-6-phosphate dehydrogenase. Finally, expression of a mutant form of PFKFB3 lacking the KEN motif was sufficient to trigger oxidative stress and apoptotic death of neurons. Together, these results demonstrate that,

by inhibiting APC/C-Cdh1, NMDAR activation stabilizes PFKFB3, hence re-programming neuronal metabolism leading to oxidative damage and neurodegeneration.

Nat Med. 10: S2-S9 (2004)

Nature Cell Biol. 11:747-752 (2009)

EMBO J. 27:2736-2745 (2008)

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C10

1-methyl-2-phenyl-1,2,3,6-tetrahydropyridine differentially dissipates mitochondrial membrane potentials in mesencephalic, striatal and cortical astrocytes

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The neurotoxin 1-methyl-2-phenyl-1,2,3,6-tetrahydropyridine (MPTP), depletes dopaminergic neurons in the substantia nigra, leading to symptoms of Parkinsonism. MPTP is used in the animal models of Parkinson's disease where it destroys dopaminergic cells mainly in the substantia nigra, leading to depletion of dopamine in the striatum. Among the proposed mechanisms for nigral neuronal death is oxidative stress and mitochondrial dysfunction. Astrocytes play a key role in clearing potentially toxic byproducts of metabolism as well as in the process that converts the MPTP to its toxic form 1-methyl-4-phenylpyridinium (MPP⁺), which acts by interfering with complex I of the mitochondrial electron transport chain (Sayre, 1989). By measuring changes in mitochondrial membrane potential ($\Delta\Psi$), we sought to establish if astrocytes in the mesencephalon are more susceptible to MPTP toxicity than astrocytes from other brain areas.

Astrocytes were cultured from the ventral mesencephalon, striatum and cortex of neonatal C57BL/6 mice. Cells were subcultured onto 25mm coverslips and incubated at 37°C in 5% CO₂ to form confluent monolayers. Cells were then treated with 500 μM MPTP for 4, 8 or 24 hours. To measure $\Delta\Psi$, cells were washed in normal HBSS and then incubated in 10 μM JC-1 for 15 minutes. JC-1 (5,5',6,6'-tetraethylbenzimidazolecarbocyanine iodide) is a ratiometric dye that accumulates in mitochondria and forms J-aggregates when membrane potential is high (normal) and remains in a monomeric form when membrane potential is low (Reers et al., 1995). Using confocal microscopy (Biorad MRC 1024) and a 63x high NA objective, a 488 nm argon ion laser line was used for excitation while emission was simultaneously collected at 522 $\pm$ 35 nm and 585 nm corresponding to peak fluorescence from the monomer (527 nm) and J-aggregate (590 nm) of JC-1 respectively. Offline image processing was carried out to combine the green and red channels of the saved confocal image stacks. Our results showed that compared to controls (with no MPTP treatment), cortical astrocytes only showed significant dissipation of $\Delta\Psi$ after 24 hours. Striatal astrocytes' mitochondria were still highly polarized after 24 hours of exposure to MPTP. Mesencephalic astrocytes were the most affected by MPTP; within 4 hours, there was a greater degree of $\Delta\Psi$ collapse than was seen in striatal astrocytes after 24 hours of treatment. By

24 hours, all mesencephalic astrocytes showed near complete dissipation of $\Delta\Psi$. We conclude that astrocytes cultured from different brain regions differ in their susceptibility to MPTP toxicity in the order mesencephalon > cortex > striatum.

Reers M., Smiley S.T., Mottola-Hartshorn C., Chen A., Lin M., Lan Bo Chen (1995) Mitochondrial membrane potential monitored by JC-1 dye. *Methods in Enzymology*, 260, pp. 406-417.

Sayre L.M. (1989). Biochemical mechanism of action of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Toxicology Letters*, 48 (2), pp. 121-149.

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C11

Mild oxidative stress regulates astrocytic Nrf2 antioxidant pathway via a Keap1-independent mechanism

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Cells capacity to counteract oxidative stress is controlled by Nuclear factor-erythroid 2-related factor 2 (Nrf2), a transcription factor and a key regulator of antioxidant and phase II detoxification genes. Nrf2 activity is subject to proteasomal degradation promoted by Kelch-like ECH-associated protein 1 (Keap1), but small molecules like tBHQ can antagonize this interaction, enabling Nrf2 activation. Previous studies have demonstrated the neuroprotective effects of Nrf2 activation in nearby astrocytes through the use of pharmacological activators or via over-expression(1,2). However, the extent to which Nrf2 in astrocytes could respond to endogenous signals to mediate neuroprotective responses was unclear. Recently our group has demonstrated that mild oxidative stress and brief ischemic episodes activate endogenous Nrf2 in astrocytes, leading to neuroprotection (3). In this study we investigate the mechanism underlying the activation of astrocytic Nrf2 by mild oxidative stress in an *in vitro* model of primary murine cortical mixed neuron/astrocyte cultures. Contrary to the established dogma, here we will report a novel mechanism through which mild oxidative stress triggers astrocytic Nrf2 activation in a manner, which is distinct from classical Keap1 antagonism via small molecular inhibitors like tBHQ. The mechanism involves direct regulation of Nrf2's transactivation properties, and can act additively to the classical tBHQ-induced pathway, suggesting that therapeutic manipulation of Nrf2 activity may be achievable even in astrocytes suffering oxidative stress.

Kraft AD, et al. (2004). Nuclear factor E2-related factor 2-dependent antioxidant response element activation by tert-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult. *J Neurosci*. 24(5):1101-1112.

Shih AY, et al. (2003). Coordinate regulation of glutathione biosynthesis and release by Nrf2-expressing glia potentially protects neurons from oxidative stress. *J Neurosci* 23(8):3394-3406.

Bell KF, et al. (2011). Mild oxidative stress activates Nrf2 in astrocytes, which contributes to neuroprotective ischemic preconditioning. *Proc Natl Acad Sci U S A*. 4; 108(1): E1-E2.

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C12

Action potential propagation in myelinated axons of central mammalian neurons relies on calcium entry at nodes of Ranvier

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High sodium channel densities at nodes of Ranvier are essential for action potential (AP) propagation in myelinated axons, safeguarding long-range signalling. Although voltage-gated calcium channels (VGCCs) are present in unmyelinated and premyelinated axons and in axon initial segments, and are thought to mediate a calcium-activated potassium current (IKCa:1,2,3), they have rarely been observed at nodes of Ranvier and little is known about their function. We have investigated the presence and functional role of activity-dependent calcium transients at the nodes of Ranvier of cerebellar Purkinje cells (PCs). While PCs express axonal VGCCs early in post-natal development, it is unknown whether they are retained post-myelination, outside of synaptic specialisations.

Using two-photon Ca²⁺ imaging in cerebellar slices of P18-P43 mice we observed spatially-restricted (~5µm), activity-dependent Ca²⁺ influx at branch points of PC axons, which are typically nodes of Ranvier. Baseline nodal [Ca²⁺] was dependent on spontaneous action potential (AP) frequency, and Ca²⁺ transient integrals correlated with the number of APs showing that nodal [Ca²⁺] is directly related to neuronal activity. Rise times of AP-evoked nodal Ca²⁺ transients were comparable to those at the axon initial segment and soma, but nodal decay times were > two-fold faster. Ca²⁺ transients were blocked by removal of extracellular Ca²⁺ and by nickel at high (400 µM, to 14 ± 6% of control, n=4) but not low (50 µM 91 ± 9% of control n=3) concentrations, implying involvement of R-type calcium channels. Other, more selective VGCC antagonists, however, were ineffective. Simultaneous patch clamp recordings of somatic APs and axonal capacitive currents arising from the propagating APs showed that local application of calcium-free solution to branch points robustly blocked action potential propagation (100% failure of axonal spikes, n=3), indicating that nodal calcium entry is essential for AP propagation in PC axons. Local application of antagonists for VGCCs (Ni²⁺/Cd²⁺) had variable effects reducing axonal AP propagation velocity and in some cases leading to propagation failure (4/13 cells). We hypothesised that these effects on propagation resulted from reduced recruitment of a IKCa. Although antagonists of SK and BK channels had no effect, local application of TRAM-34 (500 nM), which has been shown to selectively block the intermediate calcium-activated potassium channel, KCa3.1 (4), reduced the amplitude of axonal capacitive currents, indicating a slowing of the rise time of axonal APs and reduced sodium channel availability. KCa3.1 channels have recently been shown to modulate EPSP summation in cerebellar Purkinje cells (5), and these data show that they are also involved in securing cerebellar action potential output.

Lev-Ram V & Grinvald A (1986) *PNAS* 83 6551-6555

Callewaert G, Eilers J & Konnerth A. (1996) *J Physiol* 495 641-647

Bender K & Trussell L (2010) *Neuron* 61 259-271

Wulff H, Miller MJ, Hansel W, Grissmer S, Cahalan MD & Chandy KG (2000) *PNAS* 8151-8156

Engbers JDT, Anderson D, Asmara H, Rehak R, Mehaffey WH, Hameed S, McKay BE, Kruskic M, Zamponi GW & Turner RW (2011) *PNAS* 109 2601-2606

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C13

Facebook for neurons? Helping isolated neurons make appropriate connections

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When dissociated embryonic mouse striatal neurons are mixed with neurons from cerebral cortex of the same aged embryos the resulting culture reconstructs a vital part of the brain system known as the basal ganglia. In whole brain, cortical cells make connections with striatal neurons whose properties are influenced by dopamine released from the substantia nigra. The corticostriatal system has been suggested to be the seat of motor learning and habit formation. We hoped to be able to study the properties of this simple two-neuron circuit in these cultures. Because the characteristic neuronal morphology is not well developed in culture we made the cultures from different mouse lines. One of the groups of neurons (usually cortex) came from a mouse genetically modified to express GFP in all cells (the GFP was driven by human UbiquitinC promoter; Tsirigotis et al 2001) and the other from a normal mouse line.

In this kind of culture it was relatively easy to find pairs of neurons that were connected together and to study the properties of the synaptic connections. Of the pairs recorded 45% were not connected. In total 32 pairs were connected by the expected excitatory synaptic connections from cortex to striatal neurons. However, 25 pairs were reciprocally connected and a total of 53 pairs were connected in the striatal to cortical direction. This connection never forms in brains. Although we had an interesting preparation – we could not rely on the properties of this system being under the kind of control that was present in brain. So we tried to develop a culture system in which the cortico-striatal system would make the more normal one-way connections. We have developed such a preparation and in our initial experiments we have been able to show that this time the connections are more appropriate. Cortical and striatal neurons are plated in two compartments separated by a 500µm barrier that is removed after the neurons have attached to the substrate. Over the ensuing weeks the cortical neurons grow connections across the gap to reach the striatal neurons. The striatal cell activity recorded on multi-electrode arrays are driven by cortical activity and the striatal activity is almost completely silenced by cutting the connections between the cortical and striatal neurons. If we transfect the cortical cells with Channel Rhodopsin2 then the striatal cells are driven by the light induced activity in the cortical neurons. When the striatal cells are similarly transfected we have not seen any response in cortical neurons.

This method may also be useful in other situations where the sparse nature of the in vivo connections makes the study of individual pairs of connected neurons difficult, although exactly how similar such simplified systems are to those in brain needs careful study.

Tsirigotis M, Thurig S, Dube M, Vanderhyden BC, Zhang M, Gray DA (2001)

Analysis of ubiquitination in vivo using a transgenic mouse model. Biotechniques 31:120–130

We are grateful to Dr D.A. Gray for access to his transgenic mice.

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C14

Modulation of neuromuscular synaptic degeneration by activity in WldS mutant mice

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Motor axons and their terminals undergo Wallerian degeneration (WD) following nerve injury. Here we asked whether neuromuscular activity increases vulnerability of motor axons or provides protection against WD. We used WldS mice in which WD is about ten times slower than normal (Lunn et al. 1989, Gillingwater et al., 2002). Glass capillaries containing 15mM TTX were implanted under general anaesthesia (isoflurane and medical oxygen, 0.4 litres min⁻¹; Martinov and Nja 2005). A plastic cuff secured the tip adjacent to the sciatic nerve. The efficacy of nerve block was checked daily for the following 7 days by monitoring toe-pinch reflex and grip strength. Nerve stimulation in anaesthetised mice confirmed the patency of the nerve block. One week post-implant the animals were re-anaesthetised and the tibial nerve was cut. Five days post-axotomy, intracellular recordings of spontaneous MEPPs and evoked EPPs were made in isolated flexor digitorum brevis (FDB) muscles in which action potentials were abolished with µ-conotoxin (2 µM). Preconditioning with TTX-block resulted in a decrease in the level of WldS-induced synaptic protection compared with axotomised saline-treated or unoperated controls (8.57±2.51% responsive fibres, n=7, vs. 55.56±5.88% n=3, vs. 43.34±10.11% n=6 respectively, p<0.05 one way ANOVA). TTX treatment or microcapsule implantation alone did not cause any detectable damage: electrophysiological analysis showed almost all fibres responding with EPPs (87.78±7.78% n=3 TTX-blocked; 85.00±2.15% n=4 saline-treated; 91.88±2.83% n=6 unoperated controls, p>0.05 one way ANOVA). Repeated visualisation of axons in vivo in thy1.2:YFP16-WldS mice by confocal microendoscopy (Wong et al., 2009) showed no discernible effect of TTX block on the protection of axons by WldS. Preconditioning block in C57Bl6 mice also showed a reduced number of fibres responding to stimulation 12h post-axotomy compared to axotomised controls (6.25±3.75% n=4, vs. 61.67±17.56% n=4 respectively, p<0.01 unpaired t-test). To test for a direct influence of activity on synaptic degeneration, isolated FDB nerve-muscle preparations were maintained in oxygenated mammalian saline at 32 °C for 12-48 hours. Electrophysiological analysis proved that this method adequately differentiates the rates of synaptic degeneration in wild-type and WldS preparations. After 5-15h ex vivo, fewer fibres were responsive to stimulation in WT muscles than WldS (36.39±16.02% n=6, vs. 89.61±2.93% n=11, p<0.05 unpaired t-test). Intermittent high frequency (100Hz, for 1 second every 100 seconds) enhanced synaptic degeneration, in comparison to constant low frequency (1Hz) stimulation (78.75±11.21% responsive fibres, n=4, vs. 32.67±6.86% n=4, p<0.05 unpaired t-test). The data provide evidence that vulnerability of neuromuscular synapses to stimuli that trigger degeneration is selectively enhanced by axonal disuse.

Lunn ER et al. (1989). *Eur J Neurosci* 1, 27-33
 Gillingwater TH et al. (2002). *J Physiol* 543, 739-755
 Martinov VN & Nja A (2005). *J Neurosci Methods* 141, 199-205
 Wong F et al. (2009). *Mol Cell Neurosci* 42, 296-307

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C15

Nitric oxide (NO) potentiates an SK-mediated afterhyperpolarising potential (AHP) in mouse MNTB neurons

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Nitric Oxide (NO) is a highly diffusible second messenger that is generated in the CNS by synaptic activity and Ca²⁺-dependent stimulation of neuronal nitric oxide synthase (nNOS). NO signalling modulates ion channels, intrinsic excitability and synaptic plasticity. In the light of its presence in the principal neurons of the medial nucleus of the trapezoid body (MNTB), we have investigated NO-mediated modulation of Ca_vs and small conductance K⁺ channels (SK) in mediating an afterhyperpolarization (AHP). Whole-cell patch recordings were made from brain slices from P13-15 CBA mice. Slices were incubated with the inhibitor of neuronal nitric oxide synthase (nNOS) 7-nitroindazole (10μM) and pharmacological blockers used to isolate different Ca_v currents. Unpaired observations in the presence and absence of the NO-donor sodium nitroprusside (SNP, 100μM) were made to elucidate NO-dependent modulation of Apamin sensitive AHPs. Previous investigations had revealed a NO induced potentiation of Ca_v1 and Ca_v2.1 channels (Tozer *et al.* 2012), and here we show that this increase in I_{Ca} underlies the potentiation of the Apamin sensitive I_{SK} by NO. The potentiation of I_{SK} was blocked by the P/Q-type antagonist ω-Agatoxin IVA (200nM). NO significantly enhanced AHP duration evoked following postsynaptic current injections which evoked AP trains of 400Hz, for 1s (control tau, 32.7±3.9ms (n=5); NO tau, 51.9±5.2ms (n=5, p<0.05)), and the enhanced AHP duration was blocked by 100nM Apamin (tau 35.1±3.6ms (n=4, p<0.05)) (mean±SEM, student's t-test). AHPs generated following presynaptic calyceal stimulation at 400Hz (300ms duration) in the presence of NO were also blocked by Apamin. These data suggest that in addition to modulation of voltage-gated K⁺ Channels, NO also contributes to homeostatic control of excitability, by potentiation of voltage-gated calcium channels and modulation of an SK-mediated AHP.

Tozer AJ, Forsythe ID & Steinert JR. Nitric oxide signalling augments neuronal voltage-gated L-type (Ca_v1) and p/q-type (Ca_v2.1) channels in the mouse medial nucleus of the trapezoid body. *PLoS One* 7, e32256.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C16

Nitric oxide induces a hyperpolarizing shift in the half-activation voltage of HCN1, but not HCN2 dominated currents in mouse brainstem neurons

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Nitric oxide (NO) is an activity-dependent modulator of synaptic transmission in the brain; it regulates intrinsic excitability and potentially, mediates homeostasis across neuronal populations, through a process of volume transmission. The short half-life and low effective concentrations of NO makes it difficult to study its physiological roles and underlying mechanisms. Neurons in the medial nucleus of the trapezoid body (MNTB) generate NO in response to synaptic stimulation. The surrounding nuclei receive strong inhibitory projections from the MNTB, which suggests that there is also the potential for nitrergic signalling. Here we ask whether these MNTB target nuclei are modulated via NO volume transmission.

CBA/CaJ mice (P14–21) were killed by decapitation in accordance with the UK Animals (Scientific Procedures) Act 1986. Whole-cell patch recording from brainstem slices were used to study the three MNTB target nuclei: the medial and lateral superior olive (MSO & LSO) and the superior paraolivary nucleus (SPN). The hyperpolarization-activated non-specific cation conductance (IH) was studied in current-clamp and voltage-clamp mode under control conditions and following activation of nitrergic signalling.

Hyperpolarizing current injection was used to mimic an inhibitory input and showed the characteristic slow 'sag' of IH in neurons of the MSO, LSO and SPN. Hyperpolarizing voltage steps in voltage-clamp mode showed inward IH currents activating negative to -50 mV. IH currents were smaller in the MNTB compared to the target nuclei (MNTB: 4.9 ± 0.3nS; SPN: 21.3 ± 1.3nS; MSO: 18.5 ± 1.3nS; LSO: 40.3 ± 5.7). Activation time-constants at -130mV were slower in the MNTB and equally fast in the target nuclei (MNTB: 47.5 ± 2.8ms; SPN: 28.9 ± 1.8ms; MSO: 22.8 ± 1.5ms; LSO: 25.1 ± 2.9ms). Half-activation voltages were most negative in the MNTB and most positive in the SPN (MNTB: -94.9 ± 1.8mV; SPN: -87.8 ± 0.8mV; MSO: -90.3 ± 1.4mV; LSO: -88.4 ± 1.2mV). Modulation by NO was tested by perfusion of two different NO donors (100μM SNP or DEANOONOate). NO caused a significant hyperpolarizing shift of 10mV and 15mV in IH half-activation voltage in SPN and MSO neurons, respectively. This was accompanied by a slowing of the IH activation time-constants (MSO: 29.9 ± 2.3; SPN: 51.9 ± 7.8ms). NO did not affect IH peak current amplitudes in any of the tested nuclei. The NO-induced shift of half-activation voltages was unaffected by raising intracellular cGMP but was prevented by incubation with antagonists of soluble guanylyl cyclase (1μM ODQ). Immunohistochemistry shows high expression of HCN1 subunits in MSO and SPN neurons where the greatest modulation in half-activation was also observed, consistent with a conclusion that NO modulates HCN1 but not HCN2 subunits.

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C17

Initial orthostatic hypotension: effect of α_1 blockadeN.C. Lewis¹, P.N. Ainslie¹, G. Atkinson², H. Jones² and S.J. Lucas^{3,4}

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Initial orthostatic hypotension (IOH) involves a transient fall in blood pressure (BP) (systolic BP (SBP) >40 mm Hg and/or diastolic BP (DBP) <20 mm Hg) within 15 s of standing (Wieling et al., 2007). A simultaneous decline in cerebral blood flow (CBF) also occurs upon standing, and for this reason IOH is commonly associated with the onset of syncope symptoms. The α_1 (α_1) adrenergic receptor pathway plays a vital role in BP regulation during postural stress (Cooper & Hainsworth, 2002), via vasoconstriction of the systemic vasculature. The effectiveness of this response and how it may offset the decline in CBF during IOH remains to be explored. Therefore, we examined the influence of the α_1 -adrenergic receptor pathway on the development of IOH, and associated physiological responses. We hypothesized that α_1 -adrenergic blockade would lead to an inability to correct IOH and cerebral hypoperfusion, leading to rapid symptoms of pre-syncope.

Twelve normotensive humans (aged 25±1 yrs; mean±SE) attempted to complete a 3-min upright stand, 90 min after the administration of either α_1 -blockade (Prazosin, 1 mg/20 kg body weight) or placebo. Continuous beat-to-beat measurements of middle cerebral artery velocity (MCAv; Doppler), BP (finometer), heart rate, stroke volume, cardiac output and end-tidal carbon dioxide ($P_{ET}CO_2$) were obtained. Spontaneous cerebral autoregulation was assessed during supine rest using transfer function analysis. Trial differences were analysed using paired t-tests and reported as mean±SE.

Compared to placebo, the α_1 -block reduced resting SBP, MAP and DBP by 10-14% ($P \leq 0.02$); resting mean MCAv and $P_{ET}CO_2$ did not differ ($P \geq 0.28$). Transfer function analysis revealed no trial difference in gain, phase or coherence in any frequency range ($P \geq 0.17$). All subjects experienced IOH in both trials; the mean decline in SBP and DBP in the placebo trial (-46±5 mm Hg and -42±2 mm Hg) were comparable with the α_1 -block trial (-50±4 mm Hg and -39±2 mm Hg; $P \geq 0.20$), whereas the mean decline in MCAv and $P_{ET}CO_2$ during IOH were greater in the α_1 -block trial (by 12±4 cm·s⁻¹ and 4.4±1.3 mm Hg, respectively; $P \leq 0.01$). Standing tolerance during the α_1 -block trial was reduced (75±17 s vs. 180±0 s; $P < 0.001$); with MAP and MCAv and not recovering towards baseline values (difference at end-stand: -36±5 mm Hg and -25±4 cm·s⁻¹ (block); -10±3 mm Hg and -9±3 cm·s⁻¹ (placebo)).

These findings indicate that the development of IOH was not exaggerated following α_1 -blockade; however, the related decline in MCAv was greater following the α_1 -blockade. Unlike in the placebo trial, the extent of IOH and cerebral hypoperfusion did not recover resulting in pre-syncope. Collectively these findings highlight that, because of α_1 -adrenergic impairment in the capacity to increase vascular resistance upon standing, CBF was reduced below a critical threshold, eventually contributing to the onset of syncope.

Cooper VR & Hainsworth R. (2002). *Clin Sci (Lond)*, **103**, 221-226.

Wieling W et al. (2007). *Clin Sci (Lond)*, **112**, 157-165.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C18

Haemodynamic responses to muscle metaboreceptor activation in humans

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Exercise-induced increases in blood pressure (BP) are partially attributable to the activation of sensory afferents within skeletal muscle (muscle metaboreceptors). However, whether the pressor response elicited by the muscle metaboreceptors is attributable to an elevation in cardiac output (CO) or a sympathetically mediated increase in peripheral vascular resistance (TPR) remains controversial. The equivocal findings of previous studies in this area may be in part related to differences in the mode of muscle metaboreceptor activation (i.e. during or following exercise). To examine this, 10 healthy men (age 20±0.2 years, weight 78±3 kg, height 181±2 cm; mean ± SE) were studied. Participants performed 4 handgrip trials, all at 25% maximum voluntary contraction. Trial 1 consisted of isometric handgrip (IHG, 3 min) followed by post-exercise ischaemia (PEI, 3 min) to activate muscle metaboreceptors in isolation. Trial 2 consisted of IHG (3 min) followed by a recovery period under free-flow conditions (3 min). Trial 3 consisted of ischaemic rhythmic handgrip (RHG; 1 s contraction, 1 s relaxation) to volitional fatigue, to enhance muscle metaboreceptor activation during exercise. Trial 4 consisted of RHG under free-flow conditions and matched in duration to the ischaemic RHG bout in trial 3. Heart rate (HR, ECG) and beat-to-beat blood pressure (BP, Portapres) were continuously monitored. Doppler echocardiography was used to measure stroke volume (SV). CO (HR×SV) and TPR (mean BP/CO) were calculated. Statistical analyses were performed using two-way repeated-measures ANOVA with Student-Newman-Keuls post hoc tests. Both IHG trials elicited similar increases in mean BP (+29±2 and +30±3 mmHg, $P < 0.05$ vs. baseline), CO and HR (+19±3 and +19±3 bpm, $P < 0.05$ vs. baseline) in trials 1 and 2, while TPR and SV were unchanged. Mean BP (+22±3 mmHg, $P < 0.05$ vs. baseline) and TPR were significantly elevated during isolated muscle metaboreceptor activation with PEI following IHG, while HR, SV and CO were at baseline levels. Increases in mean BP (+37±4 vs. +11±2 mmHg, $P < 0.05$ ischaemic vs. free-flow RHG), CO and HR (+21±5 vs. +7±2 bpm, $P < 0.05$ ischaemic vs. free-flow RHG) were augmented with enhanced muscle metaboreceptor activation during ischaemic RHG (trial 3), compared with RHG under free-flow conditions (trial 4). SV and TPR were unchanged from baseline during RHG under ischaemic or free-flow conditions ($P > 0.05$). These data suggest that the elevated mean BP resulting from isolated activation of muscle metaboreceptors (PEI) are secondary to increases in TPR, whereas elevations in mean BP during enhanced muscle metaboreceptor activation (ischaemic exercise) can be explained by HR mediated increases in CO. Taken together, these findings support the notion that the mode of muscle metaboreceptor activation (i.e. during vs. post exercise) is an important determinant of the resultant haemodynamic response.

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Relationship of sympathetic nerve activity to peripheral vasodilator responses in young and older men: implications for blood pressure regulation and ageing

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Background: Inter-individual variability in sympathetic nerve activity (SNA) has provided important insight into integrative mechanisms contributing to blood pressure regulation in humans. In younger individuals, higher levels of SNA are balanced by lower levels of cardiac output and less adrenergic responsiveness to a given amount of noradrenaline. Older people tend to have higher SNA and higher blood pressure, but some mechanisms may act to protect older individuals against excessive pressor effects of SNA, including decreased adrenergic responsiveness compared to younger people. In the present study, we tested the hypothesis that in young men, SNA would have a restraining effect on peripheral vasodilator responsiveness, whereas in older men this phenomenon would not exist.

Methods: 12 older men (age, mean $\pm$ SEM; 64 ± 2 yrs) and 14 young men participated (age; 24 ± 2 yrs) participated in this study. Muscle sympathetic nerve activity (MSNA; peroneal microneurography), arterial blood pressure (BP; brachial intra-arterial catheter) and forearm blood flow (venous occlusion plethysmography) were measured during 5 minutes of supine quiet rest and in response to incremental intra-arterial infusion of sodium nitroprusside (NTP; 0.5, 1.0 and 2.0 $\mu\text{g}/100$ ml/min) and acetylcholine (ACH; 2, 4 and 8 $\mu\text{g}/100$ ml forearm volume/min).

Results: Systolic BP, mean BP and MSNA were higher in older (140 ± 3 mmHg; 89 ± 4 mmHg; 34 ± 2 bursts/100 heart beats) compared to young men (128 ± 2 mmHg; 80 ± 2 mmHg; 64 ± 3 bursts/100 heart beats). Baseline forearm vascular resistance (FVR) was similar in older and younger men. There was an inverse relationship of MSNA to the change in FVR in response to 1.0 $\mu\text{g}/100$ ml forearm volume/min of NTP and 4.0 $\mu\text{g}/100$ ml forearm volume/min ACH in younger men ($r = 0.80$; $r = 0.73$, respectively; $P < 0.05$). However, there was no such relationship observed in older men ($r = 0.32$, $r = 0.30$, respectively).

Conclusions: In young men, high resting MSNA appears to limit endothelial dependant and non-endothelial dependant vasodilation. However, this apparent restraining effect of MSNA does not exist in older men. A decrease in vascular alpha-adrenergic receptor sensitivity that occurs with age may explain these findings. The lack of a restraining effect of high MSNA on forearm vasodilation may partially protect healthy older men against the detrimental effects of high MSNA on the vasculature.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C20

Regional brain blood flow in man during acute changes in arterial blood gases

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Despite the importance of blood flow on brainstem control of respiratory and autonomic function, little is known about regional cerebral blood flow (CBF) during changes in arterial blood gases. We quantified: 1) anterior and posterior CBF and reactivity through a wide range of steady-state changes in the partial pressures of CO₂ (PaCO₂) and O₂ (PaO₂) in arterial blood; and 2) determined if the internal carotid (ICA) and vertebral arteries (VA) change diameter through the same range. We used near-concurrent vascular ultrasound measures of flow through the ICA and VA, and blood velocity in their downstream arteries [the middle (MCA) and posterior (PCA) cerebral arteries]. Part A (n=16) examined iso-oxic changes in PaCO₂, consisting of three hypocapnic stages (PaCO₂ = ~ 15 , ~ 20 , ~ 30 mmHg) and four hypercapnic stages (PaCO₂ = ~ 50 , ~ 55 , ~ 60 , ~ 65 mmHg). In Part B (n=10), during isocapnia, PaO₂ was decreased to ~ 60 , ~ 44 , and ~ 35 mmHg and increased to ~ 320 mmHg and ~ 430 mmHg. Stages lasted ~ 15 minutes. Intra-arterial pressure was measured continuously; arterial blood gases were sampled at the end of each stage. There were three principal findings: 1) Regional reactivity: the VA reactivity to hypocapnia was larger than the ICA, MCA and PCA; hypercapnic reactivity was similar. With profound hypoxia (35 mmHg) the relative increase in VA flow was 50% greater than the other vessels. 2) Neck vessel diameters: changes in diameter ($\sim 25\%$) of the ICA was positively related to changes in PaCO₂ (R^2 , 0.63 ± 0.26 ; $P < 0.05$); VA diameter was unaltered in response to changed PaCO₂ but yielded a diameter increase of +9% with severe hypoxia. 3) Intra- vs. extra-cerebral measures: MCA and PCA blood velocities yielded smaller reactivities and estimates of flow than VA and ICA flow. Findings respectfully indicate: 1) disparate blood flow regulation to the brainstem and cortex; 2) cerebrovascular resistance is not solely modulated at the level of the arteriolar pial vessels; and, 3) transcranial Doppler ultrasound may underestimate measurements of CBF during extreme hypoxia and/or hypercapnia.

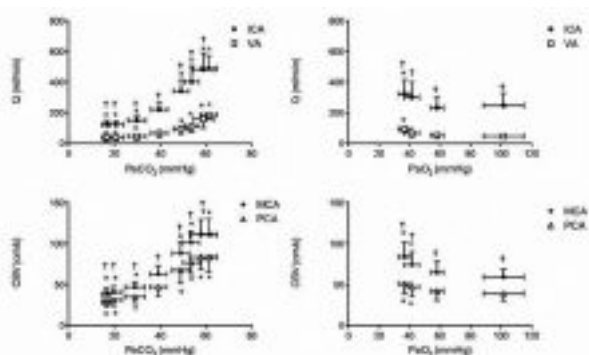


Figure 1. Blood flow (top panels; ICA and VA) and blood velocity (bottom panels; MCA and PCA) during steady state changes in arterial CO₂ (left panels) and oxygen (right panels). * Indicates difference from baseline (40 mmHg PaCO₂ or 100 PaO₂); P < 0.05. t Indicates differences between vessel flow (ICA vs. VA) or velocity (MCA vs. PCA) at a given stage; PaCO₂: P < 0.006; PaO₂: P < 0.012. All values are mean ± SD. ICA, internal carotid artery; VA, vertebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C21

Oxidative-nitrosative stress and systemic vascular function in highlanders with and without exaggerated hypoxaemia

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Background and Aims: Patients with chronic mountain sickness (CMS) are predisposed to premature cardiovascular disease and severe arterial hypoxaemia though the underlying mechanisms remain unclear (Penaloza and Arias-Stella, 2007). The present study examined if [1] CMS is characterised by exaggerated oxidative-nitrosative stress as indicated by an increased systemic accumulation of free radicals and corresponding reduction in nitric oxide (NO) bioavailability and [2] whether this was related to impaired vascular structure and function.

Methods: Thirteen male patients with primary CMS (CMS+) and 13 healthy age-matched native highlanders (CMS-) were examined at 3,600 m (La Paz, Bolivia). To place the magnitude of the oxidative-nitrosative stress response in highlanders into clearer perspective, we also examined 12 age and activity-matched healthy male lowlanders at sea-level and during acute

exposure to simulated high-altitude. A resting venous sample was obtained from a cephalic vein following a 12 h overnight fast. Oxidative-nitrosative stress was assessed using the combined application of electron paramagnetic resonance spectroscopy (ascorbate radical as a marker of global flux and spin-trapped lipid radicals as a determinant of lipid peroxidation) and (modified tri-iodide) ozone-based chemiluminescence (NO metabolites) as previously described (Bailey *et al.*, 2011). Since oxidative stress has been shown to induce vascular dysfunction (Bailey *et al.*, 2009), we also assessed flow-mediated dilatation (FMD), arterial stiffness (Alx-75) and carotid intima-media thickness (IMT).

Results: Oxidative-nitrosative stress was moderately elevated (↑ascorbate radicals, ↑lipid radicals and ↓nitrite, ↓NO) in CMS- (P < 0.05 vs. normoxic lowlanders) and comparable to that observed during acute hypoxic exposure in healthy lowlanders in whom vascular dysfunction is typically observed. Despite this, vascular function remained preserved. In contrast, oxidative-nitrosative stress was markedly exaggerated in CMS+ (P < 0.05 vs. CMS- and lowlanders) and was associated (r = 0.48 to -0.63, P < 0.05) with systemic vascular dysfunction as indicated by a lower FMD and increased Alx-75 and carotid IMT (P < 0.05 vs. CMS-).

Conclusions: These findings suggest that a moderate sustained increase in oxidative-nitrosative stress likely represents a physiological adaptive response to chronic hypoxia. However, when excessive, as in chronically hypoxaemic highlanders with CMS, it may prove maladaptive given its association with vascular dysfunction.

Clinical Trials Gov Registration # NCT01182792

Penaloza D and Arias-Stella J. (2007) *Circulation*. 2007;115:1132-1146

Bailey DM *et al.* (2009) *J Physiol*; 587: 73-85

Bailey DM *et al.* (2011) *Exp Physiol*; 96: 1196-1207

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C22

Are free radicals involved in arterial baroreflex resetting in humans during dynamic exercise?

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Increases in exercise intensity resets the arterial baroreflex (ABR) control of heart rate (HR) and mean arterial pressure (MAP) in an upward and rightward direction (Fadel, PJ and Raven, PB., 2012). This resetting is measured by changes in the operating point (OP). The mechanism for ABR resetting is unknown. We hypothesized that the antioxidant N-acetylcysteine (NAC) would decrease the OP of both HR and MAP compared to control with the same exercise intensity by reducing reactive oxygen species (ROS) centrally and in the circulation. Healthy young human volunteer subjects (n = 8, 25 ± 3.8 yrs) performed upright cycling exercise at two workloads to establish HRs of 120 (e120) and 150 (e150) bpm with and without 1200mg oral NAC. HR and beat-to-beat arterial pressure were recorded. Carotid- HR and Carotid-MAP reflex

responses were modeled using our established neck pressure and neck suction protocol during rest and exercise (Potts, JT. et al., 1993). In contrast to our hypothesis the OP of HR and MAP were significantly increased above rest in both intensities of exercise with and without NAC (2 factor ANOVA with repeated measures followed by a Newman Keuls post hoc analysis of significant main effects; HR e120 $p = 0.02$, e150 $p = 0.05$; MAP e120 $p = 0.003$, e150 $p = 0.02$) without significant interactions. However, the functional characteristics of the reflex, operating range (OR) and response range (RR), were not significantly different between treatment groups. We conclude that NAC increases production of glutathione resulting in a preservation of circulating catecholamines and increasing HR and the MAP operating point of the reflex. In order to identify whether NAC affected central sympathetic outflow requires 'in vivo' measures of muscle sympathetic nerve activity (MSNA) during rest and exercise.

Fadel PJ & Raven PB (2012). *Exp Physiol*.97, 39-50

Potts JT et al. (1993). *AJP-Heart* 265, H1928-H1938

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C23

Exercise-induced cerebral oxygenation is preserved in the trained state

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Background and Aims: It has been suggested that a decrease in brain oxygenation participates in the termination of exercise, especially in hypoxia (Subudhi et al. 2009; Vogiatzis et al. 2011). Moreover, there is growing evidence suggesting that the cerebral vasculature of aerobically fit subjects (TRAINED) is more reactive than that of their sedentary counterparts (SEDENTARY) (Ainslie et al. 2008). Despite these facts, very little is known regarding the respective responses of these groups to maximal exercise. Therefore, the primary objective of the present study was to verify if TRAINED would have a greater increase in cerebral oxygenation than SEDENTARY during exercise.

Methods: TRAINED ($n=9$) and SEDENTARY ($n=9$) responses to a standardised incremental test to volitional exhaustion were compared. Breath-by-breath gas exchanges, middle cerebral artery blood velocity (MCAv, Transcranial Doppler) and concentration in left prefrontal cortical oxyhaemoglobin (cO2Hb) (near-infrared spectroscopy) were monitored throughout the test performed on a semi-recumbent cycle ergometer. Relative changes in cO2Hb and MCAv were recorded throughout the test. Data were analysed using a 2-way repeated measures ANOVA (intensity x group) and Bonferroni corrected paired and independent samples t-tests.

Results: MCAv increased steadily in both groups from rest up to a peak at 70% of oxygen uptake at exhaustion (VO2exhaust) before decreasing back towards resting level (Figure 1A). Despite similar trends towards an increase during submaximal exercise, TRAINED MCAv was offset compared to SEDENTARY. This may be explained by the interaction effect that was identified. TRAINED cO2Hb also increased progressively throughout the submaximal intensities and continued to increase until

exhaustion. On the contrary, SEDENTARY cO2Hb did not increase significantly at any point, leading to a significant difference between groups from 40% onwards (Figure 1B). Finally, the changes in cO2Hb between rest and exhaustion were positively correlated with VO2exhaust ($n=18$, $P<0.05$).

Conclusions: These findings suggest that in the first instance the brain copes with exercise by self-regulating increased blood flow. At intensities >70% of VO2exhaust, while MCAv decreased due to the hyperventilatory-induced hypocapnia (Ogoh & Ainslie 2009), cO2Hb increased further in the TRAINED suggesting that the brain switches from more flow to more extraction. This can be seen as a protective mechanism since a hyperperfusion could potentially induce a failure of the blood-brain barrier. From a clinical point of view, this adaptive response of the 'trained brain' seems to confirm the neuroprotective effect of chronic exercise.

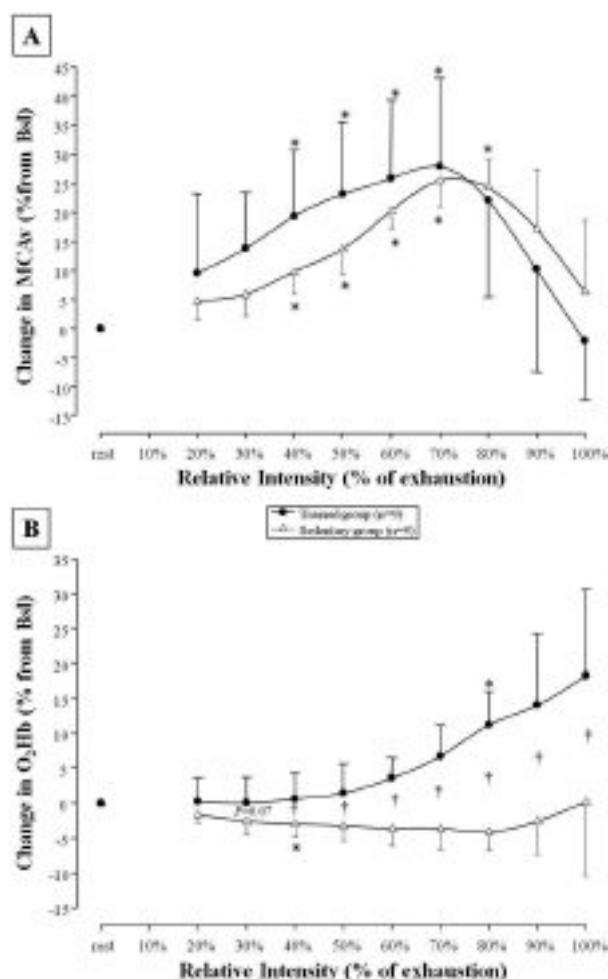


Figure 1. Percent changes from rest in middle cerebral artery blood velocity (MCAv, A) and prefrontal cortical oxyhaemoglobin (cO2Hb, B) during exercise. Values are mean $\pm$ SD. * $P<0.05$ vs. rest; † $P<0.05$ TRAINED vs. SEDENTARY.

Ainslie PN et al. (2008) *J Physiol*; 586, 4005–4010

Ogoh S & Ainslie PN (2009) *J Appl Physiol*; 107, 1370–1380

Subudhi AW et al. (2009) *J Appl Physiol*; 294, H164-171

Vogiatzis I et al. (2011) *J Physiol*; 589, 4027-4039

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C24

Dynamic cerebral autoregulation in sepsis

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Sepsis, the systemic inflammatory response to infection, is frequently complicated by brain dysfunction, which may involve disturbances in dynamic cerebral autoregulation (dCA) (Berg et al. 2011). We have previously established lipopolysaccharide (LPS) infusion as a human-experimental model of systemic inflammation that mimics the early stages of sepsis (Taudorf et al. 2007). We hypothesised that LPS infusion would impair dCA in healthy volunteers, and that patients suffering from full-scale sepsis would exhibit a similar pattern.

Nine healthy male volunteers aged 23 (mean, SD 2) years, and 16 septic patients aged 59 (mean, SD 12; one female) years were enrolled in the study. Volunteers underwent a four-hour intravenous infusion of LPS (total dose 2 ng/kg). A 20-minute recording of invasive mean arterial blood pressure (MAP) and middle cerebral artery blood flow velocity (MCAv) measured by transcranial Doppler ultrasonography was performed at 1 kHz for evaluating dCA prior to and immediately after LPS infusion in volunteers, and within 72 hours of the sepsis diagnosis in patients. Transfer function gain, phase and coherence function between MAP and MCAv were calculated in the low frequency range (0.07–0.20 Hz) (Zhang et al. 1998). A decrease in coherence and gain, and an increase in phase were interpreted to reflect improved dCA and vice versa. Data are reported as median (interquartile range).

LPS infusion induced a systemic inflammatory response with leukocytosis, fever, flu-like symptoms (all $P < 0.001$), and increases in arterial cytokine levels (Figure). This was accompanied by increased heart rate, a decrease in MAP (both $P < 0.05$), as well as a hyperventilatory response (Baseline PaCO₂: 5.4 [5.3–5.6] kPa; LPS PaCO₂: 4.8 [4.6–5.0] kPa; $P < 0.001$). A decrease in gain and coherence, and an increase in phase were observed after LPS; in patients, both coherence and phase were lower than in healthy volunteers (Table).

The cerebral haemodynamic aberrations may differ between the early and advanced stages of sepsis; contrary to our working hypothesis, the present findings indicate that dAR is improved after LPS-infusion, which is likely explained by hyperventilation, where the picture is less clear in patients.

Transfer function analysis of dynamic cerebral blood flow autoregulation after lipopolysaccharide (LPS) infusion in healthy volunteers and in patients with sepsis.

Transfer function	Healthy volunteers (baseline)	Healthy volunteers (LPS)	Patients
Gain (cm sec ⁻¹ mmHg ⁻¹)	1.17 (1.06–1.33)	0.87 (0.77–0.88)*	1.36 (0.79–1.50)
Phase (radians)	0.72 (0.66–0.84)	1.14 (1.10–1.35)**	0.22 (0.03–0.59)**
Coherence (unitless)	0.69 (0.66–0.74)	0.38 (0.33–0.64)*	0.14 (0.11–0.26)*

Data are presented as median (interquartile range). Paired comparisons between baseline and after LPS infusion were performed by Wilcoxon signed rank sum test, and comparisons between healthy volunteers and patients by means of the Mann-Whitney U-test. * $P < 0.05$ vs. Baseline; ** $P < 0.01$ vs. Baseline; † $P < 0.05$ vs. LPS; ‡ $P < 0.01$ vs. LPS.

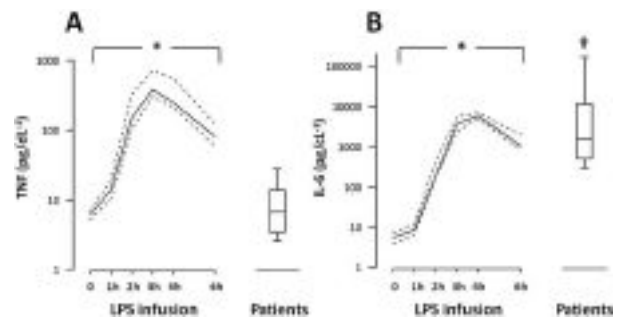


Figure. Arterial cytokine levels after lipopolysaccharide (LPS) infusion in healthy volunteers and in patients with sepsis. A blood sample was obtained from a radial artery catheter at baseline (0), and hourly during a four-hour LPS infusion, as well as 2 hours after cessation of the infusion, and within 72 hours after the sepsis diagnosis had been established in patients. A: tumour necrosis factor α (TNF); B: interleukin-6 (IL-6). Data are presented as median (black line) and interquartile range (stippled lines) in healthy volunteers, and as median (horizontal line), interquartile (box) and total (whiskers) range in patients. * Effect of time, $P < 0.001$ (Mixed model); † Different from healthy volunteers at baseline, $P < 0.001$ (Mann-Whitney U-test).

Berg et al. (2011). *J Cereb Blood Flow Metab* 31, 1532–1544

Taudorf et al. (2007). *Clin Vaccine Immunol* 14, 250–255

Zhang et al. (1998) *Am J Physiol* 274, H233–41

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C25

Liver-specific Deletion of Protein Tyrosine Phosphatase (PTP) 1B Improves Endothelial Dysfunction and Cardiovascular Alterations Associated with obesity in mice

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Obesity is tightly associated with insulin resistance and endothelial dysfunction. It is suggested that hepatic IR is sufficient to promote progression to cardiovascular disease. We showed recently that specific deletion of liver PTP1B (L-PTP1B), a negative regulator of insulin receptor, improves whole body glucose and lipid homeostasis. Thus, we propose to investigate the impact of these improvements in L-PTP1B^{-/-} mice, on cardiovascular function in the context of diet-induced obesity. Glucose tolerance test was performed on overnight fasted mice by measuring blood glucose values in tail vein bleeds using a LifeScan glucometer immediately before and at 15, 30, 60, and 120 minutes after intraperitoneal injection of glucose. Blood pressure was measured in vivo on conscious mice using a non-invasive tail cuff blood pressure monitoring system (Penlab). Cardiac function was assessed by echocardiography on anesthetized mice (Vevo 2000, Visual Sonics). Vascular function, in L-PTP1B^{-/-} and control mice, fed a chow or high fat diet (HFD) was assessed by myography technique (DMT).

Compared to control littermates, L-PTP1B^{-/-} mice had improved glucose and lipid homeostasis without changes in body mass. HFD feeding increased systolic blood pressure in both mouse groups; however, it was lower in L-PTP1B^{-/-} mice. Structure and function analysis of the left ventricle showed that HFD-feed-

ing decreased cardiac index in control mice, while L-PTP1B^{-/-} mice were fully protected. HFD feeding significantly impaired endothelium-dependent relaxation in response to acetylcholine in aortas taken from control mice compared to chow diet-fed mice, while L-PTP1B^{-/-} mice were protected. Altogether, these data indicate that targeting L-PTP1B may be useful to reduce obesity-associated cardiovascular risk in addition to diabetes.

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C26

Regulation of insulin-stimulated nitric oxide synthesis by tumour necrosis factor alpha in human aortic endothelial cells

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Type 2 diabetes is associated with increased risk of atherosclerosis, yet the molecular mechanisms that underlie this remain poorly characterised. Endothelial dysfunction, characterised by reduced nitric oxide (NO) bioavailability, is a key early event in the development of atherosclerosis [1]. Insulin has been proposed to contribute to the maintenance of vascular health, and we and others have demonstrated that insulin stimulates NO synthesis in cultured human endothelial cells via PKB/Akt-mediated phosphorylation of endothelial NO synthase (eNOS) at Ser615 and Ser1177 [2,3]. Increased levels of the pro-inflammatory cytokine tumour necrosis factor- α (TNF α) are observed in type 2 diabetes, and TNF α has previously been reported to inhibit insulin-stimulated NO synthesis and eNOS Ser1179 (equivalent to Ser1177) phosphorylation in bovine endothelial cells [2], yet the regulation of insulin-stimulated NO synthesis by TNF α in human endothelial cells remains poorly characterised. We therefore investigated the regulation of insulin-stimulated signalling pathways and NO synthesis by TNF α in cultured human aortic endothelial cells (HAECs). HAECs were preincubated with TNF α (10 ng/ml) for 0.5 or 6h prior to stimulation with insulin (15min, 1 μ M). NO synthesis and phosphorylation of insulin signalling proteins was assessed as described previously using a Sievers 280A NO meter and by immunoblotting respectively [3]. Preincubation of HAECs with TNF α for either 30min or 6h significantly inhibited insulin-stimulated NO production (40.4 $\pm$ 4.9% and 30.8 $\pm$ 15.9% respectively, n=3, p<0.05), yet only preincubation for 6h with TNF α was associated with reduced insulin-stimulated phosphorylation of Akt at Ser473 and eNOS at Ser1177 (55.0 $\pm$ 9.3% and 34.3 $\pm$ 12.6% respectively, n=4, p<0.05). As these data indicate reduced insulin-stimulated NO synthesis in the absence or reduced insulin-stimulated eNOS phosphorylation after 0.5h preincubation with TNF α , we examined the role of reactive oxygen species (ROS) in the acute action of TNF α . Incubation of HAECs with TNF α for either 0.5h or 6h significantly stimulated NADPH-dependent lucigenin chemiluminescence (414 $\pm$ 139% and 230 $\pm$ 63% respectively, n=3, p<0.05), and 30 min preincubation with the antioxidant tiron (1mmol/l) or gp91 ds-tat (50 μ M), an inhibitor of NADPH oxidase, ablated the inhibition of insulin-stimulated NO synthesis by TNF α . Taken together, these data indicate that TNF α inhibits insulin-stimulated NO synthesis via a mechanism involving the NADPH oxidase-mediated stimu-

lation of ROS and that only prolonged TNF α stimulation causes any significant downregulation of the insulin-stimulated eNOS phosphorylation via Akt. Inhibition of insulin-stimulated NO synthesis by TNF α by these mechanisms may contribute to endothelial dysfunction in subjects with type 2 diabetes.

Potenza MA et al. (2009) *Curr Med Chem* **16**, 94-112.

Kim F et al. (2001) *Am J Physiol Cell Physiol* **280**, C1057-C1065.

Ritchie SA et al. (2010) *Biochem J* **426**, 85-90.

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C27

Involvement of A_{2A} adenosine receptors in the insulin-increased L-arginine transport in human umbilical vein endothelium

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The endogenous nucleoside adenosine causes vasodilatation of human placenta vasculature [1] via a mechanism involving increased L-arginine uptake via cationic amino acid transporters 1 (hCAT-1) due to A_{2A} adenosine receptors (A_{2A}AR) activation in human umbilical vein endothelial cells (HUVEC) [2]. hCAT-1 activity and expression is increased by insulin in HUVEC [3], and A_{2A}AR activation increases insulin sensitivity in subjects with insulin resistance [4]; however, a potential A_{2A}AR involvement in L-arginine transport modulation by insulin in HUVEC is unknown [5]. The aim of this study was to determine whether insulin-stimulation of hCAT-1-mediated L-arginine transport involves A_{2A}AR in HUVEC. Methods: Primary cultured HUVEC (passage 2) from full-term normal pregnancies were used. Insulin (1 nM, 8 hours) was assayed on hCAT1 expression (SLC7A1 promoter activity (firefly/renilla luciferase reporter activity for pGL3-hCAT1⁻¹⁶⁰⁶ and pGL3-hCAT1⁻⁶⁵⁰ constructs), mRNA expression (quantitative real time PCR), protein abundance (Western blot) and L-arginine transport (0-1000 μ M L-arginine, 3 μ Ci/ml L-[³H]arginine, 1 minute, 37 °C). Assays were done in absence or presence of 4-(2-[7-amino-2-[2-furyl]-[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl-amino]ethyl)phenol (ZM-241385, 10 nM, A_{2A}AR antagonist), 2-[p-(2-carbonyl-ethyl)-phenylethylamino]-5'-N-ethylcarboxamidoadenosine (CGS-21680, 30 nM, A_{2A}AR agonist) and/or S-(4-nitrobenzyl)-6-thio-inosine (NBTL, 10 μ M, adenosine transport inhibitor). Results: Insulin and NBTL increased (P<0.05, ANOVA, n=5-10 different cell cultures) extracellular adenosine concentration, the maximal velocity (but not the apparent Km) for L-arginine transport, and hCAT-1 expression (protein and mRNA). These effects were blocked by 10 nM ZM-241385. ZM-241385-inhibited SLC7A1 reporter transcriptional activity was similar in cells transfected with pGL3-hCAT-1⁻¹⁶⁰⁶ or pGL3-hCAT-1⁻⁶⁵⁰ constructs, and comparable to the activity determined for pGL3-hCAT-1⁻⁶⁵⁰ construct in presence of NBTL + insulin. However, reporter activity was increased by NBTL only in cells transfected with pGL3-hCAT-1⁻¹⁶⁰⁶, and the ZM-241385 sensitive fraction of NBTL response was similar in absence or presence of insulin. Conclusion: hCAT-1 expression and activity is under regulation by insulin via a mechanism requiring functional A_{2A}AR in HUVEC. These findings could be determinant in diseases asso-

ciated with fetal insulin resistance, such as gestational diabetes.

Read MA. et al. (1993). Br J Pharmacol 110:454-460.

Vásquez G. et al. (2004). J Physiol 560:111-122.

González M. et al. (2011) J Cell Physiol 226:2916-2924.

Ciaraldi TP. et al. (1997) J Clin Endocrinol Metab 82:1421-1425.

Guzmán-Gutiérrez E. et al. (2011) Curr Vasc Pharmacol 9:750-762.

Study supported by CONICYT (ACT-73 PIA, AT-24100210), FONDECYT (1110977, 1120928, 11110059) Chile. EG-G, FW, PA and CS hold CONICYT-PhD fellowships. CS and PA hold Faculty of Medicine, Pontificia Universidad Católica de Chile-PhD fellowships.

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C28

Effects of maternal dietary low fat on vascular function in offspring

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Human populations exhibit temporal and cross-sectional variations in fatty acid intake. Variations in dietary saturated (butter, SFA), polyunsaturated (PUFA) and *trans* fatty acid (TFA) intakes altered cardiovascular disease risk in humans¹. In rats, maternal diets with high levels of fat induce vascular dysfunction², while maternal omega-3 PUFA deficiency is also associated with hypertension in adult offspring³. However, to date there is no information about the effect of maternal diets with sub-optimal total fat content on vascular function in the offspring.

Female rats were fed either low (LF, 3.5% w/w) or adequate (AF, 7% w/w) safflower oil (SAO, enriched in linoleic acid), hydrogenated soybean oil (HSO, enriched in TFA), butter (enriched in SFA) or fish oil (FO, enriched in eicosapentaenoic and docosahexaenoic acids) from two weeks prior to mating until offspring were weaned at day 28 onto AIN93M (4% soybean oil w/w). Vascular constriction to phenylephrine (Pe 1nM to 100µM) and endothelium-dependent relaxation to acetylcholine (ACh 0.1nM to 10µM) were measured *ex vivo* in thoracic aortae of 70 day old offspring by wire myography (n=6 per group). Statistical analysis was performed on pEC₅₀ and % maximum responses using 2-way ANOVA with Tukey's *post hoc* test.

There were no significant differences by amount or type of maternal total dietary fat in constriction of offspring aortae to Pe. There was a significant interaction (p = 0.002) effect of amount and type of fat on relaxation of female aortae to ACh, but there were no individual group differences in males or females.

These data show that low maternal dietary fat does not affect aortic constriction to phenylephrine, nor does it affect endothelium-dependent relaxation. These data suggest that, unlike maternal high fat diets, maternal low fat diets do not induce vascular dysfunction in offspring. The findings, if confirmed in humans, would inform dietary advice offered to pregnant mothers.

Reddy KS (2002). Public Health Nutr 5, 231-7

Kelsall CJ et al (2012). PLoS One (in press)

Weisinger HS et al. (2001). Nat Med 7, 258-9

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C29

Role of superoxide anion on endothelial dysfunction in an experimental model of preeclampsia

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Background: We previously described that 2ME have protective vascular effects in males and estrogen-deprived females (Bonacasa, 2008; Fenoy, 2010). Cathecol-O-methyltransferase (COMT) introduces a methyl group to 2-hydroxyestradiol to form 2-methoxyestradiol (2ME). Preeclampsia is a major obstetric problem and a significant source of maternal and fetal morbidity and mortality (Sibai, 2005), complicating an estimated 2% of all pregnancies (Duley, 2003). A key factor connecting the angiogenic imbalance with generalized endothelial dysfunction in preeclampsia has been suggested to be a reduction in the synthesis of 2ME (Kanasaki, 2008). Aim: The present study evaluated whether COMT inhibition in pregnant rats results in endothelial dysfunction as a consequence of a high levels of oxidative stress and a reduction of nitric oxide bioavailability. Methods: Pregnant Sprague Dawley rats were given entacapone (a COMT inhibitor) by gavage from the 10th to the 20th day of pregnancy at a dose of 150 mg/kg.day. Endothelium-dependent relaxation of pressurized mesenteric small arterial vessels was assessed in the absence and presence of 50 µM tempol (a superoxide anion scavenger). Nitric oxide release in aorta by electrochemical NO measurements in response to a calcium ionophore (A23187) was assessed in the absence and presence of tempol as well. Results: Vessels from entacapone-treated pregnant rats had diminished relaxations to acetylcholine compared with vehicle-treated pregnant rats. The presence of tempol improved endothelial function (Log EC₅₀ : - 7.85 + 0.27 vs. - 6.96 + 0.13). Endothelial release of nitric oxide induced by A23187 was significantly greater in aortas from vehicle-treated pregnant rats (209.8 + 17 nM) than in pregnant rats given entacapone (159 + 11 nM), and was prevented by addition of tempol (279.17 + 27 vs 212.5 + 19 nM). Conclusion: The present study suggests an important role of superoxide anion in endothelial dysfunction observed in this COMT inhibition model of preeclampsia.

Bonacasa B, et al. (2008) Maturitas; 61(4):310-6.

Fenoy FJ, et al. (2010). Nitric Oxide; 23(1):12-9.

Sibai B, et al. (2005). Lancet; 365(9461):785-99.

Duley L. (2003). Br Med Bull.; 67:161-76.

Kanasaki K, et al. (2008) Nature; 453(7198):1117-21.

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C30

Oxidative stress decreases hCAT-1 protein abundance and L-arginine transport in human fetal endothelium

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Oxidative stress plays a key role in vascular diseases such as hypertension, atherosclerosis, diabetes mellitus and cardiomyopathy. This occurs when intracellular levels of reactive oxygen species (ROS) increase, decreasing the bioavailability of nitric oxide (NO), a vasodilator synthesized from L-arginine (Sobrevia & González, 2009). This amino acid is incorporated into endothelial cells by cationic amino acid transporters (hCAT-1 and hCAT-2B) (González *et al.*, 2004). The effects of ROS on activity and/or expression of hCATs are not fully understood. Our aim was determine the effects of chronic incubation with hydrogen peroxide (H₂O₂) on hCATs expression and L-arginine transport in human endothelium.

Biological samples were obtained from normal pregnancies (ethics committee approval and informed patient consent were obtained). Human umbilical vein endothelial cells (HUVEC) were isolated by collagenase digestion (37 celsius degree) and cultured in medium 199 (M199) supplemented with 20% newborn and fetal calf sera. Cells were incubated (24 h) with H₂O₂ (10⁻¹²-10⁻³ M). The mRNA levels of hCAT-1, hCAT-2B and 28S were determined by real time PCR. The protein abundance of hCAT-1 was determined by western blot. L-Arginine uptake (100 µM) and kinetic parameters of L-arginine transport (31.25-1000 µM L-arginine, 2 µCi/mL L-[³H]arginine) was determined. Results showed that H₂O₂ (10⁻¹²-10⁻⁶ M) increased (ANOVA unpaired Student's t test, *P*<0.05, n=5-10) the mRNA levels of hCAT-1 and hCAT-2B. The protein abundance of hCAT-1 was decreased (65±13 % from control) by H₂O₂ (1 µM). H₂O₂ (0.1 µM) decreased the *V*_{max} of L-arginine transport from 1.6±0.3 (pmol/µg protein/min)(control) to 0.3±0.09 (pmol/µg protein/min), without changes in the apparent *K*_m (100-300 µM). The L-arginine transport capacity (*V*_{max}/*K*_m) was decreased from 12.8±3.2 (fmol/µg protein/min/µM) (control) to 2.3±0.3 (fmol/µg protein/min/µM) by 0.1 µM H₂O₂. In conclusion, the chronic incubation of HUVECs with H₂O₂ induces a decrease of L-arginine transport mediated by a lower expression of hCAT-1. However, the mRNA levels of hCAT-1 and hCAT-2B are increased by the same concentration and time of incubation of H₂O₂. Is possible that the oxidative stress activates a transcriptional mechanism to try to restore the cellular capacity of transport L-arginine for maintenance the cellular metabolism.

Sobrevia L & González M. (2009) *Curr Vasc Pharmacol* **7**, 467-474.

González M *et al.* (2004). *Pflugers Arch* **448**, 383-394.

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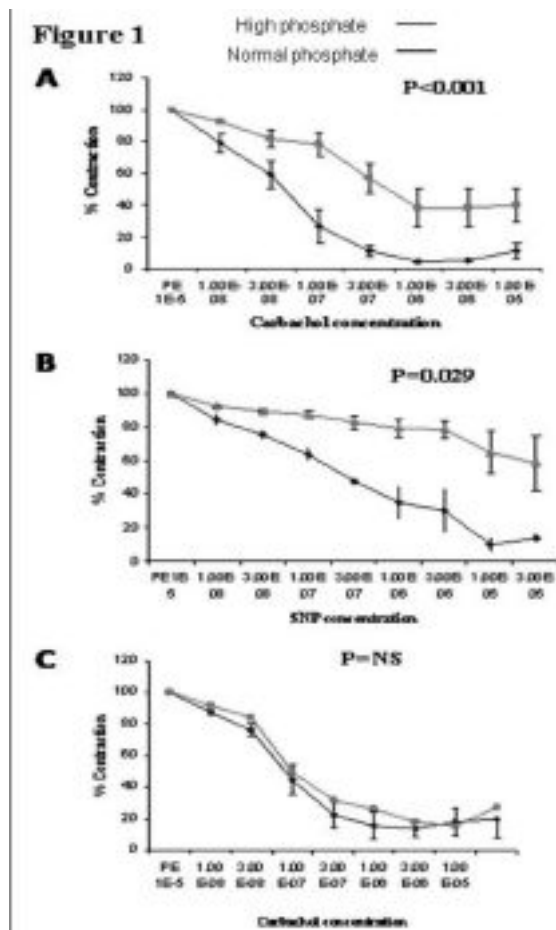
C31

Zaprinast can correct phosphate induced impaired relaxation in rat resistance vessels

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Introduction: Elevated serum phosphate is an independent risk factor for cardiovascular disease although the mechanism of action is unclear. This study looks at the effect of altered phosphate concentration in rat resistance vessels and rat smooth muscle (SMCs) and endothelial cells (ECs). Methods: 12 week old male WKY rats were sacrificed (schedule 1). 3rd order resistance vessels were dissected from the mesentery and incubated at 4°C for 16 hours in physiological saline solution (PSS) with normal (1.18mM) or high(2.5mM) phosphate concentration. Vessels were mounted on a wire myograph, normalized and assessed for the presence of an intact endothelium. Vasoconstriction response to increasing concentrations of phenylephrine (PE) and vasorelaxation response to increasing concentrations of carbachol were measured in the presence and absence of a phosphodiesterase 5 inhibitor, zaprinast. Vessels were then pre-constricted a second time with PE, again in the presence and absence of zaprinast, and vasorelaxation response to sodium nitroprusside (SNP) was measured. Concentration-response curves were constructed for each vessel for PE, carbachol and SNP +/- zaprinast. Area under the curve was calculated for each vessel and comparisons were made between the vessels in high and normal phosphate PSS and +/- zaprinast using a Student's t test in SPSS. SMCs and ECs were grown in normal (0.5mM) and high (3mM) phosphate medium and used at passage 6-8. Cell lysates were obtained and Western blot was performed for total and phospho eNOS expression. Calcium concentration was measured in the cells by epifluorescence with FURA 2 AM. Results: Vessels in high phosphate relax less well in response to carbachol and SNP than those in normal phosphate (*p*<0.001 and *p*<0.05; Figure 1A and B). The contractile response to PE was similar and unaltered by the addition of zaprinast. The addition of zaprinast significantly improved the relaxation response of vessels to carbachol and SNP in both high and normal phosphate concentration PSS. This was most marked in the vessels in high phosphate (*p*<0.05) resulting in no significant difference between the relaxation response to carbachol and SNP between vessels in normal and high phosphate (Figure 1C). Expression of eNOS was lower in ECs but unchanged in SMCs grown in high phosphate compared with those in normal phosphate. Intracellular Ca²⁺ concentration was not significantly different in cells in high phosphate medium (228.3±21.97nM vs 278±22.44nM). Conclusion: Elevated phosphate decreases both endothelium dependent and independent vasorelaxation, possibly secondary to altered cGMP signaling. These effects seem to be independent of changes in calcium. These experiments mimic a uraemic state and may offer an explanation for elevated serum phosphate as a cardiovascular risk factor.



Darlinda's Charity for Renal Research

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C32

A new slow releasing, H₂S generating compound, GYY4137 relaxes human and rat pregnant myometrium

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To reduce pre-term delivery, new pathways and drugs that reduce uterine contractility are of interest. Hydrogen sulfide (H₂S) is produced in vivo from L-cysteine, by cystathionine β -synthase (CBS) and cystathionine γ -lyase (CBE). At least two enzymes degrade H₂S; thiosulfate sulphur transferase (TST) and Thiol S- methyltransferase. Thus H₂S will be regulated within cells. NaHS, which releases a rapid bolus of H₂S, reduces myometrial contractility. However it is not clear if an H₂S-generating system is present throughout gestation or if more physiological modes of H₂S production can affect contractility. We have therefore: (1) examined throughout gestation, the effects on myometrial contractility of GYY4137 (GYG), a novel compound that slowly releases H₂S, (2) compared GYG effects to those of NaHS in rat and human tissues, (3) investigated effects on Ca signalling, and (4) determined the myometrial expression of enzymes governing tissue H₂S levels. Methods: Myometrial strips from biopsies obtained, with consent, from women undergoing term caesarean sections or hysterectomy, or non-pregnant (NP), 14, 18 or 22 day (term) pregnant and labour-

ing rats were studied. Effects on contractility in response to GYG (1nM-1mM, Cayman chemicals) and 1mM NaHS were examined. Immunohistochemistry and western blotting were used to examine the expression of CBS, CSE and TST. Results: There was a significantly greater inhibitory effect of GYG and NaHS in pregnant compared to NP rat and human myometrium. The inhibitory effects of GYG and NaHS on contractility increased throughout gestation; however, in labouring myometrium neither GYG nor NaHS could reduce contraction. Thus at day 14, 18, 22 day and labouring, 1 mM GYG reduced amplitude (100%, control) to $78 \pm 10\%$, $52 \pm 13\%$, $38 \pm 6\%$ and $106 \pm 5\%$, $n=6$, mean + sem. In term pregnant human myometrium the reduction by 1 mM GYG was $40 \pm 16\%$, $n=5$, and by NaHS was $9 \pm 2\%$, $n=7$. GYG's effects were dose dependent with an EC₅₀ of $0.7 \pm 2 \mu M$. Underlying calcium transients were decreased in the presence of GYG (amplitude and frequency, $n=4$, $p < 0.01$, day 22). Immunohistochemistry showed CBS and CSE in human and rat myometrium ($n=3-7$) and preliminary Western blot data indicates increased expression in NP compared to term myometrium ($n=4$). TST was not found in the myometrium ($n=4$ pregnant human and rat). Conclusions: NaHS and GYG produce uterine relaxation in a dose-dependent manner. Their effects increased throughout gestation, perhaps due to changes in H₂S removal rates, which we found not to be via TST. Our data suggest that H₂S contributes to uterine quiescence until labour onset. GYG decreased Ca transients, suggesting it affects L-type Ca channels, perhaps via sulphydration of residues. These data suggest that H₂S is an attractive target for therapeutic manipulation of human myometrial contractility and drugs such as GYG will be effective.

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Thirst perception, plasma osmolality and plasma arginine vasopressin concentration in dehydrated and oral saline loaded subjects

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Infusion of hypertonic saline produces a gradual increase in thirst appreciation throughout a wide range of plasma osmolality (Thompson et. al., 1986) as well as increase in plasma AVP concentration (Philips et. al., 1985). Plasma AVP concentration may be estimated using thirst perception and/or plasma osmolality values (Obika et. al., 2011). In this investigation, we further examined the changes in thirst perception, TP, plasma osmolality, Posm, and plasma arginine vasopressin concentration, PAVP, in dehydrated and oral saline loaded subjects.

Forty (40) healthy subjects between the ages of 18-30 years were divided into 4 groups of 10 subjects (5 females and 5 males) each. Subjects in group A (dehydrated) were subjected to 18 hr fluid restriction, while those in B, C, and D orally ingested 7.1 ml/kg of 0.9%, 1.8% and 2.7% NaCl solutions respectively. TP was rated using the Visual Analogue Scale, VAS. Estimation of Posm was done using the standard formula: $2(Na^+ + K^+) + Urea + Glucose$ and was also calculated from TP (Thompson et. al., 1986). PAVP was calculated from TP (Igbokwe and Obika, 2008) and Posm, Thompson et. al. (1986). The results in females showed that there was no significant difference between the measured and calculated Posm at 0

min, in groups A and D, whereas, values for group B (278.8 ± 4.2 vs 292.0 ± 2.9 mOsm/kgH₂O) and C (282.6 ± 4.1 vs 295.6 ± 2.5 mOsm/kgH₂O) differ significantly ($p < 0.05$). At 90 min, the calculated Posm was significantly higher than the measured Posm in group D (283.6 ± 2.1 vs 312.0 ± 0.8 mOsm/kgH₂O, $p < 0.05$) only. In male subjects, there was no significant difference between the measured and calculated Posm in all the groups at 0 min, and at 90 min in groups A and B, while the calculated Posm was significantly higher ($p < 0.05$) than the measured Posm in groups C (283.0 ± 4.6 vs 302.4 ± 3.3 mOsm/kgH₂O) and D (275.6 ± 4.8 vs 307.2 ± 2.6 mOsm/kgH₂O). In both male and female subjects, there was a significant ($p < 0.05$) and positive correlation between TP and measured Posm at 0 min, but not at 90 min. PAVP values correlated significantly ($p < 0.05$) with Posm and TP at 90 min only in group A. When group A was compared to group B, the changes in Posm were significant ($p < 0.05$) in the males at 0 min (298.0 ± 1.6 vs 281.0 ± 4.4 mOsm/kgH₂O), and at 90 min (303.0 ± 1.8 vs 270.6 ± 11.0 mOsm/kgH₂O), whilst in the females the changes were only significant at 90 min (304.4 ± 3.5 vs 285.0 ± 4.3 mOsm/kgH₂O). In conclusion, the relationship between TP, Posm and PAVP may not be maintained during oral saline loading, probably due to body fluid changes.

Igbokwe, VU and Obika, LFO. (2008). Afr. J. Biomed. Res., 11: 39-46.

Obika, LFO., Amabebe, E and Idu, FK. (2011). Proc. Physiol Soc., 24. C07 and PC07.

Phillips, PA., Rolls, BJ., Ledingham, JG., Forsling, ML and Morton, J. (1985). Am. J. Physiol., 248: R645-R650.

Thompson, CJ., Bland, J., Burd, J and Baylis, PH. (1986). Clin. Sci., 71:651-656.

We acknowledge the cooperation of our subjects in these experiments.

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C34

Using a combined spiking and secretion model to investigate osmotic signal encoding and hormone secretion response in hypothalamic vasopressin neurons

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The magnocellular vasopressin neurons of the supraoptic nucleus and the paraventricular nucleus of the hypothalamus project axonal terminals to the posterior pituitary, where they secrete vasopressin hormone into the blood, acting at the kidneys to reduce water loss, as part of the homeostatic system regulating osmotic pressure. The neurons respond to synaptic input and depolarising currents generated by osmotic pressure, with increasing activity generating a distinctive phasic firing pattern, consisting of long bursts and silences lasting tens of seconds. We have previously modelled this phasic spike firing mechanism, and have now integrated our spike model with a model of the spike triggered calcium driven secretion mechanism. The secretion mechanism is highly non-linear, showing both frequency facilitation, and fatigue. The secretion response is reduced after about 20s of intense stimulation, as the readily releasable pool of vasopressin vesicles is depleted, and can be reversed by 20-30s of quiescence. Our secretion model uses a simple representation of the calcium dynamics driving vasopressin release, synthesis, and transport

to reproduce these effects and fit in vitro secretion data. We suggest that vesicles synthesised in the cell body are transported to a reserve pool, before being transported to the readily releasable pool docked at the cell membrane and use this to explain the observed dynamics. Combined with the spike model we have begun to investigate the relation between phasic firing and the secretion response, studying both individual neurons, and as a population. Conventionally the purpose of phasic firing is to optimise to secretion response per spike, by mixing intense stimulation with silent periods allowing recovery. We reproduce this effect with the model but also show that it gives no great advantage over non-phasic cells, and suggest that the sequence of pools is more important to buffer and maintain response while the central vasopressin store is being depleted. We also show that acting within an asynchronous population, phasic firing serves to give a much more linear response to increasing osmotic input compared to non-phasic model cells, matching the linear relationship observed in vivo.

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Gender dimorphisms in the plasma IL-10, TNF- α and IL-6 levels of spontaneously hypertensive rats: the role of the renin-angiotensin system

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Angiotensin I-converting enzyme (ACE) inhibitors exert their pleiotropic anti-inflammatory effects on patients with arterial hypertension or congestive heart failure. However, the effects that ACE inhibitors have on pro-inflammatory and anti-inflammatory cytokines levels in male and female SHR still remain unclear. So, this study was designed to investigate the interaction between the renin-angiotensin system (RAS) and sex hormones and the effect that this interaction has on inflammatory biomarker (interleukin (IL)-10, IL-6, TNF- α) levels in spontaneously hypertensive rats (SHR) rats. The procedures were carried out in compliance with the guidelines for the ethical use of animals in scientific research and were approved by the Ethical Committee of the Federal University of Espírito Santo. All of the surgical procedures were carried out under ketamine (70 mg/kg i.p.) and xylazine (10 mg/kg i.p.) anesthesia. Rats were divided into 4 experimental groups (n: 7) that consisted of female and male SHR: sham + vehicle (SV), sham + enalapril (SE; 10 mg/kg body weight), castrated + vehicle (CV), and castrated + enalapril (CE). The treatments began 21 days after castration and lasted for 30 days. The systolic blood pressure, plasma cytokine levels and angiotensin I-converting enzyme (ACE) activity of the rats were measured by tail-cuff, ELISA and fluorimetry, respectively. Values are means $\pm$ S.E.M., compared by ANOVA. The IL-10 ($p < 0.05$, SVF: 16.4 ± 1.1 pg/ml vs SVM 12.8 ± 1.2 pg/ml), TNF- α ($p < 0.05$, SVF: 16.6 ± 1.2 pg/ml vs SVM: 12.8 ± 1 pg/ml) and IL-6 ($p < 0.05$, SVF: 10.3 ± 0.2 pg/ml vs SVM: 7.2 ± 0.2 pg/ml) levels were higher in female

rats than male rats. The ovariectomy procedure reduced the levels of all of the cytokines. The orchiectomy reduced the IL-6 concentrations and increased the IL-10 concentrations in the male rats. Castration changed the previous pattern of response, eliminated the differences in inflammatory cytokine levels between the males and females and reduced the IL-10 levels in females. Enalapril equilibrated the cytokine levels of the male and female groups and increased the IL-10 concentration in all of the treated groups. However, the pro-inflammatory cytokine concentrations did not change in the castrated groups. In conclusions, sexual dimorphism was observed in the plasma cytokine levels of the SHR. ACE inhibition eliminated the sexual dimorphism observed in hypertensive animals, a finding that demonstrates the influence of RAS on the levels of these cytokines.

CNPq, CAPES and FAPES

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Moderate maternal salt intake leads to hypertension and hypernatraemia in the offspring: a novel role for the developmental induction of colonic sodium transport

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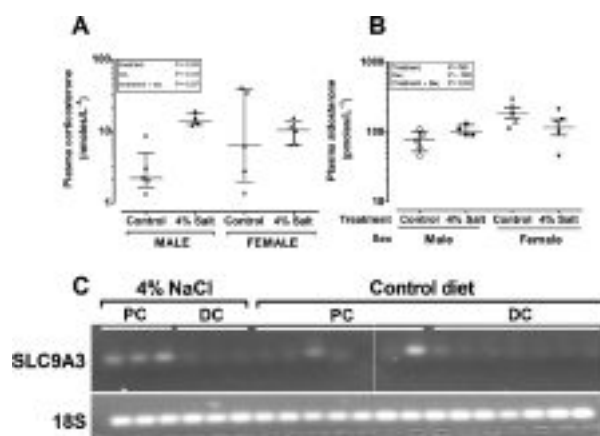
Introduction: Increased maternal salt intake can lead to hypertension in the offspring. Programmed changes to the renal handling of electrolytes in the adult offspring may underpin this developmental effect. This study tested using in vitro and in vivo models whether moderate maternal salt intake alters fetal kidney development and subsequent structure and function later in life.

Methods: Sprague Dawley rats were randomly divided into 2 dietary groups; 1) control diet (CD) fed purified chow and tap water and 2) Salt diet (SD) fed purified chow + 4% NaCl. Animals were fed the diets before during and after (to weaning) gestation. At day 20, a proportion (n=10/diet) was euthanased for biofluid (plasma and amniotic fluid) collection. Remaining litters (n=10 dams/diet) were weaned onto standard chow diet until 9 weeks of age when a proportion (n=5-6 per treatment*sex) were implanted with radiotelemetry probes (TA11PA-C40, Data Sciences Int, USA) for cardiovascular recording or fed chow or salt-diet for 5 days and renal function assessed in metabolic crates. Data are mean [s.e.d] and were analysed by linear mixed-effects models (Genstat v14, VSNi, UK).

Results: In vitro murine kidney expansion was marked attenuated (a 1-fold difference) by a 50 mosmole increase in media osmolality (data not shown), but in vivo no effect on fetal kidneys was observed – likely reflecting no difference in fetal plasma osmolality (e.g. fetal plasma: CD, 298 vs. SD, 301 [2] mosmoles/kg water). Salt diet revealed sex-specific programming of blood pressure; SD-males were hypertensive, SD-females were hypotensive relative to CD (mean = 111; treatment × sex effect size = 25 [6] mm Hg). All SD offspring had increased plasma osmolality (SD, 350 vs. CD, 322 [10]

mosmoles/kg water) and ECF Na⁺ (e.g. for SD males, 173 vs. CD males, 147 [6] mmoles/L). Renal salt retention was not different between treatment groups under baseline and salt-loaded conditions but SD offspring had significantly increased plasma corticosterone and proximal colon abundance of SLC9A3 (Fig) - the major glucocorticoid-inducible mechanism for colonic Na⁺ reabsorption.

Conclusions: Maternal high salt intake can have a lifelong effect on offspring blood pressure and Na⁺ retention, an effect apparently resident in the proximal colon and not the kidneys.



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The effects of *Syzygium aromaticum*-derived oleanolic acid on kidney function of male Sprague-Dawley rats and on kidney cell lines

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Studies indicate that *Syzygium spp* derived oleanolic acid (OA) enhances renal function of streptozotocin (STZ)-induced diabetic rats as evidenced by its reversal of the previously reported inability of the kidney to excrete Na⁺ in these animals¹. Accordingly this study monitored renal fluid and electrolyte outputs in conscious male Sprague-Dawley administered with OA twice every third day for five weeks. The study also investigated the effects of OA on proximal tubular Na⁺ handling in rats fed standard rodent chow supplemented with lithium chloride (12 mmol kg⁻¹ dry weight) for 48 h prior to experimentation in order to raise plasma lithium to measurable concentrations without affecting renal Na⁺ or water excretion² in an effort to establish tubular effects of the triterpene. Thereafter, the animals were anaesthetized and the right jugular vein was cannulated to allow intravenous infusion of 0.077M NaCl. The urinary bladder was also cannulated via an incision in the lower abdomen. After a 3½ h equilibration period, urine samples were taken every 30 min over the 4h post-equilibration period of 1h control, 1h 30 min treatment and 1h 30 min recovery periods; blood samples were taken once per hour for the measurement of electrolyte and clearance marker concentrations.

Li clearance is widely used to assess proximal tubular function of the mammalian kidney³. Glomerular filtration rate (GFR) was assessed by creatinine clearance. Cytotoxicity of OA on kidney and liver cell lines was assessed by the MTT and comet assays. Statistical comparison of the differences between the control means and experimental groups was performed with GraphPad InStat Software (version 4.00, GraphPad Software, San Diego, California, USA), using one-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparison test. A value of $p < 0.05$ was considered significant. OA increased Na^+ excretion of conscious male Sprague-Dawley rats from week 3 to week 5. By the end of the 5th week experimental period, OA treatment significantly reduced ($p < 0.05$) plasma creatinine concentration of STZ-induced diabetic rats with a concomitant elevation in GFR. Acute OA infusion was also associated with increases in fractional excretion of sodium (FE_{Na}) and lithium (FELi) in anaesthetized rats in the absence of significant changes in GFR. The MTT assay studies demonstrated that OA increased the metabolic activity of kidney and liver cell lines. Taken together with previous observations that OA increases GFR in streptozotocin induced diabetic rats, this study implicates the proximal tubule in OA-evoked increases in urinary Na^+ output. Key words: Renal function; diabetes mellitus; Syzygium aromaticum; oleanolic acid; triterpenes

Mapanga RF, Tufts MA, Shode FO & Musabayane CT (2009). Ren Fail; 31(6):481-491.

Thomsen K & Shirley DG (1997). Nephron Physiol 77:125-138.

Shalmi M, & Thomsen K (1989). Ren Physiol Biochem ; 12:273-280.

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C38

BACE1: a novel regulator of neuronal glucose metabolism

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Alzheimer's Disease (AD) is the most common cause of dementia and currently there is no cure or means to slow disease progression. This represents a clear, unmet medical need in light of the ageing population worldwide. While much of the current AD research focuses on the hallmark pathologies (amyloid plaques and neurofibrillary tangles), their appearance is extremely end stage and therapeutic interventions to alleviate them have failed to halt symptom progression. It may therefore be beneficial to look at earlier changes, with reduced glucose metabolism among the earliest defects seen in the AD brain (1). While the exact mechanism of neuronal energy supply remains debated, it is known that the primary substrates are glucose and lactate. Indeed, glucose is a required substrate for a number of neuronal functions, such as induction of long-term potentiation (2) and the consolidation of short to long-term memories. Evidence suggests the aspartyl protease β -site Amyloid precursor protein Cleaving Enzyme 1 (BACE1) as a key enzyme in the progression of AD. Previous work from our laboratory has also observed a role in metabolism, with BACE1 knock out mice displaying enhanced glucose disposal and insulin sensitivity (3).

This study aimed to investigate the role of BACE1 in neuronal glucose metabolism. We utilised wild type SH-SY5Y neuronal cells stably overexpressing either an empty vector or BACE1. Radiolabelled 2-deoxyglucose uptake and glucose oxidation assays were performed to observe the effect of BACE1 on utilisation of primary neuronal energy sources. All data expressed as mean $\pm$ standard error of the mean, and statistical significance determined by Student's t-test.

Stable overexpression of BACE1 resulted in numerous derangements to neuronal metabolism, significantly impairing the utilisation of preferential substrates, lactate and glucose (uptake reduced to 67 ± 5 per cent, $p < 0.0001$ and oxidation reduced to 74 ± 4 per cent, $p < 0.01$ compared to control). As well as this fundamentally compromised substrate use, BACE1 overexpressing cells displayed an inability to respond to metabolic challenge. There were also significant reductions in the activity of key metabolic enzymes hexokinase (HK, activity reduced to 75 ± 6 per cent, $p < 0.01$) and pyruvate dehydrogenase (PDH, reduced to 69 ± 8 per cent, $p < 0.05$). This impairment in glucose use was effectively attenuated through application of ketone bodies.

These data suggest that BACE1 overexpression alters neuronal metabolism towards ketone bodies, which in the short-term can overcome impaired glucose and lactate utilisation. However, this adaptation leaves cells unable to respond effectively to further challenge or perform energy intensive tasks. This further supports the idea that regulation of BACE1 can alter glucose homeostasis.

Craft S., Zallen G., and Baker L. D. (1992) Glucose and memory in mild senile dementia of the Alzheimer's type. J Clin Exp Neuropsychol 14: 253-267

Yousef F. F., Manswell S. and Homeward L. (2009) Effect of acute changes in glucose concentration on neuronal activity and plasticity in the rat hippocampus. West Indian Med J. 58: 410-416

Meakin P. J., Harper A. J., Hamilton D. L., Gallagher J., Mcneilly A. D., Burgess L. A., Vaanholt L. M., Bannon K. A., Latcham J., Hussain I., Speakman J. R., Howlett D. R. and Ashford M. L. J. (2012) Reduction in BACE1 decreases body weight, protects against diet-induced obesity and enhances insulin sensitivity in mice. Biochem J 441: 285-296

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The effect of two different high fat diets (fish oil vs. anhydrous milk fat) on markers of energy homeostasis in rats

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High dietary fat intake is considered to be a factor in the development of obesity. However, evidence suggests not all dietary fats are equally obesogenic. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been suggested to stimulate processes that reduce body weight and specifically prevent the deposition of visceral adipose tissue in rodent models. However, these findings are inconsistent and the

comparator fats used have not been representative of typical Western dietary fats. This study was designed to determine whether rats fed a high fat diet based on fish oils, high in EPA and DHA, were less likely to develop obesity than those fed on a diet based on anhydrous milk fat (rich in the saturated and monounsaturated fatty acids common in the Western diet). Male Wistar rats were *ad libitum* fed high fat diets with 40% of energy from fish oil (HF-FO) or anhydrous milk fat (HF-AMF) (n=9-10 per group) for 12 weeks. Diets were matched for energy density, protein, carbohydrate and fat content. Food intake and body weights were measured daily. At the end of the study, trunk blood was taken by cardiac puncture for the determination of plasma thyroid hormone concentrations. Uncoupling protein-1 (UCP-1) mRNA expression was measured in brown adipose tissue by Northern blot. Magnetic resonance imaging (MRI) was used to assess adipose tissue volumes in subcutaneous and visceral depots (n=5 per group). Generalised estimating equations were used to compare body weight and energy intake changes between groups and unpaired t-tests used for all other data.

The HF-AMF group had a significantly increased energy intake compared to rats fed the HF-FO diet ($p<0.001$). Despite this difference in energy intake, there was no significant difference in body weight between the groups over the course of the study ($p=0.80$). Similarly, there was no significant difference in total, visceral or subcutaneous adipose tissue between the two groups ($p=0.50$, $p=0.15$ and $p=0.92$ respectively). UCP-1 mRNA expression was not significantly different between the two groups ($p=0.99$). Circulating thyroxine (T4) was found to be significantly lower in the HF-FO diet group ($p=0.04$) although no difference was observed in tri-iodothyronine (T3) concentrations ($p=0.39$). These data suggest that a HF-AMF diet may promote energy overconsumption compared to an isocaloric HF-FO diet, but this does not appear to be associated with an increase in body weight or adiposity. This may suggest a greater metabolic efficiency in the HF-FO group, or a decreased energy expenditure (perhaps indicated by the reduced T4). It is necessary to measure energy expenditure to confirm this. Although Western diets rich in fats such as AMF have been hypothesised to favour the development of obesity compared to diets rich in EPA and DHA, this data suggests that this was not the case in this model.

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Selective ablation of Peptide YY cells in adult mice reveals a role in beta-cell survival

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Background and aims: Peptide YY (PYY) is a gut hormone synthesized by enteroendocrine L-cells in the gut. It is also present in the pancreas where in the adult PYY immunoreactivity is restricted to a major subpopulation of β -cells, a subpopulation of the β -cells and pancreatic polypeptide (PP) cells. PYY gene deletion has demonstrated it plays a key role in body weight regulation without obvious alterations in glucose homeostasis. However, ablation of peptides using germ line knock-

out approaches can lead to compensatory adaptive changes. So we developed a transgenic mouse expressing diphtheria toxin receptor (DTR) specifically in PYY cells. This allowed the conditional ablation of PYY-expressing cells throughout the body following administration of a single dose of diphtheria toxin (DT) (40ng/g i.p.) to PYY-DTR mice.

Results: Body weight and food intake were similar between wild-type and PYY-DTR mice. DTR was expressed by tissues that express PYY; gastrointestinal tract, islets and brainstem. In the pancreas dual labelling demonstrated DTR staining was not likely to be expressed by more than a minority of β -cells (less than 5%). Administration of DT lead to a 97% reduction in colonic PYY concentrations and cell number and resulted in severe weight loss, but food intake was unaffected. DT-treated PYY-DTR mice had polyuria, suggesting the development of diabetes. In agreement with this these mice were hyperglycaemic, hypoinsulinaemic and had reduced pancreatic insulin content. DT also resulted in dose dependent and significant reductions in PYY and insulin concentrations in isolated islets. Our data are consistent with PYY-expressing cells providing a trophic signal(s) for β -cell maintenance. If true, pharmacological replacement with this factor(s) would be expected to rescue insulin loss *in vivo*. Administration of either PP (Y4 receptor agonist, i.p minipump) or PYY3-36 (Y2 receptor agonist, i.p minipump) did not rescue the loss of pancreatic insulin following DT administration. However, a novel long-acting PYY analogue (given i.p.) with high affinity for both Y1 and Y2 receptors significantly reduced the loss of insulin content and β -cell number. Administration of this analogue also ameliorated the hyperglycaemia and insulin loss induced by streptozotocin.

Conclusion: These studies suggest that PYY may play a role in the regulation of β -cell maintenance and survival via the Y1/2 receptor. This could have important implications for identifying novel therapies for the prevention and treatment of β -cell loss in diabetes mellitus.

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Iron-induced switching of a receptor mediated transferrin uptake in epithelial cells

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Classically, the iron binding protein transferrin (Tf) is responsible for delivery of iron into the majority of cell types. Receptor-mediated endocytosis of holo-transferrin into cells is via the ubiquitously expressed transferrin receptor 1 (TfR1). However, some cell types, including enterocytes, glial cells, placental cytotrophoblasts and kidney proximal tubule epithelia, also express the megalin/cubilin receptor complex, that has affinity for Tf and thus offer an alternative means of receptor mediated cellular Tf uptake.

Multi-ligand receptors, megalin/cubilin are suggested to be the mediators of Tf uptake in the proximal tubule (Kozyraki, et al. 2001). However in mice, the TfR1 has been shown to localise at the apical membrane of the proximal tubule epithe-

lia (Zhang, et al. 2007). The aim of this study was to determine the role of TfR1 and megalin/cubilin complex to cellular transferrin delivery. Experiments were performed in vitro, using a cell line (WKPT-0293 Cl.2 (WKPT)) derived from the proximal tubule of rats. Ligands of megalin/cubilin, receptor associated protein (RAP) and Tf were used for uptake studies.

RT-PCR showed that WKPT cells contain mRNA transcripts encoding TfR1 and megalin. Furthermore under iron restriction (100 μ M desferrioxamine (DFO)) TfR1 mRNA levels increased and megalin mRNA decreased after 40hr of iron restriction. Conversely, iron enrichment (100 μ M ferric ammonium citrate) decreased TfR mRNA and elevated megalin mRNA levels.

Ligand uptake studies showed that under control conditions dsRED conjugated RAP (dsRED-RAP) and alexa488 conjugated Tf (Tf-488) were internalised. In control cells, pre-binding of unconjugated RAP (his-RAP), inhibited dsRED-RAP and Tf-488 uptake, suggesting megalin/cubilin to be the predominant mechanism of Tf uptake under normal conditions.

Under iron restriction, uptake of Tf-488 markedly increased in comparison to control treated cells, whilst no qualitative change in dsRED-RAP uptake was observed. Pre-binding of his-RAP inhibited most dsRED-RAP uptake. Interestingly, pre-binding of his-RAP had no detectable effect on the uptake of Tf-488, suggesting that TfR1 is the primary mechanism of Tf uptake under iron restriction.

These data strongly suggests that megalin and TfR1 are differentially regulated by iron. Furthermore, megalin/cubilin under iron replete conditions are the dominant means by which Tf is endocytosed. However, upon iron restriction, TfR1 switches to become the principal receptor for Tf endocytosis. These data potentially provide an insight in to the interplay of transferrin binding receptors in the control of cellular iron balance.

KOZYRAKI, R., FYFE, J., VERROUST, P. J., JACOBSEN, C., DAUTRY-VARSAT, A., GBUREK, J., WILLNOW, T. E., CHRISTENSEN, E. I. & MOESTRUP, S. K. (2001) Megalin-dependent cubilin-mediated endocytosis is a major pathway for the apical uptake of transferrin in polarized epithelia. *Proc Natl Acad Sci U S A*, 98, 12491-6.

ZHANG, D., MEYRON-HOLTZ, E. & ROUAULT, T. A. (2007) Renal Iron Metabolism: Transferrin Iron Delivery and the Role of Iron Regulatory Proteins. *J Am Soc Nephrol*, 18, 401-406.

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C42

Farnesoid X receptor exerts antisecretory actions on colonic epithelium – a new target for development of antidiarrhoeal drugs?

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Cl⁻ secretion, the main driving force for intestinal fluid secretion, can become dysregulated in conditions of disease, leading to the onset of diarrhoea. While such diseases, which include irritable bowel syndrome and infectious diarrhoeas, constitute a huge health and economic burden to society, currently available medications are unsatisfactory and new, more specific, therapies are required. Although we have previously

shown that, at physiological concentrations, colonic bile acids prevent epithelial Cl⁻ secretion, the mechanisms involved remain unclear. Here we investigated a potential role for the nuclear bile acid receptor, farnesoid X receptor (FXR), in regulation of intestinal secretory responses and as a new target for development of antidiarrhoeal drugs.

GW4064 (5 μ M for 24 hrs) was used to activate the FXR. Cl⁻ secretion was measured as changes in short-circuit current across T₈₄ cell monolayers or muscle-stripped mouse colonic tissues in Ussing chambers. Protein expression was measured by western blotting.

GW4064 treatment activated the FXR and inhibited responses to the Ca²⁺- and cAMP-dependent secretagogues, carbachol (CCh) and forskolin (FSK), to 56 $\pm$ 5 (p < 0.001) and 60 $\pm$ 5% (p < 0.001) of controls, respectively (n=15). GW4064 (10 μ M) also inhibited responses to the naturally-occurring secretagogues, cholera toxin (0.1 μ g/ml) and deoxycholic acid (500 μ M) to 81 $\pm$ 6 (n=7, p < 0.05) and 64 $\pm$ 10% (n=10, p < 0.05) of controls, respectively. Similar to its effects in cultured epithelia, intraperitoneal injection of GW4064 to mice (50 mg/kg; n=11) attenuated CCh- and FSK-stimulated secretory responses in colonic tissues. However, jejunal sodium-dependent glucose co-transport and colonic ENaC currents were not decreased, suggesting the effects of FXR activation to be specific for secretory processes. Furthermore, GW4064 treatment dramatically inhibited the severity of symptoms in a mouse model of diarrhoeal disease (n=10). In experiments to determine molecular mechanisms involved, we found that GW4064 decreased CFTR-mediated Cl⁻ currents in T₈₄ cells to 68 $\pm$ 5% (n=9; p < 0.01) of control values, and this was associated with a 33 $\pm$ 9% decrease in CFTR protein expression (n=4; p < 0.01). GW4064 also inhibited Na⁺/K⁺-ATPase activity to 61 $\pm$ 10% of controls (n=6; p < 0.05). However, while GW4064 did not alter expression of the Na⁺/K⁺-ATPase, it did increase expression of a regulatory protein, FXR3, to 166 $\pm$ 16% (n=8, p < 0.01) of control values.

These data reveal novel antidiarrhoeal actions of FXR in colonic epithelial cells. These effects are mediated by direct inhibition of multiple components of the Cl⁻ secretory pathway, without altering key absorptive processes. Our data suggest that FXR agonists may be useful in treating secretory diarrhoeas associated with common intestinal disorders.

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Inflammatory cytokines downregulate the Na⁺/H⁺ exchanger 3 (NHE3) interacting PDZ-domain protein PDZK1 in ulcerative colitis patients, colitis mice and in Caco-2bhe cells: Link to inflammation-associated NHE3 dysfunction

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Introduction: We have previously reported a dysfunction of the major intestinal sodium absorptive transporter, the Na⁺/H⁺ exchanger NHE3, in the surface colonocytes of patients with

ulcerative colitis, despite normal expression and membrane localization. Objective: In this study, we searched for potential mechanism for this inflammation-associated functional defect in NHE3 transport. Material and Methods: Realtime PCRs, Western blotting, immunohistochemistry, ileal loop experiments and pH fluorometry using pH sensitive BCECF dye. Results: In three immunologic mouse models for colitis (Rag2 KO CD45RBhigh and IL-10 KO) and Crohn's disease (TNF ARE +/-), and in biopsies from patients with active UC, we found no change in the mRNA expression of NHE3 or NHERF1, but a dramatic decrease in PDZK1, in the respective inflamed segments. This was confirmed at the protein level. Fluid absorption in isoflurane-anesthetized mice in vivo, and acid-activated NHE3 activity in vitro decreased significantly in the inflamed segments, despite normal immunohistochemical NHE3 staining in the brush border membrane. In PDZK1 heterozygote mice, where enterocyte PDZK1 protein content was reduced by > 50%, acid-activated NHE3 activity decreased to a similar degree in colonocytes compared to inflamed mice. PDZK1 knockdown in stably NHE3-transfected Caco-2bbe similarly caused a strong reduction in NHE3 activity, suggesting a causal relationship between PDZK1 downregulation and NHE3 dysfunction. In Caco-2bbe cells treated with cytokines, significant down regulation in PDZK1 mRNA and protein expression was observed. Conclusion: The data demonstrate a marked decrease in the PDZ-adaptor protein, PDZK1, in inflamed murine and human intestine, and a dysfunction of NHE3 activity that is similar in inflamed enterocytes and in those with genetic downregulation of PDZK1. PDZK1 downregulation during inflammation may thus be one factor responsible for inflammation-associated NHE3 dysfunction

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Metformin reduces transepithelial glucose permeability and inhibits the hyperglycaemia associated growth of *Staphylococcus aureus* in the airway

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Glucose in the airway surface liquid (ASL) is normally maintained at a low concentration compared to the blood (~12.5 x lower). Raised blood glucose elevates ASL glucose (1), which increases the risk of respiratory infection, particularly with methicillin-resistant *Staphylococcus aureus* (2). We investigated the relationship between basolateral/blood glucose concentration and apical/luminal *S. aureus* growth using an in vitro model of human airway epithelium and an in vivo mouse model and tested whether metformin (drug used to lower blood glucose) affected this relationship.

H441 epithelial cells grown at air-liquid-interface were inoculated with 5x10⁵ CFU/cm² *S. aureus* (8325-4) on the apical surface and incubated for 7 hours. The transepithelial electrical resistance (TEER) of H441 monolayers was significantly reduced and basolateral to apical paracellular glucose flux across H441-monolayers (assessed using ¹⁴C-L-glucose) was

greatly enhanced following co-incubation with *S. aureus* (P<0.05 and P<0.0001 respectively, n=8). A 12h pre-treatment of H441 cells with metformin (1mM) partially attenuated the fall in TEER and decreased transepithelial glucose flux (P<0.001 and P<0.0001 respectively, n=8). Apical *S. aureus* growth (determined by Miles-Misra) increased with basolateral glucose concentration (10, 20, 40mM). However, no correlation was seen in metformin treated co-cultures, and *S. aureus* growth was significantly impaired (P<0.05, n=8). Metformin treatment had no effect on bilateral ¹⁴C-D-glucose uptake in H441 monolayers or any direct effect on *S. aureus* growth in culture.

6-10 week old wild type C57BL/6 (WT) or db/db (leptin receptor deficient) mice were treated with intraperitoneal PBS or 40mg/kg metformin for two days prior to intranasal inoculation with 10⁷CFU *S. aureus*. Mice were culled 24h post infection and bronchoalveolar lavage (BAL) collected. The db/db mice had significantly higher blood glucose concentrations than WT mice (P<0.01, n=9). Metformin had no effect on blood glucose levels in either the WT or db/db mice. Untreated db/db mice had more *S. aureus* in their BAL than WT mice (P<0.001, n=9). Metformin treatment significantly reduced numbers of *S. aureus* in the BAL of db/db mice compared to PBS treated db/db mice (P<0.01, n=9). Metformin had no effect on *S. aureus* CFU in the BAL of WT mice.

These results indicate that metformin treatment inhibits *S. aureus* growth in the airways by reducing the paracellular diffusion of glucose into the ASL under hyperglycaemic conditions.

Baker EH et al. (2007) *J Appl Physiol* **102**, 1969-1975.

Philips BJ et al. (2005) *Thorax* **60**, 761-764.

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The effects of maternal dietary exposure on the placental transcriptome

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Aims: Feeding pregnant rats a low protein diet has been shown to have deleterious effects on placental development and induce an altered phenotype in the adult offspring. The aim of this study was to investigate the effects of maternal dietary restriction on the expression of the whole placental transcriptome in order to identify molecular processes involved. Methods: Rats were fed one of three diets: control (C, 18% casein), protein restricted (PR, 9% casein) or 30% reduction in total food intake (UN) from conception. On day 18 of gestation dams were culled and the placentas collected. The placental transcriptome (6 pooled placentas per dietary group) was analysed by Oxford Gene Technology (OGT, Oxford, UK) using an Agilent 14879 whole rat genome expression microarray (4 X 44K) G4131F (Agilent Technologies Inc., USA). Altered gene networks were identified using gene ontology analysis (GeneSifterTM; www.genesifter.net; VizX Labs LLC, Seattle, USA). Molecular pathways identified by the microarray analysis were investigated further using quantitative real-time PCR to measure transcript levels for specific genes (6 placentas per dietary group). Data were normalised using the endogenous control gene cyclophilin and analysed by one-way analysis of variance with Bonferroni's post hoc test.

Results: 4264 genes differed (>1.5-fold change) between placentas of PR and C dams (1631 increased, 2633 decreased). 4575 genes differed between placentas of UN and C dams (2004 increased, 2571 decreased). Gene ontology analysis showed that maternal PR and UN induced changes (Z score >2) in the gene ontology pathways: detection of stimulus, signal transducer activity and lipid metabolic processes were increased, while developmental processes and ion transport were decreased. PCR revealed that fatty acid transport protein (slc27a5), lipoprotein lipase and thioredoxin-interacting protein mRNA levels were increased in UN compared to C and PR placentas ($P<0.05$), whereas peroxisome proliferator-activated receptor (PPAR) alpha mRNA levels were increased in UN compared to PR placentas ($P<0.05$). PPAR delta mRNA levels were unchanged.

Conclusions: This study demonstrates that an altered maternal diet can affect the expression of the placental transcriptome with some similarities between dietary exposures. Lipid metabolism and PPAR signalling were identified as key pathways in the placental response to altered total maternal nutrition. The effects are specific to the dietary challenge which suggests that the placenta may act as a nutrient sensor. By making specific changes in gene expression in response to a particular maternal diet the placenta may alter its function in order to adapt fetal development to suit that environment.

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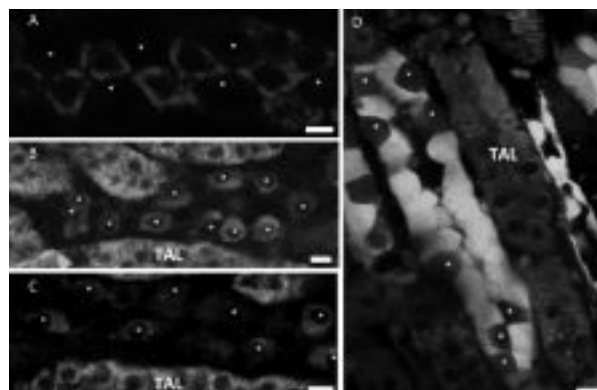
Fluorescence imaging reveals differences in mitochondrial function along the collecting duct

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The collecting duct (CD) consists of two different cell populations, principal cells (PCs) and intercalated cells (ICs), which have distinct solute transport functions. Given that transport and metabolism are closely coupled in the nephron, we hypothesise that there are intrinsic differences in mitochondrial function between PCs and ICs; this has not previously been investigated due to a lack of appropriate technology. It has been demonstrated that mitochondrial function can be measured *in situ* in intact rat kidney slices using confocal and multiphoton microscopy (1); we applied this approach to investigate our hypothesis. Adult male Sprague-Dawley rats were killed by cervical dislocation (in accordance with the Animals (Scientific Procedures) Act 1986). Kidneys were removed immediately, decapsulated and placed in oxygenated ice-cold physiological saline solution. Serial 200 μm -thick coronal kidney slices were cut using a vibratome slicer (2) and fluorescent dyes were loaded using a re-circulating perfusion system gassed with 95% O₂ 5% CO₂. Slices were imaged with a Zeiss LSM 510 NLO axiovert microscope, coupled to a tunable Coherent Chameleon laser and remained viable for up to 4h, confirmed by calcein-AM dye uptake. The two cell types have distinct morphological differences, which facilitated their individual identification. Antibodies to label PCs and mitochondria (aquaporin 2 and ATP5 β respectively) confirmed differences in cell morphology and revealed apical distribution

of mitochondria in ICs. Marked heterogeneity was observed in mitochondrial signals along the CD. Mitochondrial membrane potential, as measured using the lipophilic cation tetramethylrhodamine methyl ester (TMRM), was higher in PCs (mean TMRM signal: 34.45 A.U. $\pm$ 1.43) than ICs (16.82 $\pm$ 0.28, $n=3$, $p=0.009$). The differential mitochondrial potential observed was not altered by the addition of dye efflux inhibitor verapamil or the ATP-synthase inhibitor oligomycin indicating that the signal seen was not artefactual. Autofluorescence signals emitted by NADH and FAD²⁺ (respective substrates for complexes I and II in the respiratory chain) were both higher in ICs than in PCs ($p=0.03$ and $p=0.056$ for NADH and FAD²⁺ respectively). Reactive oxygen species production was measured using fluorescent dihydroethidium (HET) and a higher signal detected in ICs. Glutathione, an important intracellular antioxidant, was measured using monochlorobimane (MCB), which forms a fluorescent adduct with glutathione. Once MCB signal intensities had reached a steady state (40 minutes), glutathione levels were higher in PCs (mean MCB fluorescence 170.84 $\pm$ 10.53 A.U.) than in ICs (43.73 $\pm$ 10.96) ($n=3$, $p=0.006$). In summary, fluorescence imaging reveals intrinsic differences in mitochondrial function between PCs and ICs, which may be important in understanding physiology and patho-physiology in this nephron segment.



Imaging mitochondrial function *in situ* in the collecting duct. A) TMRM signal was higher in mitochondria of principal cells, B,C) Signals from the respiratory chain substrates NADH (B) and FAD²⁺ (C) were higher in intercalated cells, D) Glutathione levels measured using monochlorobimane were higher in principal cells. (asterisk = intercalated cells, TAL = thick ascending limb, scale bars = 10 μm).

Hall AM, et al: J Am Soc Nephrol 2009;20:1293-1302

Crawford C, et al: Acta Physiol 2011;202:241-251

MRC

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Dissociation of structure from function in the distal renal tubule of mice lacking 11 β -hydroxysteroid dehydrogenase type 2

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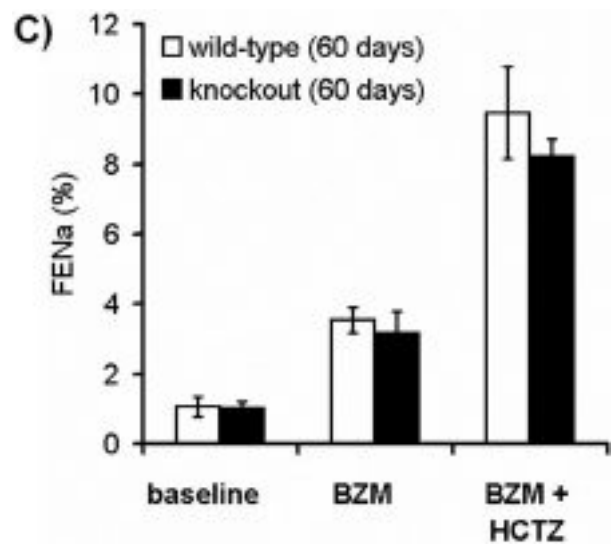
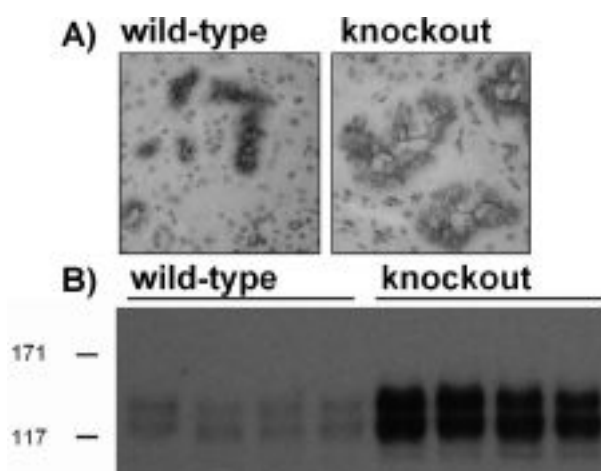
Intro. The renal distal convoluted tubule (DCT) participates in the regulation of renal sodium (Na) excretion and blood

pressure. DCT epithelial cells exhibit structural plasticity, a classic example being the hypertrophy observed in rodents treated with loop diuretics, which is associated with increased rates of Na transport in the DCT (1). However the relationship between epithelial structure and function in hypertensive, salt-retaining states has not been characterised (notwithstanding indirect evidence of increased Na transport in the hypertrophic DCT seen in mouse models of Gordon's syndrome (2,3)). DCT hypertrophy has been observed in mice lacking 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) (4) and we hypothesised that in this context it would be associated with increased Na transport in the DCT, contributing to the hypertensive phenotype.

Methods. Male 11 β -HSD2 knockout mice and wild-type controls were sacrificed at one of two ages (60 or 120-150 days). Right kidneys were formalin-fixed and the DCTs labelled by immunohistochemistry using an antibody to the NaCl co-transporter, NCC. Left kidneys were homogenised to prepare protein used in Western blots probed with anti-NCC. Urinary Na excretion was determined in separate cohorts. Mice were anaesthetised with thiobutabarbital (167mg/kg I.P.) and catheters inserted into the jugular vein, carotid artery (for blood pressure recording and blood sampling) and bladder. They were given a continuous I.V. infusion of saline containing 0.25% FITC-inulin. After 60 mins equilibration, fractional Na excretion (FENa, urinary Na excretion expressed as a fraction of filtered Na) was determined during serial 40 min sampling periods taken at baseline, following the epithelial Na channel blocker benzamil (BZM, 2mg/kg I.V. bolus then 1mg/kg/hr infusion) and following BZM plus the NCC inhibitor hydrochlorothiazide (HCTZ, 2mg/kg bolus). The incremental response to HCTZ, Δ FENa provided an index of Na transport in the DCT. Values are mean $\pm$ SEM compared by unpaired t-test.

Results. Knockout mice exhibited hypertrophy and hyperplasia in the DCT at both ages (figA) and had more abundant NCC protein (figB; n=8, p<0.05). Despite this, Δ FENa was not statistically different from wild-type mice (figC; knockout vs WT at 60 days: 5.06 $\pm$ 0.78 vs 5.93 $\pm$ 1.45; at 120-150 days: 5.73 $\pm$ 1.36 vs 6.55 $\pm$ 0.66; n=6-8 each group).

Conclusion. There is dissociation of structure from Na transport function in the DCT of 11 β -HSD2 knockout mice. This surprising finding contradicts the prevailing hypothesis that structural changes in the renal tubular epithelium are associated with changes in transport function (1) and has implications not only for the regulation of renal Na excretion but also our general understanding of the relationship between structure and function in transporting epithelia.



Ellison DH et al. (1989). *J Clin Invest* **83**(1), 113-26.

Lalot MD et al. (2006). *Nat genetics* **38**(10), 1124-32.

Yang SS et al. (2007). *Cell Metab* **5**(5), 331-44.

Bailey MA et al. (2008). *J Am Soc Nephrol* **19**(1), 47-58.

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Response of salt-sensitive and salt-resistant adult Nigerian subjects to amiloride

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The activity of the epithelial sodium channel (ENaC) has been related to salt-sensitivity especially in Liddle Syndrome, a severe form of salt-sensitive hypertension (Furuhashi et al., 2005). The selective ENaC blocker, amiloride, has been shown to be effective in reducing blood pressure not only in Liddle's syndrome (Botero-Velez et al., 1994) but also in blacks with T594M polymorphism of ENaC (Baker et al., 2002). Every population is heterogenous for salt-sensitivity. It is therefore of interest whether amiloride will block the channel in salt-resistant individuals as well as salt-sensitive persons especially as amiloride has been suggested as a sole anti-hypertensive agent in blacks (Baker et al., 2002). Baseline blood pressure was measured in 22 normotensive (NT) and 42 age-matched hypertensive (HT) subjects earlier divided into salt-sensitive (NT = 11; HT = 22) and salt-resistant (NT = 11; HT = 20) subgroups (Elias et al., 2011). Subjects were salt-loaded with 200mmol Na⁺/day for 5 days. Blood pressure was thereafter measured to determine the effect of the salt-load. After one week wash-out period, subjects ingested a combination of the salt-load and 5mg amiloride tablets daily for 5 days. The effect of the combination on blood pressure was determined. Ethical clearance for this study was obtained from the College of Medicine, University of Lagos. Data are presented as mean $\pm$ S.E.M., compared with ANOVA and appropriate post-hoc tests. Baseline systolic blood pressure (SBP) and diastolic blood pressure (DBP) were similar (p>0.05) in the salt-sensitive (SBP 121.5 $\pm$ 2.8 mmHg; DBP 80.9 $\pm$ 0.8 mmHg) and salt-resistant (SBP

120.7±2.4 mmHg; DBP 80.2±1.6 mmHg) NT subjects and among the salt-sensitive (SBP 144.7±3.6 mmHg; DBP 92.9±2.4 mmHg) and salt-resistant (142.9±4.8 mmHg; DBP 99.2±2.0 mmHg) HT subjects. Following the salt-load, blood pressure changed significantly ($p<0.01$) (+7.7±2.6% SBP; +11.6±2.1% DBP) among the salt-sensitive NT subjects but marginally ($p>0.05$) (+0.6±1.9% SBP; -0.5±2.6% DBP) among the salt-resistant NT subjects. Similarly, there were significant ($p<0.001$) pressor responses to the salt load among the salt-sensitive (+7.8±1.7% SBP; +11.6±1.7% DBP) but not among the salt-resistant (SBP +2.1±1.7%; DBP -2.2±1.4%) HT subjects. Salt plus amiloride-loading led to significant reduction in SBP among salt-sensitive subjects ($p<0.001$) (-9.9±2.1% NT; -12.9±2.0% HT) and salt-resistant subjects ($p<0.05$) (-6.5±2.0% NT; -10.0±1.5% HT) compared with salt-loading alone. Similarly, the combination significantly reduced DBP among salt-sensitive ($p<0.001$) (-13.9±3.4% NT; -10.4±2.8% HT) subjects but reduced DBP in salt-resistant (-7.1±1.7%, $p<0.001$) HT subjects only. The effect of ENaC blockade by amiloride are similar among salt-sensitive and salt-resistant subjects. These results suggest that salt-resistance does not preclude treatment of hypertension with amiloride.

Baker EH, Duggal A, Dong Y et al., (2002). Hypertension 40, 13-17

Elias SO, Azing EC, Umoren GA et al., (2011). Nig Qt J Hosp Med 21, 85-91

Furuhashi M, Kitamura K, Adachi M et al., (2005). J Clin Endocrinol Metab 90, 340-344

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A custom-built fast scanning stimulated emission depletion microscope with a large field of view

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In classical microscopy the resolution is limited to ~200-300 nm due to Abbe's diffraction limit. In the past two decades several techniques emerged to overcome this limit, among which the Stimulated Emission Depletion (STED) microscopy has attracted wide interest (1). In STED microscopy, two beams are required: one excites the sample as in classical confocal microscopy, while the other one, with a donut-shape profile, de-excites the fluorophores by stimulated emission, which avoids the fluorescence emission. Only the tiny volume located at the center of the donut will emit fluorescence. The size of this emitting spot defines the resolution of the STED images, and strongly depends on the quality and the intensity of the STED beam that is critical to have zero energy at the center of the donut.

We built a resonant scanning STED microscope, which achieves a lateral resolution in biological samples of ~30 nm in a large field of view of ~40 x 40 µm acquired at ~10 nm/pixel. To acquire images of ~4000 x 4000 points at a speed of 16,000 lines per second, we used an 8 KHz resonant scanning mirror synchronized with a 96 MHz (10.4 ns/point) AD converter. Light was collected by a fast and ultrasensitive photomultiplier (H7422-PA-40, Hamamatsu Photonics) (for more details see: <http://www.anes.ucla.edu/sted/index.html>).

Potassium and calcium channels in cardiomyocytes and in isolated cardiac mitochondria were immunolabeled with specific antibodies and Atto-647N as the secondary antibody. Sprague-Dawley rats or C57BL/6 were used for organelle/cell isolation and protocols received institutional approval. At fast scanning speed, the photobleaching of samples was considerably reduced allowing repeated imaging of the same area, as well as the acquisition of a stack of planes for 3D reconstruction. With this microscope, we have uncovered a discrete distribution of BK channels within a single mitochondrion, and a distinct array of L-type calcium channels and ryanodine receptors in cardiomyocytes in control and after heart failure. For mitochondrial BK channels, a cluster size histogram revealed a peak at ~50 nm (500 individual clusters from three independent mitochondrial preparations). In conclusion, we have constructed a STED microscope that allows the detailed analysis of protein clusters using a large field of view.

Hell and Wichmann, Opt. Lett. 19:780-782, 1994

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Changes in ionic currents in respiratory neurons cause sympathetic overactivity in chronic intermittent hypoxic rats

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Sympathetic activity is increased during late-expiratory (late-E) phase in chronic intermittent hypoxic (CIH) rats. We evaluated the mechanisms involved in the CIH-induced changes in electrophysiological properties of medullary respiratory and pre-sympathetic neurons. At 11th day after CIH, the rats were deeply anesthetized with halothane and decerebrated and then placed in the recording chamber for *in situ* working heart brainstem preparation of wistar rats. We performed simultaneous recordings of respiratory and sympathetic nerves with intracellular or whole cell patch-clamp of respiratory and pre-sympathetic neurons of ventrolateral medulla. In CIH rats, the decrease in the frequency discharge of Böttinger post-inspiratory [post-I, (n=28)] neurons reduced the post-I activity of cervical vagus nerve (n=10). The overactivities of augmenting expiratory [aug-E, (n=55)] and pre-Böttinger pre-inspiratory [pre-I, (n=30)] neurons increased the late-E abdominal (n=28) and pre-I hypoglossal (n=20) nerves activities. Due to this changes, the frequency discharge of pre-sympathetic neurons (n=27) and thoracic sympathetic activity (n=30) were enhanced time-locked with AbN late-E activity. However, CIH produced changes in the input resistance and excitability, independent of synaptic transmission, only in the pre-I (179±13 vs 253±22 MΩ; 25±3 vs 47±2 pA; $p<0.05$; n=15) and post-I neurons (355±15 vs 250±31 MΩ; 71±3 vs 51±2 pA; $p<0.05$; n=17). These changes were accompanied by increase in the inward riluzole-sensitive persistent sodium current (at -50 mV: 156 ± 15 vs 72 ± 20 pA, $p<0.05$; n=6) and in the 4-aminopyridine-sensitive component of transient outward potassium current (at 60 mV: 405 ± 75 vs 210 ± 77 pA; $p<0.05$; n=9) in the CIH pre-I and post-I neurons, respectively. These data describe novel mechanisms underlying sympathetic over-

activity, which critically depend on changes of ionic currents of medullary respiratory neurons of CIH rats.

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Chronic intermittent hypoxia induces hypertension and alters lumbar sympathetic control of haemodynamic responses to airway occlusion

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Obstructive sleep apnoea (OSA), the most common sleep-related breathing disorder, is characterised by repetitive airway collapse during sleep. OSA is associated with cardiovascular morbidity and recognised as an independent risk factor for daytime hypertension. Sympathetic activity is commonly increased in OSA patients and animal models exposed to chronic intermittent hypoxia (CIH). Studies of the relationship between CIH, hypertension and sympathetic activity focus on renal sympathetic activity primarily and report sympathetic hyperactivity; however the lumbar sympathetic chain (LSC) has been relatively neglected. We hypothesised that CIH would induce systemic hypertension and sectioning of the LSC would cause a greater drop in mean arterial blood pressure (MAP) and a greater increase in femoral vascular conductance (FVC) compared to sham controls. We also hypothesised that CIH exposure would alter the LSC contribution to haemodynamic responses during brief tracheal occlusions.

Age matched adult male Wistar rats (317±6g) were exposed to CIH (n=8) consisting of 90s hypoxia (5% O₂ nadir)/210s normoxia cycles, or sham (n=8) treatment (normoxia), for 8h/day for 2 weeks. Under urethane anaesthesia (1.5g/kg, administered via intraperitoneal injection), rats were tracheotomised and vascular cannulations inserted. Both LSC and were prepared for transection at the L2-L3 ganglia to removal central drive to the lower lumbar sympathetic nervous system. Hindlimb flow was measured at the femoral artery with an ultrasonic flow probe. Data is presented as Mean ± S.E.M and analysed by two-way ANOVA.

CIH exposure significantly increased MAP (93.5±2.8 vs 81±1.4 mmHg; $p=0.0006$) and sectioning of the LSC caused a drop in MAP which was not different in CIH and sham animals ($p=0.1463$). Sectioning of the LSC resulted in a large increase in FVC ($p<0.0001$) which was again not found to be different between groups ($p=0.0689$). In response to tracheal occlusion, CIH exposure significantly affected the MAP ($p<0.001$) and FVC ($p<0.001$) response with the LSC intact. After sectioning of the LSC, MAP and FVC responses to occlusion in CIH treated and sham animals were not significantly different ($p>0.05$). Heart rate response to occlusion, despite a CIH induced tachycardia ($p=0.0013$), was not different in sham and CIH treated animals, with the chain intact or cut ($p>0.05$).

Haemodynamic responses to apnoeic events are altered after CIH exposure with the LSC intact. In response to obstructive apnoea, CIH treated animals exhibit a smaller drop in MAP and FVC does not increase as much compared to the sham response. We conclude that whilst CIH treatment alters the lumbar sympathetic response during hypoxic events facilitat-

ing maintenance of a steady blood pressure and perfusion to the hindlimb, the lumbar sympathetic outflow does not contribute significantly to CIH induced hypertension.

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Insights into vagal ganglionic processing from the von Bezold-Jarisch reflex in the rat

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Loss of cardiac vagal tone is a feature of many cardiovascular disorders including heart failure. The cardiac ganglia are an important potential site for regulation of vagal drive to the heart, where loss of transmission may translate to loss of vagal tone: this has been shown in a heart failure model (1). However, how cardiac vagal postganglionic neurones (CVNs) process ongoing and reflex activity has hitherto been difficult to investigate. We recently characterised CVN responses to a number of cardiorespiratory reflexes (2), and now extend this work to include the von Bezold-Jarisch reflex (vBJR), a vagally-mediated, cardioprotective reflex activated during myocardial ischaemia.

Male Wistar rats (P18-25, n=12) were anaesthetised with 5% Halothane (until decerebration) for surgical set up of the working heart-brainstem preparation. The atria were opened, stabilised and the cardiac vagal ganglia exposed (2). Intracellular recordings were made from CVNs with sharp electrodes (65-140M Ω , 0.5M KCl), and classified as tonic or phasic firing neurones depending on their response to depolarising pulses (220ms, 1Hz, 1nA). CVN responses to activation of chemo- (50 μ l, 0.03% NaCN i.a.), baro- (+20-50mmHg), diving (100 μ l, ~10°C ACSF to the nose) and vBJR activation with phenylbiguanide (PBG, 200 μ g ia) were assessed.

vBJR activation produced robust bradycardias (-84±27 bpm, n=14). Intracellular recordings were made from 14 CVNs (10 phasic, 4 tonic). All tonic cells had spontaneous EPSPs and 1 had spontaneous action potentials. vBJR and chemoreflex responses were recorded in all 4 tonic cells, on 3 of which other reflexes were also tested.

All tonic cells exhibited augmented EPSP and/or spike rates in response to chemo- (+7±4Hz, n=4), baro- (+8±4 Hz, n=3), diving (+13±1 Hz, n=2) and vBJR (+16±10 Hz, n=4) activation. Notably, the increase in EPSP/spike frequency was ~2.5x higher during the vBJR than the chemoreflex despite similar bradycardias (-98±29 vs -98±35bpm). Intense vBJR activation could also produce a distinctive pattern with a depolarising envelope of summing EPSPs. Reflex activation had no effect upon phasic cells (putative interneurones). PBG produced no change in EPSP frequency in preparations that had lost brainstem reflex function, and there was no evidence of direct depolarisation or activation of local cardiac ganglionic circuits.

These data show that vBJR activation excites a common pool of vagal postganglionic neurones shared with the other tested cardiorespiratory reflexes. We found no evidence of recruitment of ganglionic interneurones, nor for direct effects of PBG on the ganglion cells. The striking pattern of excitation of CVN by vBJR activation means that the intrinsic integrative prop-

erties of the ganglion cells are likely to be important in determining the magnitude of the end organ response.

Bibevski S, Dunlap ME. Ganglionic mechanisms contribute to diminished vagal control in heart failure. *Circulation*. 1999;99:2958-63

McAllen RM, Salo LM, Paton JF, Pickering AE. Processing of central and reflex vagal drives by rat cardiac ganglion neurones: an intracellular analysis. *J Physiol*. 2011;589:5801-18

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C53

The A-current as a low-pass filter of burst activity in sympathetic preganglionic neurones

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The intrinsic properties of sympathetic preganglionic neurones (SPNs) are important in determining the output characteristics of the sympathetic nervous system. The potassium A-current (I_A) is prominent in SPNs, and in other systems has been shown to regulate firing frequency and after-hyperpolarisation (AHP) shape [1]. Sympathetic vasomotor discharge is known to be bursty, and one rhythm is entrained to the central respiratory pattern generator output. We have shown that in spontaneously hypertensive rats (SHRs) there is an increase in the amplitude of respiratory-sympathetic coupling [2] and hypothesise that this may be due to changes in I_A .

To test this hypothesis we have built a NEURON [3] model. By fitting our A-current parameters to detailed experimental data [4], the model exhibited physiological responses similar to those of SPN *in vivo*, and steady-state curves that well-fit data for the normotensive Wistar (WKY) rat. Features of the I_A that could provide a SHR response were investigated; one particular parameter, maximal conductance density g_{KA} , was identified as being capable of turning the WKY cellular response profile into that seen in SHR with increased excitability [5].

To better understand the effect of altered g_{KA} on cell excitability we first examined action potential morphology and found small, biologically minor, changes in threshold (-0.24mV) spike amplitude (+0.24mV) and half-width (+0.2ms) for the SHR strain. The AHP amplitude (WKY=9.87mV vs SHR=5.23mV) was markedly reduced in the SHR (but not duration).

We next generated a frequency-modulated synaptic input into our SPN to mimic the effect of a respiratory entrained drive, with inter-event intervals (IEI) between 980ms and 20ms, and quantified the influence of g_{KA} on the output characteristic. Within bursts, the proportion of successfully transmitted EPSPs significantly decreased in the WKY (SHR=0.99±0.01, WKY=0.6±0.1, $p<0.001$) and it was noted that the higher frequency component was specifically attenuated. Examination of EPSP shape (1Hz stimulation) showed that increasing I_A shortened EPSP duration ($\tau_{SHR}=28ms$, $\tau_{WKY}=13ms$) with little effect on amplitude (decreased by <3%). This change in EPSP decay had a major effect on EPSP summation at higher frequencies in the Wistar model (IEI<60ms; 2.5-fold decrease in EPSP amplitude gain; SHR=4.3±0.1mV, WKY=1.2±0.8mV)

which, when combined with the augmentation in AHP amplitude, acts to low-pass filter the sympathetic output.

Our data shows that a reduction of g_{KA} in SHRs would result in a reduced filtering of respiratory-modulated (and indeed any high frequency) input, and therefore an amplification of respiratory-sympathetic coupling. These modelling data are consistent with I_A playing a major role in determining SPN excitability as a tuneable low pass filter, thus regulating sympathetic output.

[1] J. A. Connor and C. F. Stevens (1971). *J. Physiol.*, 213, pp 31-53.

[2] Simms et al. (2009). *J Physiol* 587.3, pp 597-610.

[3] Hines, M.L. and Carnevale, N.T. (2003). 2nd ed, edited by M.A. Arbib. Cambridge, MA: MIT Press, pp 769-773.

[4] Whyment et al. (2011). *Neuroscience* Volume 178, pp 68-81.

[5] Julian FR Paton, Alexey O Stalbovskiy and Anthony E Pickering (April 6, 2010). Increased intrinsic excitability of muscle vasoconstrictor sympathetic preganglionic neurones in neonatal spontaneously hypertensive rats. *FASEB J*, 24:809.13.

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C54

Characterisation of cells in the postnatal neurogenic niche surrounding the central canal of the mammalian spinal cord

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The area surrounding the central canal of the mammalian spinal cord is known to contain two cell types: ependymal cells and cerebrospinal fluid contacting cells (CSFCCs). It has been defined as a neurogenic niche due to the presence of a subpopulation of ependymal cells that have characteristics of neural stem cells and the presence of CSFCCs that appear to be immature neurones. To date the function and characteristics of both these cell types are poorly understood, this needs to be rectified if we are to comprehend how the neurogenic niche functions. This study used electrophysiology and immunohistochemistry to characterise ependymal cells and CSFCCs. For whole cell patch clamp electrophysiology, Wistar rats or C57/b6 mice (P11-21) were anaesthetised by I.P administration of sodium pentobarbitone (60 mg/kg), the spinal cords dissected and 300 μ m slices cut. Recordings were made in current clamp, whilst dyes were added intracellularly. Recordings revealed that ependymal cells were a uniform population with a resting membrane potential of $-75.7 \text{ mV} \pm 0.7$ (SE, $n = 63$), an input resistance of $100.1 \text{ M}\Omega \pm 15.2$ ($n = 63$) and no spontaneous or evoked activity. Dyes revealed a subpopulation of cells that possessed dorsally projecting fibres. Based on electrophysiology, the CSFCCs were categorised into four subtypes: subtype 1 ($n = 7$) had similar electrophysiological properties to ependymal cells but were morphologically defined as CSFCCs, subtypes 2 ($n = 19$), 3 ($n = 95$) and 4 ($n = 44$) were defined by their ability to produce a depolarisation, a spike or 2 or more spikes, respectively, in response to the injection of positive current. For immunohistochemistry, adult C57/b6 mice were anaesthetised by I.P administration of sodium pentobarbitone (60mg/kg), perfused with 4 % paraformaldehyde and the spinal cord was removed and sectioned. CSFCCs were identified by rabbit anti-PKD2L1. Rat anti-5HT revealed that 58 ± 17 % of CSFCCs had 5HT-positive terminals in close apposi-

tion and rat anti-substance P revealed that $52 \pm 7\%$ of CSFCCs had substance P-positive terminals in close apposition. Electrophysiology, however, showed that neither 5HT ($n = 4$, mouse; $n = 9$, rat) nor substance P ($n = 12$, rat) had an effect on the input resistance or membrane potential of CSFCCs. As positive controls, CSFCCs were shown to respond to GABA ($n = 4$, mouse; $n = 17$, rat) and nearby interneurons in lamina X were shown to respond to 5HT ($n = 3$, rat) and substance P ($n = 4$, rat). The results to date are only the beginning of the characterisation of these cells; this must be built on to determine whether CSFCCs are capable of maturing and integrating with existing circuits. Understanding this area to the point where we can manipulate both ependymal cells and CSFCCs could lead to beneficial treatments for numerous spinal cord pathologies.

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C55

Myenteric descending serotonergic nerve pathways excite submucosal neurons but inhibit surrounding glial cells during the murine colonic migrating motor complex

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Aims: To determine whether myenteric descending serotonergic interneurons that project to the submucous plexus activate glial cells as well as secretomotor neurons between and during the colonic migrating motor complex (CMMC; Okamoto et al., 2012).

Methods: Mice (C57) were anesthetized with isoflurane (1-2%) and euthanized by cervical dislocation, as approved by our Institutional Animal Ethics Committee and according to oNIH guidelines. The whole colon was removed, opened, threaded through a partition, and pinned with the mucosa uppermost. A small section of mucosa was removed to reveal submucosal ganglia that were loaded with Fluo-4-AM. Ca^{2+} transients were recorded at 340C. The CMMC, which was detected by tissue movement, was evoked by brushing the mucosa at the anal end.

Results: S100 β positive SGCs were surrounded by nNOS, but not calretinin, positive nerve varicosities. S100 β ($\sim 15\%$; $n=5$), nNOS ($\sim 8.5\%$; $n=5$) and calretinin (83% ; $n=3$) staining revealed populations of submucosal neurons. Submucosal neurons were spontaneously active, whereas, SGCs exhibited no detectable activity between CMMCs (duration 23s). Just preceding the CMMC, submucosal neurons (including S100 β , nNOS and calretinin positive neurons) exhibited fast Ca^{2+} transients ($1.6 \pm 0.1\text{Hz}$) superimposed on a sustained rise in Ca^{2+} (duration: $20.8 \pm 2.5\text{s}$; $n=7$) that occurred after $1.7 \pm 0.2\text{ s}$. In contrast, evoked Ca^{2+} transients in SGCs (S100 β positive) exhibited two phases: an early phase after $5.5 \pm 1.3\text{s}$ consisting of slow, but sometimes barely detectable, rise in Ca^{2+} that occurred during the CMMC (duration $\sim 24\text{s}$), and a later pronounced peak of Ca^{2+} after $36.4 \pm 4.3\text{s}$ ($n=6$) that followed the CMMC. Ondansetron ($1\text{-}2\mu\text{M}$; 5-HT $_3$ antagonist; $n=4$) significantly reduced the duration ($7.1 \pm 2.4\text{s}$, $p<0.01$) and frequency ($0.7 \pm 0.1\text{Hz}$, $p<0.01$) of the evoked Ca^{2+} transients in submucosal neurons. However, following ondansetron, SGCs exhibited pronounced Ca^{2+} transients after a latency of $9.3 \pm 0.3\text{s}$,

which started during the CMMC. Similar effects were observed following L-NA ($100\mu\text{M}$) or when TTX ($1\mu\text{M}$) was placed in the oral chamber, which separated it from the recording chamber. **Conclusion:** Myenteric descending serotonergic neurons activate submucosal secretomotor neurons via 5-HT $_3$ receptors just prior to the CMMC. In contrast, SGCs are normally inhibited as a consequence of activity in these descending neurons, possibly because they also cause the release of nitric oxide from nNOS positive submucosal neurons.

Okamoto T, Bayguinov PO, Broadhead MJ, Smith TK (2012) Ca^{2+} transients in submucosal neurons during the colonic migrating motor complex in the isolated murine large

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C56

Synaptic responses in the inferior olive to projections from the neocortex

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The olivocerebellar circuit is involved in co-ordinating movements and in motor learning. Long-range inputs converge on a particular component of the circuit, the inferior olive (IO), which in turn projects via climbing fibres to Purkinje cells in the cerebellum. The output from inferior olive neurons is manifest as a complex spike, which is important for computations carried out by Purkinje cells. Inferior olive neurons receive inputs from the deep cerebellar nuclei and from the periphery via the spinal cord. Anatomical studies have suggested that neurons in the neocortex also project to the inferior olive, but their physiological role remains unclear. We virally transduced neurons of the neocortex with an adeno-associated virus expressing channelrhodopsin-2 (AAV-ChR2), with the aim of isolating neocortical inputs in to the inferior olive. Mice (5-6 weeks) were anesthetized with isoflurane (2%) while placed in a stereotaxic frame. Bilateral injections of AAV-ChR2-Venus ($0.5\mu\text{l}$) were made in to the neocortex (AP: $1.1\text{-}1.6\text{ mm}$, ML $\pm 1.1\text{-}1.2\text{ mm}$, DV -1.0 mm to bregma), and the animals allowed to recover for 3 weeks. After the recovery period, mice were anesthetized using the same procedure, their brains removed and $200\mu\text{m}$ coronal IO sections were cut. Whole-cell recordings were then performed, and the responses of olivary neurons to optical stimulation of the transduced axons from neocortex measured. We find that light-evoked responses, specific to projections from the neocortex, can be reliably invoked in IO 3 weeks post-injection. Responses to neocortical input consist of a fast depolarizing component (peak $1.77 \pm 0.09\text{ mV}$ $n=6$) followed by a large and relatively slow hyperpolarizing component (peak $-2.54 \pm 0.49\text{ mV}$ $n=6$). Minimal stimulation results in all-or-none responses ('1V' 2ms light pulse: slope $0.82 \pm 0.26\text{ mV/ms}$ $n=6$) with a low probability of release (0.35 ± 0.09). These data provide evidence of functional connections between the neocortex and inferior olive. Understanding how these inputs affect neural computations in olivary neurons will contribute to understanding motor co-ordination.

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On the role of reciprocal inhibition in locomotion rhythm generation - Brown's half centre hypothesis revisited a century later

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Background: Brown's half centre hypothesis proposed a century ago (Brown, 1911; Brown, 1914) emphasized the importance of reciprocal inhibition in the generation of locomotor rhythms. However, pharmacological blockade of reciprocal inhibition or lesion of commissural connections in all vertebrates has led to a widely accepted notion that reciprocal inhibition is not required in locomotor rhythm generation and neuronal pace-maker properties have been suggested to mediate the rhythms (Li, 2011). We use two new methods to suppress reciprocal inhibition on millisecond scales in the *Xenopus* tadpole swimming circuit to re-investigate the role of inhibition in locomotor rhythm generation.

Methods: Procedures for producing tadpoles comply with UK Home Office Animals Act 1986 and have received local ethical approval. *Xenopus* tadpoles were anaesthetised with 0.1% MS-222 (3-aminobenzoic acid ester), immobilised with 10 μ M α -bungarotoxin and dissected to expose the spinal cord. The two methods we used for rapidly suppressing reciprocal inhibitory interneuron (cIN) activity are 1) Optogenetics: we expressed the light-sensitive reverse proton pump archaerhodopsin-3 (ArCh) (Chow et al., 2010) on one side of the nervous system by injecting its cRNA into one blastomere at the two cell stage of embryo development. We then specifically inhibited that side of the nervous system by shining yellow light (585 nm) to activate ArCh. 2) Injecting large negative DC into single excitatory interneurons (dINs) which are electrically coupled and directly excite cINs during swimming.

Results: We found that acutely inhibiting activity on one side could instantly stop swimming rhythms on both sides (median time for yellow light inhibition: 0.19 seconds or 2 swimming cycles, n=149 trials in 8 tadpoles; for -DC injections: 0.19 seconds or 2 cycles, n=107 trials in 13 dINs). Analysing changes in synaptic drives on the un-inhibited side showed that reciprocal inhibition was specifically weakened to $4.4 \pm 2.6\%$ (IPSC size normalised to control, Wilcoxon Signed Rank test, n=11, p<0.001) in the last swimming cycle, leading to reduced dIN rebound firing reliability (from control of 99.6 ± 0.4 to $0.4 \pm 0.4\%$, Wilcoxon Signed Rank test, n=11, p<0.001). dIN activity drives tadpole swimming circuit and their rebound firing provides one mechanism to maintain normal tadpole swimming rhythms (Soffe et al., 2009; Li, 2011).

Conclusion: Our data thus support Brown's original proposal that reciprocal inhibition is required in locomotor rhythm generation.

Brown, T.G. (1911) The intrinsic factors in the act of progression in the mammal. Proceedings of the royal Society of London. Series B, 308-319.

Brown, T.G. (1914) On the nature of the fundamental activity of the nervous centres; together with an analysis of the conditioning of rhythmic activity in progression, and a theory of the evolution of function in the nervous system. J Physiol, 48, 18-46.

Chow, B.Y., Han, X., Dobry, A.S., Qian, X., Chuong, A.S., Li, M., Henninger, M.A., Belfort, G.M., Lin, Y., Monahan, P.E. & Boyden, E.S. (2010) High-performance genetically targetable optical neural silencing by light-driven proton pumps. Nature, 463, 98-102.

Li, W.-C. (2011) Generation of Locomotion Rhythms Without Inhibition in Vertebrates: The Search for Pacemaker Neurons. Integr Comp Biol, 51, 879-889.

Soffe, S.R., Roberts, A. & Li, W.C. (2009) Defining the excitatory neurons that drive the locomotor rhythm in a simple vertebrate: insights into the origin of reticulospinal control. J Physiol, 587, 4829-4844.

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Release parameters at single site synapses between rat cerebellar interneurons investigated in vitro with laser photolysis of Ca-DM-nitrophen

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Calcium release by photolysis has been applied to study mechanisms of release at large, multisite synapses (1,2). However, synapses made on the dendrites of central neurons, comprising a single or few release sites, may differ from these and may also show differences from one to another. Their small size is a technical impediment to studies of transmitter release. We describe here the application of laser photolysis to release Ca²⁺ in presynaptic terminals to study the parameters of vesicular release at single site connexions between cerebellar molecular layer interneurons (MLI).

MLI have advantages for studies of this kind. Synapses between MLI show evidence of multivesicular release of GABA at single contacts (3). They are electrically compact, show a high likelihood of synaptic connection in recorded pairs, and have autoreceptors and autapses permitting single cell studies. Laser photolysis of DM-nitrophen 4 was used to release Ca²⁺ in the presynaptic terminal in paired whole cell recordings. The presynaptic interneuron was filled with Alexa 594 and Oregon Green Bapta 5N and the postsynaptic with Alexa 488. To find putative sites of connection and for mapping their morphologies, the preparation was illuminated alternately with 470/40 nm and 573/30 nm light from LED light sources, and imaged with an EMCCD camera through dual band filters. Photolysis of DM-Nitrophen-Ca was with a diode laser at 405 nm with controlled intensity, duration and frequency of exposure. The laser spot was set to 3 μ m to illuminate single connexions and the preparation positioned with a piezo-controlled platform with resolution <0.25 μ m. Ca²⁺ release was monitored by EMCCD imaging at 200-1000 Hz with 470/40 nm excitation and 535/40 nm emission. The method permits the timing and number of release events to be monitored postsynaptically with controlled pulses of presynaptic Ca²⁺ release.

Data were analysed by measuring intervals between the laser pulse and the postsynaptic events in successive 1 s sweeps separated by 90 s. The distribution of intervals, fitted with maximum likelihood (5), showed two exponential components with

time constants of 4 ms and 320 ms, and 60% of events were in the early component. This corresponds to a mean of 1.76 events /stimulus in the fast component. Corrections were applied for missed second and subsequent events due to post-synaptic receptor saturation, giving an estimate of the mean readily releasable pool of 2.0 /stimulus. The number of events varied from sweep to sweep at the same laser energy, up to a maximum observed of 4, and the probability of release is estimated as 0.7. The distribution of first latencies showed an initial silent period of 0.5 ms followed by a rapid rise to a maximum release rate in 1 ms, consistent with highly cooperative activation by photoreleased Ca²⁺.

1. Bollmann, J. H., Sakmann, B., and Borst, J. G. (2000). Calcium sensitivity of glutamate release in a calyx-type terminal. *Science* 289, 953-957.

2. Schneggenburger, R., and Neher, E. (2000). Intracellular calcium dependence of transmitter release rates at a fast central synapse. *Nature* 406, 889-893.

3. Auger, C., Kondo, S., and Marty, A. (1998). Multivesicular release at single functional synaptic sites in cerebellar stellate and basket cells. *J. Neurosci.* 18, 4532-4547.

4. Kaplan, J. H., and Ellis-Davies, G. C. (1988). Photolabile chelators for the rapid photorelease of divalent cations. *Proc. Nat. Acad. Sci. USA* 85, 6571-6575.

5. Colquhoun, D., and Sigworth, F. J. (1995) Analysis of single ion channel data. In *Single Channel Recording*, 2nd ed. (Eds: Sakmann, B., Neher, E.). Plenum Press, New York, pp. 483-587. DCProgs (D. Colquhoun and I. Vais: available at www.ucl.ac.uk/Pharmacology/dc.html).

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20 Hz, 2 min trains was also assessed, comparing first and subsequent trains applied one hour later. Data are expressed as mean $\pm$ SE, n (NMJs) from 6-15 preparations and compared by Student's t-test (means) or 2-way ANOVA (trains). As previously (Fong et al, 2010), 1ng/ml TGF- β 2 significantly increased amplitudes of mEPPs (0.50 ± 0.06 mV, 14 to 0.70 ± 0.03 mV, 22; $p < 0.01$) and EPPs (24.3 ± 1.9 mV, 14 to 28.5 ± 1.1 mV, 22; $p < 0.05$). Conversely, TGF- β 2 neutralising antibody reduced the size of mEPPs (0.32 ± 0.04 mV, 15; $p < 0.02$) and EPPs (18.2 ± 1.5 mV, 15; $p < 0.02$). TGF- β 2 significantly increased EPP amplitudes throughout a train ($n = 15$ & 6; $p < 0.0001$). Strikingly, in control muscles given a second train 1 hr later EPPs were also larger ($n = 8$, $p < 0.0001$), and not significantly different from a first EPP train in TGF- β 2 ($p = 0.3$). The effect of repeated trains was abolished by neutralising antibodies to TGF- β 2 ($n = 5$ & 8; $p = 0.8$), while repeated trains in TGF- β 2 produced only a small further increase ($n = 15$, $p = 0.02$). These data indicate nerve activity trains release endogenous TGF- β 2 from postsynaptic muscle fibres that enhances subsequent neurotransmission. Thus, the data indicate TGF- β 2 is an activity-dependent retrograde modulator of synaptic transmission.

Fong SW et al. (2010). *Proc Natl Acad Sci* 107, 13515-13519.

McLennan IS & Koishi K (2002). *Int J Dev Biol* 46, 559-567.

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Evidence that endogenous TGF- β 2 is released by activity as a retrograde modulator of synaptic transmission

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We are examining the role of Transforming Growth Factor- β 2 (TGF- β 2) at mammalian synapses. TGF- β 2 is the most highly expressed cytokine in the healthy adult mammalian nervous system. It is particularly expressed in skeletal muscle fibres at neuromuscular junctions (NMJs), while motor terminals express the high affinity receptor for TGF- β 2 binding (McLennan & Koishi, 2002). We recently showed TGF- β 2 enhances neuromuscular transmission by increasing loading of neurotransmitter into synaptic vesicles (increasing quantal size; Fong et al, 2010). It also increases evoked postsynaptic potentials, but reduces the number of vesicles released per action potential (lower quantum content). These effects occur with 60 min pre-incubation at 1ng/ml. Thus, TGF- β 2 acutely increases both efficacy (bigger potentials) and efficiency (fewer vesicles required). However, it is still unclear if endogenous TGF- β 2 can be released as a retrograde modulator of neuromuscular transmission.

Adult Sprague-Dawley rats were humanely killed by cervical dislocation (Schedule 1, ASPA 1986) and the amplitudes of spontaneous (miniature end-plate potentials, mEPPs) and nerve-stimulation evoked ACh release (EPPs) recorded from NMJs in excised hemidiaphragm/phrenic nerve preparations with sharp intracellular microelectrodes. μ -conotoxin GIIIB blocked muscle contraction. EPP amplitude rundown during

C60

Investigation of Pax6-dependent gene networks controlling progenitor cell proliferation in the developing telencephalon

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Pax6 encodes a highly conserved transcriptional regulator that contains two DNA binding domains, the paired domain (PD) and homeodomain (HD). In mammals, Pax6 is widely expressed in a complex spatiotemporal pattern during the development of the eye, olfactory bulbs and central nervous system and plays important roles in pattern formation, cell fate determination and cell cycle progression in these regions. Here, we characterized a Pax6-dependent signaling pathway through which Pax6 controls progenitor cell proliferation in the developing telencephalon. Comparison of cell cycle parameters between Pax6^{+/+} and Pax6^{sey/sey} progenitors suggested that correct levels of Pax6 are crucial in regulating progenitor cell proliferation. To address the possible molecular basis of the cell cycle defect observed in Pax6^{sey/sey} embryos, the expression of a number of cell cycle genes was analyzed by qPCR in the lateral cortex of Pax6^{+/+} and Pax6^{sey/sey} embryos, which confirmed the significantly altered expression levels of these genes. Of these, *Cdk6* was further identified as a direct target of Pax6 and the interaction of putative binding sites with Pax6 protein was confirmed by EMSA *in vitro* and by qChIP *in vivo*. In addition, the functional role of these Pax6 binding sites, through which Pax6 represses the transcription of *Cdk6*, was further evaluated by luciferase assays. Activation of *Cdk6* is required for pRb phosphorylation as well as induction of the pRB/E2F pathway, and in turn promotes the G1-S cell-cycle

transition. An increase in pRb phosphorylation accompanied by changes in pRb subcellular distribution and up-regulation of E2F downstream targets were observed in the cortex of *sey/sey* embryos. In contrast, reduced Cdk6 expression and pRb phosphorylation was found in HEK293 cells overexpressing Pax6. Collectively, these findings provided new insight into the molecular mechanism of Pax6-dependent regulation of progenitor cell proliferation in the developing telencephalon.

Estivill-Torres, G., Pearson, H., van Heyningen, V., Price, D. J. and Rashbass, P. (2002). Pax6 is required to regulate the cell cycle and the rate of progression from symmetrical to asymmetrical division in mammalian cortical progenitors. *Development* 129, 455-66.

Sansom, S. N., Griffiths, D. S., Faedo, A., Kleinjan, D. J., Ruan, Y., Smith, J., van Heyningen, V., Rubenstein, J. L. and Livesey, F. J. (2009). The level of the transcription factor Pax6 is essential for controlling the balance between neural stem cell self-renewal and neurogenesis. *PLoS Genet* 5, e1000511.

Dehay, C. and Kennedy, H. (2007). Cell-cycle control and cortical development. *Nat Rev Neurosci* 8, 438-50.

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Direct pharmacological monitoring of the developmental switch in NMDA receptor subunit composition

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NMDARs are tetrameric assemblies containing two GluN1 and two GluN2 subunits where the identity of the latter determine many of the key biophysical, pharmacological and downstream signalling properties of NMDARs. In the forebrain GluN2A and GluN2B NMDARs predominate and therefore identifying how these NMDAR subtypes contribute to various physiological and pathophysiological roles is key for our understanding of glutamatergic function. Antagonists that are sufficiently selective to block preferentially GluN2A- over GluN2B NMDARs are few in number. Recently a high throughput screen of a chemical library (Bettini *et al.* 2010) identified a novel antagonist, TCN 201, that showed selectivity for GluN2A over GluN2B NMDARs. We have characterized the nature of the antagonism produced by TCN 201 and used it to monitor the switch in NMDAR subunit composition in developing cortical neurones. Two-electrode voltage-clamp recordings were made from *Xenopus laevis* oocytes expressing recombinant GluN2A or GluN2B NMDARs. The block of GluN2A NMDARs by TCN 201 was dependent on the concentration of glycine. At a glycine concentration of 10 μ M, TCN 201 blocked GluN2A currents by $82 \pm 1\%$ ($n = 12$) whereas a lower block of $51 \pm 1\%$ ($n = 8$) was observed at a higher glycine concentration of 30 μ M. Conversely, the blockade by TCN 201 was independent of the glutamate concentration used to evoke GluN2A NMDAR-mediated currents. Importantly TCN 201 (10 μ M) gave minimal block of GluN2B currents ($3 \pm 1\%$, when the glycine concentration was either 10 μ M or 30 μ M). Schild analysis showed that TCN 201 was a potent antagonist of GluN2A NMDARs with a K_b value of 70 nM; however the nature of this antagonism was not competitive as assessed by a non-linear Schild plot. We assessed the ability of TCN 201 to block NMDAR-mediated currents in rat cultured cortical neurones. At days *in vitro* (DIV) 7-10 NMDAR currents were strongly blocked by ifenprodil (80

$\pm 3\%$, $n = 7$), indicating that most NMDARs contained only GluN1 and GluN2B NMDAR subunits. At this stage TCN 201 gave only modest block of NMDAR currents ($5 \pm 2\%$). The sensitivity of the currents to ifenprodil decreased in older (DIV 14-18) cultures ($57 \pm 5\%$, $n = 9$) or in younger (DIV 7-10) neurones over-expressing the GluN2A subunit ($24 \pm 3\%$, $n = 6$), while TCN 201 gave stronger block ($47 \pm 4\%$ and $16 \pm 3\%$ respectively). A negative correlation between ifenprodil and TCN 201 block was observed ($R^2 = 0.91$).

Our data demonstrate that TCN 201 allows the monitoring of the developmental switch in NMDAR subunit composition. Future studies will use this and a related antagonist, TCN 213 (McKay *et al.* 2011) combined with complementary genetic approaches to investigate the physiological and pathophysiological roles played by NMDAR subtypes.

Bettini E, *et al.* (2010). Identification and characterization of novel NMDA receptor antagonists selective for NR2A- over NR2B-containing receptors. *J Pharmacol Exp Ther.* 335: 636-644.

McKay S, *et al.* (2011). Direct pharmacological monitoring of the developmental switch in NMDAR subunit composition using TCN 213, a GluN2A-selective, glycine-dependent antagonist. *Brit J Pharmacol.* DOI: 10.1111/j.1476-5381.2011.01748.x

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C62

Heteromeric kainate receptors have a fixed subunit stoichiometry

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Kainate-type ionotropic glutamate receptors (iGluRs) assemble primarily as heteromeric complexes at glutamatergic synapses. Despite this, little is known about their basic biophysical and structural properties. Here, we investigate the relationship between the functional behavior of recombinant heteromeric GluK2/GluK5 kainate receptors (KARs) and their subunit stoichiometry. Using outside-out patch electrophysiology, we observed that homo- and heteromeric receptors differed in their responsiveness to the agonist, AMPA, and had differing decay kinetics in response to brief applications of L-glutamate. Interestingly, the association between these two features was positively correlated with the degree of GluK2/GluK5 heteromerization. To elucidate whether this relationship is due to a fixed or variable subunit copy number, we interrogated stoichiometry directly by using single-molecule fluorescent step detection. We have made significant modifications to the original method which was based upon expressing GFP-tagged subunits in *Xenopus laevis* oocytes and manually counting the number of step-wise changes in fluorescence intensity due to photobleaching (Ulbrich and Isacoff, 2007). Our new approach has adapted the technique for mammalian cells and we have also eliminated user-bias by developing a fully-automated single-molecule fluorescence counting program which we have called Progressive Idealization and Filtering or PIF. Using this approach, the tetrameric and pentameric stoichiometry of homomeric GluK2 ($n=1312$ spots)

and $\alpha 1$ glycine receptors ($n=1186$ spots) respectively were correctly identified. In both cases, the GFP maturation rate (pm) was determined to be 53%, indicating that maturation is fluorophore-dependent. Interestingly, best fits of the data were obtained by assuming a single photobleaching spot could contain two protein complexes. Applying this same method to heteromeric GluK2/GluK5 KARs revealed that these subunits assemble with a fixed stoichiometry of 2:2 ($n=1659$ spots). Similar to previous results, pm was 53% and best fits of the data were obtained by assuming multiple receptor complexes could occupy a single resolvable spot. Since prior work on NMDA-type iGluRs has shown that they are also obligate heteromers, our work suggests that this may be a common feature of all heteromeric iGluRs.

Ulbrich MH & Isacoff EY (2007). Subunit counting in membrane-bound proteins. *Nat Methods* 4, 319-321.

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Mutant mice lacking Synapse Associated Protein 102 (SAP102) display defects in thalamocortical axon development and dendritic spine frequency

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Mutations in the SAP102 gene, which encodes SAP102 have been found in families with a history of X-linked mental retardation (1). SAP102 is a member of the membrane associated guanylate kinase (MAGUK) family of synaptic scaffolding molecules that link NMDA receptors to downstream signalling targets in the post-synaptic density. MAGUKs are hypothesised to create microdomains of intracellular signalling pathways through mediating protein-protein interactions. SAP102 directly binds to both the GluN2 subunit of the NMDA receptor and SynGAP, a synaptic Ras-GTPase activating protein (3). Genetic disruption of either cortical NMDA receptors (2) or SynGAP display cortical defects in early postnatal development (2). Therefore, we tested the hypothesis that SAP102 regulates early patterning of, and neuronal connectivity within, the mouse somatosensory cortex (S1) using a mouse model with a genetic deletion of the Sap102 gene. Sap102 $-/-$ mice and their littermate controls were anaesthetised with a lethal dose (0.025 ml/g, i.p.) sodium pentobarbital and perfused transcardially. The brain was removed and sectioned for immunohistochemistry, Golgi-Cox staining method or carbocyanine dye (DiI) labelling. All data are represented as means $\pm$ S.E.M. and analysed using a two-tailed t test. Sap102 $-/-$ show a reduction in body and brain mass throughout postnatal development, however laminar thickness of S1 is unchanged. Cortical map formation in Sap102 $-/-$ mice is grossly normal although thalamocortical axon (TCA) patch size is reduced. This reduction in TCA patch size is cannot be explained by a reduction in total brain size, as this defect is significant when nor-

malised to neocortical area (Sap102 $+/y$ 0.0052 $\pm$ 0.00057, SAP102 $-/y$ 0.0036 $\pm$ 0.00036, $n=9,6$ respectively, $p<0.05$). DiI labelling reveals an increase in mediolateral extent of TCA branching indicating that the decrease in TCA patch size is not the result of reduced TCA length. Synapse formation was investigated in Sap102 $-/y$ mice by comparing the distribution of dendritic spines in different cell types. We find the loss of SAP102 causes cell-type specific defects on the density of dendritic spines in the S1. On layer IV stellate cells dendritic spine density was increased in Sap102 $-/y$ mice compared to controls (Sap102 $+/y$ 8.52 $\pm$ 0.46, SAP102 $-/y$ 10.13 $\pm$ 0.33, $n=5$, $p<0.05$) whereas spine density was decreased on the apical shaft of layer V pyramidal cells as they pass through layer IV (Sap102 $+/y$ 6.02 $\pm$ 0.76, Sap102 $-/y$ 8.36 $\pm$ 0.54, $n=12,11$ respectively, $p<0.05$). Together these data indicate that SAP102 plays a key role during cortical development in the mouse, a time that corresponds to embryonic development in humans. Furthermore, the loss of SAP102 results in cell specific phenotypes in layer IV suggesting heterogeneous roles for the protein during cortical development.

(1) Tarpey, P. et al. (2004) *Am. J. Hum. Genet.* 75:318-324

(2) Li, H., & Crair, M. (2011) *Ann. N.Y. Acad. Sci.* 1225:119-129

Kim, M. et al (2004) *Neuron* 46:745-760

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Divergent circuit changes partially re-balance cortical layer IV thalamocortical responses in a mouse model of Fragile X syndrome

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Fragile X Syndrome (FXS) is the most common heritable form of intellectual disability and autism. It is caused by a single gene mutation and symptoms include cognitive impairment, seizures, deficits in sensory gating and abnormal sleep. Dissecting the pathophysiology of sensory cortical activity helps explain sensory phenotypes in FXS and offers insights into higher cognitive dysfunction. Studies in the Fmr1-KO mouse model of FXS show developmental abnormalities in cortical neuronal physiology. In particular, altered plasticity at the thalamocortical (TC) synapse was shown in somatosensory cortex during postnatal days 6-12 (P6-12)(1). Between these ages TC responses undergo coordinated developmental maturation in wild-type (WT) mice. (2,3). Appearance of local feed-forward inhibition (FFI), modulation of cell intrinsic properties and synaptic kinetics sharpen the temporal resolution of cortical TC responses by controlling synaptic integration in cortical layer IV (LIV) excitatory neurons. We sought to examine these processes in juvenile Fmr1-KO mice. Intrinsic membrane and synaptic properties of Fmr1-KO (P10-11) excitatory neurons were investigated using electrophysiology in a TC slice preparation (4). LIV excitatory neurons (WT: $n=33$, KO: $n=37$) displayed increased input resistance with unaltered whole-cell capacitance. Effective membrane time constant, τ_m was therefore longer (KO: 55 $\pm$ 3 vs. WT: 39 $\pm$ 4ms, $P<0.002$). Values are mean $\pm$ S.E.M., compared by t-test. Electrical stimulation in ventrobasal thalamus evoked feed-forward EPSP-IPSP sequences in excitatory neurons (WT: $n=30$, KO: $n=31$). Strength of FFI

was quantified as IPSC/EPSC peak ratio (I/E). TC FFI was enhanced in Fmr1-KOs (I/E ratio, KO: 5.0 ± 0.5 vs. WT: 3.9 ± 0.5 $P < 0.05$, Mann-Whitney test). Latency and kinetics of FFI EPSC-IPSCs were additionally prolonged. Furthermore, repetitive TC stimulation at behaviorally relevant frequencies (5-50Hz) induced exaggerated short-term depression (STD) of both EPSCs and IPSCs in Fmr1-KOs. We used a conductance-based ball-and-stick model with depressing TC and FFI inputs to investigate interaction of these phenotypes on the temporal integration window at TC synapses. At WT FFI ranges (I/E < 3), longer τ_m dominated and a broad temporal integration window was observed in the Fmr1-KO model across a range of input frequencies. Increasing FFI to ranges recorded in Fmr1-KOs ($3 < I/E < 10$) stabilised and then further sharpened the integration window relative to matched WT simulations. Individual rescue in silico of τ_m , synaptic kinetics or STD to WT levels could not return improve TC function and exacerbated temporal resolution defects. These data support the conclusion that multiple divergent circuit alterations, including stronger feed-forward inhibitory drive interact in juvenile Fmr1-KOs to partially re-tune TC temporal discrimination.

Harlow, E.G. et al. *Neuron*, 2010(65):385-398.

Critical Period Plasticity Is Disrupted in the Barrel Cortex of Fmr1 Knock-out Mice.

Daw, M.I., Ashby, M.C. and Isaac, J.T.R. *Nature Neuroscience* 2007(10):453-461.

Coordinated recruitment of latent fast spiking interneurons into the layer IV barrel cortex circuit enables rapid development of thalamocortical feed forward inhibition.

Chittajallu, R. and Isaac, J.T.R. *Nature Neuroscience* 2010(10):1240-8.

Emergence of cortical inhibition by coordinated sensory-driven plasticity at distinct synaptic loci.

Agmon, A. and Connors, B.W. *Neuroscience* 1991(41) 365-79 Thalamocortical responses of mouse somatosensory (barrel) cortex in vitro.

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C65

The nitric-oxide dependent antifibrillatory effects of vagus nerve stimulation in the rabbit ventricle involve cGMP-dependent mechanisms

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Introduction

We have previously shown that vagus nerve stimulation (VNS) increases effective refractory period (ERP) and ventricular fibrillation threshold (VFT) [1], via a nitric oxide (NO) dependent mechanism [2] that is independent on acetylcholine [3]. NO can activate soluble guanylyl cyclase [sGC] to produce cyclic guanylyl monophosphate (cGMP) and modulate cardiac function. The aim of this study was to investigate if the effects of VNS-NO pathway on ERP and VFT were mediated via cGMP mechanisms.

Methods

Adult New Zealand White rabbits (n=7, 2.5-4kg) were pre-sedated with an i.m mixture of ketamine (10mg/kg), medetomidine hydrochloride (0.2mg/kg) and butorphanol (0.05mg/kg). Following sedation, animals were heparinised (1000IU, i.v.) and humanely killed with an overdose of sodium pentobarbitone (160mg/kg, i.v.). Hearts were removed and perfused in constant flow Langendorff mode with ventricular

electrophysiology measured using contact electrodes. ERP were measured using single extrastimulus protocol (300ms cycle length). VFT was the minimum current needed to induce sustained VF with burst pacing (30x30ms). These were measured at baseline [BL] and with: 1) NO donor (Sodium Nitroprusside [SNP], 100 μ M), 2) sGC inhibition (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one [ODQ], 10 μ M), 3) vagus nerve stimulation [VNS, 10Hz] and (4) VNS or SNP in the presence of ODQ. Data are mean $\pm$ SEM, Students T-Test, $P < 0.05$ compared with BL.

Results (see Table):

SNP and VNS both increased ERP and VFT. The effects of SNP were preserved during sGC inhibition, whilst the effects from VNS were inhibited.

Conclusion

Cyclic GMP is involved in the protective effects of VNS-NO pathway against VF but concurrent cGMP independent mechanisms could also be at work.

Table

	BL	SNP	BL	ODQ	BL	SNP&ODQ
ERP	124.8 $\pm$ 2.9	139.0 $\pm$ 4.6 *	136.4 $\pm$ 1.8	131.4 $\pm$ 4.2	130.0 $\pm$ 5.0	148.2 $\pm$ 9.5 *
VFT	4.6 $\pm$ 1.0	11.6 $\pm$ 2.4 *	3.9 $\pm$ 0.8	3.1 $\pm$ 0.9	2.7 $\pm$ 1.4	7.3 $\pm$ 1.9 *
	BL	VNS	VNS&ODQ			
ERP	135.0 $\pm$ 2.0	155.8 $\pm$ 4.9 *	141.3 $\pm$ 5.4			
VFT	3.0 $\pm$ 0.9	6.8 $\pm$ 1.7 *	3.4 $\pm$ 1.4			

* $P < 0.05$

Ng GA, Brack KE, Patel VH, Coote JH. Autonomic modulation of electrical restitution, alternans and ventricular fibrillation initiation in the isolated heart. *Cardiovasc Res* 2007;73(4):750-760.

Brack KE, Patel VH, Coote JH, Ng GA. Nitric oxide mediates the vagal protective effect on ventricular fibrillation via effects on action potential duration restitution in the isolated rabbit heart. *J Physiol* 2007;583(Pt 2):695-704.

Brack KE, Coote JH, Ng GA. The protective effect of vagus nerve stimulation against ventricular fibrillation is independent of muscarinic receptor blockade. *Card Re* 2011;91(3):437-446.

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C66

Altered GABA_A and NMDA receptor subunit expression in the Hypothalamic Paraventricular Nucleus: A possible cause of sympathoexcitation?

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The autonomic nervous system plays an essential role in cardiovascular regulation, and sympathetic overactivation is characteristic of cardiovascular disease, e.g. hypertension¹. In pregnancy, sympathoexcitation is vital for normal foetal development².

The hypothalamic paraventricular nucleus (PVN) is an important site for integration of cardiovascular inputs and autonomic regulation³. GABA elicits inhibitory effects within the PVN through the GABA_A receptor, whereas glutamate has an excitatory effect through the NMDA receptor³. Under normal conditions, GABAergic inhibition suppresses autonomic outflow from this nucleus. However in hypertension, glutamatergic activity is potentiated, while GABAergic inhibition is attenuated.

ated⁴. This study investigates whether GABA_AR and NMDAR subunit alterations in the PVN are associated with hypertension and/or pregnancy.

Experiments conformed to the Animals (Scientific Procedures) Act, 1986. Western blot analysis was performed on PVN micropunches from female Wistar (n=3), female spontaneously hypertensive (SHR; 14wk, n=3) and pregnant Wistar rats (E19, n=3). The protein extract was probed with antibodies against the GluN1, -2A or -2B subunits of the NMDAR or the α 1, α 2, α 5, β 1, β 3 subunits of the GABA_AR. Analysis of protein band density was performed using ImageJ. For immunohistochemical analysis, animals were perfuse-fixed with 4% paraformaldehyde and the brain sectioned to 40 μ m. Sections were then probed for the same receptor subunits as above. The number of cells immunoreactive for each subunit was counted in the PVN.

There was a significant increase ($P<0.05$) in expression of the GluN2A subunit in the SHR compared with normotensive and late-term pregnant rats. Cell counts revealed this increase to be confined to the medial, dorsal cap and posterior parvocellular subnuclei of the PVN.

There was a significant reduction in GABA_A α 1 ($P<0.05$) expression in the SHR and late-term pregnant rat compared with normotensive controls. A significant reduction in α 5 ($P<0.001$) subunit expression was also observed in the SHR compared to both normotensive and late-term pregnant rats.

We have shown that in the SHR there is a significant decrease in GABA_AR α 1 and α 5 subunit expression, and a significant increase in NMDAR GluN2A expression. The increase in GluN2A expression correlates with regions of the PVN known to influence the cardiovascular system. Conversely, late-term pregnancy is associated with a decrease in GABA_AR α 1 subunit expression only.

We propose that altered subunit expression may explain why sympathetic regulation in hypertension and pregnancy differs from that in a "normal" physiological state.

Grassi et al (2009) J Nephrol: 22; 190-195

Kvachina et al (2009) Physiol & Behavior: 97; 171-179

Li & Patel (2003) Acta Physiol Scand: 177; 17-26

Biancardi et al (2010) J Comp Neurol: 518; 567-585

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Investigation of cardiorespiratory responses to activation of cardiac nociceptors in the decerebrate, arterially-perfused *in situ* mouse preparation

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Introduction: Nociceptive information from the heart is transmitted via sympathetic and vagal cardiac afferents and the involvement of these pathways in the development of cardiac pain is incompletely understood. This dual innervation of the heart and other thoracic viscera, and their convergence in central integrating centres, make it challenging to decipher the mechanisms of cardiac pain, which is exacerbated by the

practical issues of accessing the heart, especially in unanaesthetised animals. Given the potential benefit of investigating nociception in transgenic mouse lines, there is a surprising lack of mouse models of cardiac pain. We have extended the established working heart-brainstem preparation (WHBP; Paton 1996), a decerebrate, arterially-perfused *in situ* mouse model, to investigate cardiac nociception.

Methods: Adult mice were anaesthetised with 5% halothane (until decerebration) for surgical set up of the WHBP. The descending aorta was cannulated with a double lumen catheter and retrogradely perfused with carbogenated (5% CO₂, 95% O₂) Ringer's. Cardiac nociceptors were activated by bolus perfusion of capsaicin (1-100 μ M) over the epicardial surface of the heart. Simultaneous recordings were made of perfusion pressure, heart rate, phrenic nerve activity and somatic EMG of inter-scapular muscles. Data are expressed as mean $\pm$ SEM or median [quartile ranges].

Results: Application of capsaicin to the epicardial surface of the heart elicited rapid, reproducible responses in cardiorespiratory, somatic and vasomotor outflows. Capsaicin produced a tachycardia (10.2 [4.9 – 22.9] bpm, n=9) and attenuated respiratory sinus arrhythmia (n=8). This was usually accompanied by a small pressor response (1.8 $\pm$ 0.2 mmHg, n=4) consistent with sympatho-activation. Capsaicin also caused immediate bursts of somatic EMG activation (n=4). The respiratory changes were consistent within a preparation but varied across animals and consisted of tachypnoea (187 $\pm$ 13% of control, n=4), transient apnoea followed by tachypnoea (n=3) or a transient apnoea alone (3.4 $\pm$ 0.9s, n=2). Interestingly, vagotomy prevented the tachycardia and also abolished the pressor and respiratory components of the responses to capsaicin (n=2). Conclusions: Epicardial application of capsaicin elicited characteristic patterns of cardiorespiratory and vasomotor changes that suggest sympathetic activation and vagal withdrawal. Our preliminary data suggest that cardiac vagal afferent transmission plays an important role in generating this co-ordinated response. This novel mouse model of cardiac pain also offers the opportunity to study genetically modified strains in combination with recordings of cardiac vagal or sympathetic nerves to determine mechanisms.

Paton JF (1996). J Neurosci Methods 65: 63-68.

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Wenckebach rhythms during human atrial tachyarrhythmias: role of AV nodal recovery and dual pathway physiology

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The atrioventricular (AV) node is the specialized conduction system, which electrically connects the atria and the ventricles. Under critical fast atrial rates, as observed during atrial tachyarrhythmias, the AV node filters atrial beats, producing a variety of abnormal AV conduction rhythms. Among these, Wenckebach rhythms are characterized by a progressive prolongation of the AV conduction time leading to a blocked beat,

which repeatedly occurs when the atrial cycle length becomes shorter than the node functional refractory period (1). Despite the significant clinical importance of the phenomenon, the mechanisms and nodal determinants underlying AV nodal Wenckebach periodicity remain elusive (2,3).

To investigate the dynamical features of Wenckebach rhythms in humans at spontaneous high atrial rates, we analyzed 29 episodes of spontaneous or pacing-induced atrial flutter (AFL) (4), covering a wide range of high atrial rates (cycle lengths from 145 to 270 ms). Atrial electrograms and ECG signals were recorded in patients during an electrophysiological study after light sedation with diazepam (10 mg i.m.). Wenckebach patterns of n:m order (i.e., repeating cycles of n atrial and m ventricular activations) were identified by application of firing sequence (5) and surrogate data analysis to atrial and ventricular activation series. In order to disclose the role of different nodal properties in the generation of Wenckebach patterns, data analysis was complemented with computer simulations by a difference-equation AV model, including recovery and dual pathway properties.

Wenckebach rhythmicity in AFL patients was characterized by: i) the presence of n:m rhythms with decreasing conduction ratios ($m/n=0.34\pm0.12$ to 0.23 ± 0.06 , $p<0.01$) at shorter atrial cycle lengths ($AA=236.3\pm32.4$ to 172.6 ± 17.8 ms, $p<0.01$), ii) the ordering of rhythms at changing atrial cycle length according to the Farey sequence, with $(n+N):(m+M)$ rhythms between n:m and N:M rhythms, iii) the appearance of high-order alternating Wenckebach rhythms (type A), such as 6:2, 10:2 and 12:2, associated with large ventricular oscillations (amplitudes of 407.7 ± 150.4 ms) in five episodes. Simulations showed that a single pathway AV model, described by a monotonically decreasing recovery curve, predicted the existence and Farey sequence ordering of different Wenckebach patterns, with the exception of higher-order alternating Wenckebach rhythms (type A). The latter rhythms were, instead, predicted by the dual pathway model and originated from an alternate propagation of the impulse in the two pathways.

These results support the idea of an intimate connection between nodal recovery and dual pathway physiology in the generation of AV Wenckebach patterns at high atrial rates in the intact human AV node (2,3).

Rosenblueth A (1958). *Am J Physiol* 194, 491-494.

Zhang Y and Mazgalev TN (2011). *J Cardiovasc Electrophysiol* 22, 1256-1262.

Billette J and Tadros R (2011). *J Cardiovasc Electrophysiol* 22, 1263-1265.

Ravelli F et al. (1994). *Circulation* 89, 2107-2116.

Glass L et al. (1980). *J Theor Biol* 86, 455-475.

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The ventilatory response to carbon dioxide in experienced scuba divers during rest, exercise and hypoxia

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Hypercapnia related symptoms are not uncommon during scuba diving ranging from a mild to severe headache to unconsciousness. Scuba divers have been found to have a lowered ventilatory response to CO₂ (Florio *et al.*, 1979). To gain more understanding of the adaptive response to diving we investigated the ventilatory response to CO₂ in hyperoxia, hypoxia, and during exercise amongst experienced scuba divers and matched controls. Three studies were performed comparing the ventilatory response to CO₂ of experienced scuba divers with non-diving matched controls. The first study measured the ventilatory recruitment threshold (VRT) during CO₂ rebreathing in hyperoxia of experienced divers (n=10) and controls (n=10); the second study investigated the CO₂ sensitivity in rest and exercise using CO₂ rebreathing in hyperoxia at a workload designed to mimic the load of diving with scuba divers (n=11) and controls (n=11). The third study examined the respiratory drive of scuba divers (n=10) and controls (n=10) whilst breathing four different gas mixtures (ambient air; 25% O₂/6% CO₂; 13% O₂; 13% O₂/6% CO₂) aimed to assess the combined response to hypercapnia and moderate hypoxia. Statistical analysis of data was performed by ANOVA. Experienced divers possessed a higher VRT ($P<0.05$) related to 7%^{ins}CO₂ ($pCO_{2cap}=53.20\pm2.20$ mm Hg) than controls with VRT at 6%^{ins}CO₂ ($pCO_{2cap}=44.72\pm1.74$ mm Hg). Exercise at a load typical for diving was found to have no effect on the ventilatory sensitivity to CO₂ in divers (rest: 1.49 ± 0.33 ; exercise: 1.22 ± 0.55 [l/min x mmHg⁻¹]) and controls (rest: 2.08 ± 0.71 ; exercise: 2.05 ± 0.98 [l/min x mmHg⁻¹]) while differences in sensitivity remained between the groups ($P<0.05$). Inspiration of the four test gas mixtures revealed there was no contribution of the tested oxygen pressures to the difference in ventilatory sensitivity to CO₂ between divers and controls. Divers possess a lower ventilatory response to CO₂ which was not affected by exercise or the tested oxygen pressures suggesting an adaptation of central CO₂ sensitivity.

Florio, J.T., Morrison, J.B. and Butt, W.S. (1979). Breathing pattern and ventilatory response to carbon dioxide in divers. *Journal of Applied Physiology: Respiratory Environmental Exercise Physiology*, 46, 1076-1080.

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C70

LKB1 expression in carotid body type I cells is required for the ventilatory response of mice to hypoxia but not hypercapnia

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Hyperventilation is triggered by arterial hypoxia and hypercapnia[1]. The response to hypercapnia is well characterised, requires carbonic anhydrase and is initiated by CO₂/H⁺-depend-

ent activation of carotid body type I (CB1) cells and of neurones within the respiratory centre of the brain stem[1]. The ventilatory response to hypoxia requires CB1 cells, but their mechanism of activation by hypoxia is enigmatic. We have proposed that CB1 activation by hypoxia is mediated by the AMP-activated protein kinase (AMPK), consequent to inhibition of mitochondrial oxidative phosphorylation[2]. Activation of AMPK by metabolic stress is triggered by increases in ADP and AMP and requires the upstream kinase LKB1. LKB1 seems to be constitutively active, with ATP displacement by ADP/AMP at one site on the AMPK γ -subunit causing a conformational change that promotes phosphorylation of the catalytic α -subunit of AMPK and inhibits its dephosphorylation[3]. AMPK may also be activated by the Ca^{2+} -dependent kinase CaMKK β [3]. However, its low basal activity only permits activation by AMP/ADP when $[\text{Ca}^{2+}]_i$ is elevated - e.g. upon CB1 activation by hypoxia and hypercapnia. Using transgenic mice, we tested the effect on ventilatory responses to hypoxia and hypercapnia of global CaMKK β deletion and conditional deletion of *Lkb1* in CB1 cells; the latter being induced by crossing *Lkb1*^{fl/fl} mice with mice expressing Cre recombinase under the control of the tyrosine hydroxylase (TH) promoter, thus deleting *Lkb1* specifically in cells expressing TH, including CB1 cells. *Lkb1* and CaMKK β deletion was confirmed by genotyping and RT-PCR. Using whole-body unrestrained plethysmography we then assessed the ventilatory response to hypoxia (8% O_2) and hypercapnia (7% CO_2) in these mice. Hypoxia induced a regular increase in breathing frequency ($\approx 55\%$), tidal volume ($\approx 35\%$) and minute ventilation ($\approx 90\%$) in wild type (C57/BL6) and TH-Cre mice. By contrast, in *Lkb1* knockouts (KOs) hypoxia induced a paradoxical fall in breathing frequency (peak $\approx -27\%$). Moreover, despite an increase in tidal volume ($\approx 50\%$) the increase in minute ventilation ($\approx 30\%$) by hypoxia was also markedly attenuated relative to control. In marked contrast, exposure of *Lkb1* KOs to hypercapnia induced a response which was comparable to that observed in wild type and TH-Cre mice with an increase in breathing frequency ($\approx 65\%$), tidal volume ($\approx 17\%$), and minute ventilation ($\approx 83\%$). Contrary to the effects of *Lkb1* deletion, CaMKK β KO was without effect on either the ventilatory response to hypoxia or hypercapnia. We conclude that the LKB1 but not the CaMKK β signalling pathway mediates the ventilatory response to hypoxia, and that neither is required for the ventilatory response to hypercapnia without hypoxia.

Ward, S. A. Physiology of Breathing I. Surgery, 2004. 22(8): p. i-v.

Wyatt, C. N. et al. AMP-activated protein kinase mediates carotid body excitation by hypoxia. J Biol Chem. 2007. 282(11): 8092-8098.

Hardie, D. G. AMP-activated protein kinase - an energy sensor that regulates all aspects of cell function. Genes Dev., 2011. 25: p. 1895-1908.

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Placental growth factor knockout mice demonstrate elevated pulmonary hypertension and increased vessel leak

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Rational - Chronic lung diseases, including chronic obstructive pulmonary diseases are among the leading causes of mortality worldwide. The pathogenesis of chronic lung disease is not fully understood, however chronic hypoxia is commonly seen in this setting. Our laboratory has recently demonstrated hypoxia-induced angiogenesis in the adult rat and mouse lungs. Placental growth factor (PlGF), a homologue of VEGF, is known to play a role in systemic angiogenesis, however little is known of its role in the pulmonary vasculature. Elucidating the distinct roles of the VEGF family particularly PlGF in hypoxia-induced angiogenesis could provide possible therapeutic strategies for treatment of such lung diseases where tissue loss is apparent.

Methods - Adult male wild-type and placental growth factor knockout (PlGF^{-/-}) mice were exposed to hypoxic (FiO₂ 0.10) or normoxic (FiO₂ 0.21) conditions for 3 weeks. Following the exposure period, the animals were deeply anaesthetised (60mg.kg⁻¹ sodium pentobarbitone, intraperitoneally) and killed by exsanguination. 1) Alterations of the different growth factors (VEGF-A and VEGF-D) and their receptors (soluble VEGFR-1, soluble VEGFR-2, neuropilin-1 and membrane bound VEGFR-2) at protein level were assessed using ELISA assays and western blot analysis. 2) Isolated, ventilated perfused preparation was used to assess pulmonary vascular resistance and vascular permeability. 3) Lungs were removed post mortem and perfused and fixed at standard pressures for structural analysis of vascular and airway parameters using stereological methods.

Results - We have demonstrated differential regulation of the VEGF family members in response to hypoxia in PlGF^{-/-} mice. Loss of PlGF resulted in a significant increase in pulmonary vascular resistance indicating pulmonary hypertension compared to Wt mice. Increased vascular permeability was observed in PlGF^{-/-} compared to Wt mice following 24hrs of hypoxic conditions indicating increased vascular leak. PlGF^{-/-} mice also have significantly attenuated alveolar epithelial surface area compared to Wt controls.

Conclusion - VEGF family members were differentially regulated in PlGF^{-/-} mice suggesting a potential compensatory mechanism in the adult lung. PlGF^{-/-} mice demonstrated worse pulmonary vascular resistance, reduced epithelial surface area and increased vessel leak compared to wild-type mice suggesting an important role for PlGF in maintenance of normal vascular function. Taken together these data suggest a potentially important therapeutic target for the treatment of hypoxic lung disease.

Supported by the Health Research Board, Ireland.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C72

The effect of age on pharyngeal calibre: a mechanism for obstructive sleep apnoea?

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Rationale: The prevalence of obstructive sleep apnoea (OSA) increases with age; in 50-60 year olds it is 31%, compared to 17% in 30-40 year olds⁽¹⁾. The mechanism for the age-related increase in OSA is unclear. Risk factors could include an enlarged tongue, soft palate and adipose deposits lateral to the pharynx⁽²⁾. The aim of this study was to test the hypothesis that aging *per se* was associated with a reduction in the upper airway morphology in older (>60 years) males with no co-morbidity, compared to younger (<40 years) healthy males.

Methods: A cross-sectional, observational study was carried out on 16 healthy younger males (mean + sd, age: 28.4 ± 4.2 years) and 10 healthy older males (age: 67.2 ± 5.2 years). Groups were matched for body mass index (Younger: 27.0 ± 4.3 vs Older: 27.3 ± 1.8 kg/m²; $p=0.8$) and neck circumference (Younger: 39.9 ± 2.2 vs Older: 40.3 ± 1.4 cm; $p=0.5$). All participants had an apnoea-hypopnoea index (AHI) <5 events/hour and were not subjectively sleepy. Mean pharyngeal cross-sectional area (APmean), pharyngeal volume (Pvolume), glottis cross-sectional area (AG) and pharyngeal length (Plength) were measured using acoustic reflection between the mouth and epiglottis, over 40 breaths. Measurements were made in the evening (17:00-20:00) while awake, in a supine posture after 5 minutes of lying still and breathing quietly. Differences between groups were tested for significance using 1-tailed independent samples t-tests ($p=0.05$).

Results: Pharyngeal dimensions are shown in Figure 1. AG and APmean were greater in the older group ($p=0.002$ and $p=0.03$ respectively). Pvolume also appeared to be greater in the older group but this failed to reach significance ($p=0.06$). Plength was not different between groups ($p=0.4$).

Summary: The pharyngeal calibre of the older healthy males was significantly greater than younger healthy males. These findings do not support our hypothesis that aging is associated with a reduction in the calibre of the upper airway. We speculate that the larger pharynx in the older people was protective of OSA.

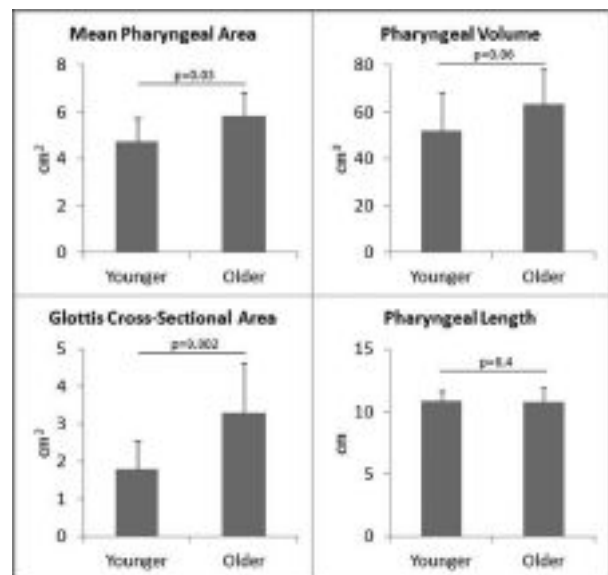


Figure 1. Pharyngeal dimensions of younger and older healthy males.

Young T *et al.* (1993). *NEJM* **328**, 1230-1235.

Schwab RJ *et al.* (2003). *Am J Respir Crit Care Med* **168**, 522-530.

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C73

Yes-associated protein (Yap) expands activated muscle stem cells

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Yap is a transcriptional co-factor known to regulate the proliferation, differentiation and/or survival of embryonic, intestinal, neuronal and epidermal stem cells as well as hepatic oval cells. We have recently shown that Yap is expressed in skeletal muscle, C2C12 myoblasts (Watt *et al.*, 2010) and in activated satellite cells, the resident stem cells of skeletal muscle. The aim of this study was to investigate the effect of perturbing Yap protein levels on satellite cell proliferation and fate. Mouse muscle fibres with satellite cells retained in their niche were cultured *ex vivo* in suspension or the satellite cells were removed from the muscle fibre and plated as satellite cell-derived myoblasts (Collins & Zammit, 2009). Cells were infected with either retroviruses to overexpress wildtype hYAP1 or constitutively active hYAP1 S127A or with a lentivirus to knock down Yap via shRNA. Proliferation was then assessed with EdU or IdU assays, and cell fate was investigated using Pax7, MyoD and myogenin immunocytochemistry.

We found that wildtype hYAP1 and hYAP1 S127A significantly increased the proliferation of satellite cells in their niche and satellite cell-derived myoblasts. By contrast Yap knock down reduced the proliferation of satellite cell-derived myoblasts by ~40%. Wildtype hYAP1 and constitutively active hYAP1 S127A

maintains cells more as activated satellite cells (Pax7+/MyoD+) whilst preventing their differentiation as judged by myogenin and myosin heavy chain expression.

These data suggest that Yap expands the pool of activated satellite cells and inhibits myogenic differentiation. Thus Yap might contribute to the expansion of activated satellite cells in response to muscle injury or muscle growth stimuli and could potentially be targeted to better expand satellite cells *in vitro* prior to transplantation.

Watt KI et al. (2010). *Biochem Biophys Res* 393, 619-624.

Collins CA & Zammit PS (2009). *Methods Mol Biol* 482, 319-330.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C74

Acute amino acid intake does not alter mitochondrial function in older adults

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Coronary heart disease (CHD) is the main cause of mortality amongst adults in the U.S. Plasma hypertriglyceridaemia, an independent risk factor for CHD, is more prevalent in older adults, making older adults an at-risk group for CHD. Amino acid (AA) intake has been shown to lower both hepatic and plasma triglyceride (TG) concentrations in older adults (Børsheim et al., 2009). We have also shown that acute AA intake increases hepatic very low density lipoprotein (VLDL) TG and apolipoprotein B-100 secretion (Børsheim et al., in preparation). Furthermore, very recent data from our group have shown that ¹³CO₂ breath enrichment, measured during a constant infusion of U-¹³C₁₆-palmitate, is increased with AA intake relative to a basal period (Hurren et al., in progress). Collectively, our observations suggest that acute AA intake increases hepatic TG secretion while seemingly increasing fatty acid (FA) uptake and oxidation, presumably within skeletal muscle. However, whether this apparent increase in FA oxidation is the result of altered skeletal muscle mitochondrial function is not known. Here we investigated the impact of acute AA intake on skeletal muscle mitochondrial function in older adults.

Five older adults (mean±SD: 63.3±7.8 years) with elevated plasma TG concentrations (2.16±0.95 mmol/l) participated in this study. Skeletal muscle biopsies were obtained before and after acute AA intake (21g of AAs in total: 1g ingested every 10 minutes for 3.5h). Mitochondrial function was assessed in permeabilised myofibres with the sequential addition of pyruvate (5mM), malate (2mM), glutamate (5mM), palmitoyl-carnitine (150µM), ADP (5mM) and succinate (10mM). VO₂ values were normalized to citrate synthase (CS) protein content.

State 3 (ADP-dependant) respiration was not different following AA intake (mean±SEM: 0.74±0.04 vs 0.66±0.09 pmol/(sec*mg)/µg CS, figure 1). The ability of the mitochondria

to oxidise the FA palmitate (palmitoyl-carnitine) did not change following AA acid intake (0.76±0.04 vs 0.74±0.09 pmol/(sec*mg)/µg CS, figure 1). In addition, maximal mitochondrial oxidative respiration determined following the addition of succinate was not altered by AA acid intake (1.09±0.04 vs. 1.12±0.09 pmol/(sec*mg)/µg CS, figure 1).

Here we provide evidence that acute AA intake does not alter skeletal muscle mitochondrial function in older adults. Apparent changes in palmitate oxidation following acute AA intake are therefore likely to be the result of increased intracellular FA availability and oxidation *in vivo*. However, whether long-term AA supplementation alters mitochondrial function in older adults warrants investigation.

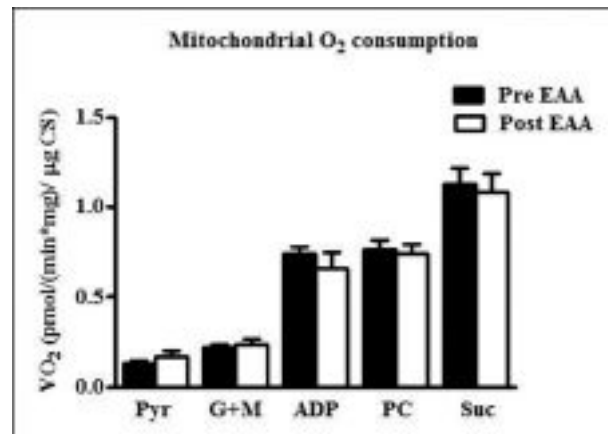


Figure 1. Mitochondrial VO₂ corrected for citrate synthase (CS) protein content following the addition of pyruvate (Pyr), glutamate and malate (G+M), ADP, palmitoyl-carnitine (PC) and succinate (Suc). Values are group means±SEM (n=5).

Børsheim et al, 2009. *Nutrition* 25: 281-8.

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C75

Causes of the reduced *in vivo* specific tension of skeletal muscle in old age

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Skeletal muscle weakness in old age is associated with increased risk of falling, mobility problems and greater mortality risk. Muscle weakness can occur due to reduced number of sarcomeres in parallel, which is represented by the physiological cross sectional area (PCSA); reduced ability to fully activate the muscles and/or reduced strength per unit muscle mass, also known as specific tension. In young (22 ± 1 years) and older (72 ± 1 years) men (n = 36) and women (n = 35) we used magnetic resonance imaging (MRI) and ultrasonography to determine the quadriceps PCSA as: muscle volume / fascicle length. Electrically-induced contractions were used to measure activation capacity during maximal voluntary contractions.

In vivo specific tension was calculated as: (external torque / patellar tendon moment arm) / PCSA multiplied by the cosine of the fascicle angle of pennation. Data are reported as Mean $\pm$ SEM. Compared with young, the older subjects were 37% weaker (torque corrected for activation capacity was 246.5 ± 11.7 vs 156.2 ± 7.1 Nm in young and older, respectively); PCSA was 27% lower (204.5 ± 7.3 vs 149.6 ± 5.9 cm² in young and older, respectively), and specific tension was 17% lower (Fig 1; all $P < 0.0005$). These effects were similar in men and women. Muscle biopsy samples showed similar fibre-type composition and type I fibre cross sectional area in young and older, but 26% smaller type II fibres in older subjects ($P < 0.0005$). MRI pixel intensity and collagen stain both indicated higher levels of connective tissue in older subjects. It is concluded that quadriceps in vivo specific tension is lower in older people. If, as reported, type II fibres have a higher specific tension, the atrophy of these fibres might contribute to about half the observed change in specific tension of the whole muscle; the remainder appears to be due to an accumulation of connective tissue.

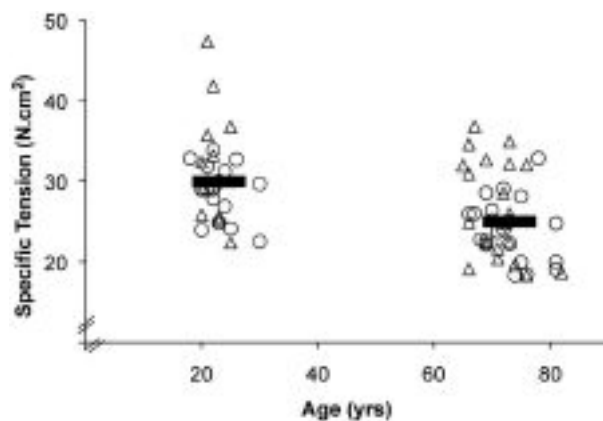


Figure 1: Quadriceps in vivo specific tension. Individual data points are shown for men (circles) and women (triangles). The mean for young and older groups are shown as black bars.

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C76

High-intensity interval training increases insulin stimulated glucose uptake in skeletal muscle but has no effect on uptake in liver, pancreas and visceral fat tissue

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Introduction

High-intensity interval training (HIT) has been shown to increase insulin sensitivity over two weeks and six sessions of training (1-2). Adaptations in skeletal muscle have been reported previously (3-5), but it is not known whether HIT affects glucose metabolism in abdominal tissues.

Methods

We measured glucose uptake in skeletal muscle, liver, pancreas and visceral fat tissue using FDG PET -method during euglycaemic hyperinsulinaemic clamp in eight healthy, sedentary,

middle aged men (mean $\pm$ SD, age: 47 ± 5 years, BMI: 26 ± 2.9 kg $\times$ m⁻², VO_{2max} : 34 ± 4 ml $\times$ kg⁻¹ $\times$ min⁻¹) pre and post two weeks and six sessions of HIT (Gibala method: 4×30 s repeated Wingate tests with 4 minutes of recovery). In addition, oral glucose tolerance test was performed.

Results

Two weeks of HIT increased VO_{2max} by 4.7 % (from 34 ± 4 to 35.5 ± 4 ml $\times$ kg⁻¹ $\times$ min⁻¹, student paired t-test, $p = 0.019$). Training had no effect on fasting plasma glucose and insulin levels, but there was a tendency for reduced serum free fatty acid concentration (pre: 0.46 ± 0.14 vs. post: 0.33 ± 0.09 mmol $\times$ l⁻¹, $p = 0.054$).

HIT training increased insulin stimulated glucose uptake in m. quadriceps femoris by 38 % (from 44 ± 11 to 60 ± 18 μ mol $\times$ kg⁻¹ $\times$ min⁻¹, $n = 5$, $p = 0.004$), but had no significant effect on whole body insulin sensitivity (pre: 39.1 ± 11.4 vs. post: 43.4 ± 16.3 μ mol $\times$ kg⁻¹ $\times$ min⁻¹, $p = NS$) and no influence on glucose uptake in liver (24.7 ± 11.5 vs. 25 ± 11.3 μ mol $\times$ kg⁻¹ $\times$ min⁻¹, $n = 6$, $p = NS$), pancreas (26.6 ± 5.1 vs. 26.1 ± 6.7 μ mol $\times$ kg⁻¹ $\times$ min⁻¹, $n = 6$, $p = NS$) and visceral fat tissue (9.9 ± 5.2 vs. 8.7 ± 5 , μ mol $\times$ kg⁻¹ $\times$ min⁻¹, $n = 6$, $p = NS$) in healthy men.

Conclusion

We conclude that although HIT is a promising exercise method for health, our results suggest that further investigation is needed to see if low-volume intermittent training improves insulin activity and metabolic control on whole body level, or exclusively in skeletal muscle.

Babraj et al. (2009). BMC Endocr Disord 9, 3.

Richards et al. (2010). J Physiol 588, 2961-2972.

Burgomaster et al. (2005). J Appl Physiol 98, 1985-1990.

Gibala et al. (2006). J Physiol 575, 901-911.

Gibala et al. (2009). J Appl Physiol 106, 929-934.

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C77

The acute effects of beclomethasone on force in mouse skeletal muscle fibres are both dose and fibre type dependent

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Glucocorticoids (GC) are steroid hormones released from the adrenal gland in response to physical and emotional stress. GCs have high anti-inflammatory (glucocorticoid) and insignificant water retention (mineralocorticoid) properties. Therefore, they are widely used in the management of diverse pathological conditions including asthma, rheumatoid arthritis, ulcerative colitis and bacterial meningitis. However, their use is limited because of several side effects including a myopathy, the cause of which is still poorly understood (Stahn and Buttgeriet, 2008). Like other steroid hormones, GCs have both genomic (chronic) and non-genomic (acute) actions. Although the chronic effects of GCs in skeletal muscle have been the subject of numerous previous studies (Schakman et al., 2008), little is known about their acute effects. The primary aim of this study was to investigate the acute effects of beclomethasone, a synthetic GC, in isolated intact mouse fast and slow twitch skeletal muscle fibre bundles.

The experiments were performed at 20°C on small skeletal muscle fibre bundles isolated from the extensor digitorum longus (a fast-twitch muscle) and soleus (a slow-twitch muscle) of adult mice. The mice were killed as recommended by the Animals (Scientific Procedures) Act 1986, UK and the experiments conformed to the local animal welfare committee guidelines. Force was measured in the presence and absence of various concentrations of beclomethasone (125, 250 and 500nM) as previously described in Hamdi and Mutungi (2010). In some experiments, the glucocorticoid receptor (GCR) inhibitor mifepristone was added and the expression of the GCR in the fibre bundles was determined.

Treatment of the fibre bundles with beclomethasone increased maximum isometric tension (P_0) in the slow twitch fibre bundles without significantly affecting that of the fast twitch ones. The increase was maximal at 250nM beclomethasone and was completely abolished by pre-treating the fibre bundles with 10µM mifepristone. Thus, 250nM beclomethasone led to a $16.3 \pm 0.08\%$ ($n = 5$ fibres, S.E.M) increase in P_0 in the slow twitch fibres but only to a $0.8 \pm 0.1\%$ ($n = 5$ fibres) increase in the fast-twitch fibres. Examination of GCR expression showed that both fibre types expressed the cytosolic form of the receptor and that its concentration was higher in the slow twitch than in the fast twitch fibres. In contrast, the receptor was found in the membrane fraction of the slow twitch fibre bundles only. These findings suggest that the acute effects of GCs are mediated through a membrane GCR and that at low doses beclomethasone, administered soon after stress, may be protective in slow-twitch skeletal muscle fibres.

Crossland et al. (2010). *J Physiol* 588, 1333-1347

Hamdi M, Mutungi G (2010). *J Physiol* 588, 511-525

Schakman H, Gilson O, Thiessen JP (2008). *J Endocrinol* 197, 1-10.

Stahn C, Buttgeriet F (2008). *Nature Clinical Practice* 4, 525-533

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C78

Motor unit firing characteristics of the knee extensors following acute accentuated eccentric load

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Short term overload of the eccentric phase of a contraction leads to superior strength gains than conventional contractions despite a matched workload (3). Adaptations from these interventions have been shown to come from increases in IIX fibre cross sectional area (2). Initially, however it is likely that these strength adaptations will occur from increases in neural drive (4). Therefore, it is possible that there is an acute neural response from the larger motor units to eccentric overload that contributes to this stimulus. Accordingly, it was the purpose of this study to investigate motor unit firing rates following an acute accentuated eccentric load (AEL) protocol in comparison to constant load (CL) protocols. Additionally, these interventions were performed at 2 different eccentric velocities (full knee extension to flexion: 4 or 2 seconds). Healthy, resistance trained males ($n=10$) attended the laboratory on 4 separate occasions: they completed 3 concentric repetition

maximum contractions (3RM) following a warm up. They then performed a maximal isometric voluntary contraction (MVC) followed by a submaximal 10 second contraction (70% of MVC) during which high density surface electromyography (1) recorded firing rate of all motor units from the vastus lateralis. Then, they either did CL (2s), AEL (2s), CL (4s) or AEL (4s) in random order. AEL and CL consisted of eccentric load at 120% and 85% of 3RM respectively and both with a concentric load of 85% of 3RM where they were encouraged to contract maximally. The contractions consisted of 3 sets (3 minute recovery) of 3 repetitions. Then the participants repeated the MVC and submaximal 10 second contraction. Values are mean + SD and compared by ANOVA. For all 4 interventions no alterations were shown in MVC following the exercise, neither was there any differences in concentric velocity during the contractions. The only changes observed were following the fast (2s) interventions during the submaximal isometric contractions where the larger motor units recruited (represented by the final third in order of recruitment), significantly ($p < 0.05$) declined following AEL (2s) in comparison to CL (2s) (AEL: pre: 10.8 ± 2.9 post 8.8 ± 1.7 pps vs CL: pre 10.3 ± 2.9 post 11.2 ± 2.2 pps), whereas no changes were observed in the smaller motor units. In addition, no alterations were shown for the number of motor units recruited for any of the interventions. In conclusion, it is likely that the larger motor units fatigued following the faster acute eccentric overload intervention without any maximal force capacity decrements. It may be this initial stimulus that contributes to the superior adaptation of eccentric overload previously reported.

De Luca CJ and Hostage EC. Relationship between firing rate and recruitment threshold of motoneurons in voluntary isometric contractions. *J Neurophysiol* 104: 1034-1046, 2010.

Friedmann-Bette B, Bauer T, Kinscherf R, Vorwald S, Klute K, Bischoff D, Muller H, Weber MA, Metz J, Kauczor HU, Bartsch P and Billeter R. Effects of strength training with eccentric overload on muscle adaptation in male athletes. *Eur J Appl Physiol* 108: 821-836, 2010.

Hortobagyi T, Devita P, Money J and Barrier J. Effects of standard and eccentric overload strength training in young women. *Med Sci Sports Exerc* 33: 1206-1212, 2001.

Selvanayagam VS, Riek S and Carroll TJ. Early neural responses to strength training. *J Appl Physiol* 111: 367-375, 2011.

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C79

In the absence of vision muscle receptors contribute to body representation

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The sense of body ownership, a sense of which things belong to our body, is presumably generated from sensory information. This sense can be manipulated using tactile stimuli to induce an illusion of ownership over a rubber hand (Botvinick and Cohen, 1998). We recently showed that muscle receptor signals can also be used to manipulate the sense of body ownership, using a passive movements stimulus (Walsh et al., 2011). When muscle receptor signals are manipulated to induce an illusion of ownership over a plastic magician's finger, subjects report that they feel that the plastic magician's finger is their finger, and that the perceived position of their finger moves closer to the plastic finger. However, it is unclear

if muscle receptors contribute to the sense of ownership on their own or whether vision is required.

We further developed this plastic finger illusion to exclude any contribution from seeing the finger move. Both of the subject's hands were covered and the experimenter used the subject's left index and thumb to passively hold and move a plastic finger that was coupled to the subject's right finger. The coupling placed the plastic finger 12 cm above the subject's finger and ensured that any movement of the proximal interphalangeal joint of the plastic finger or the subject's finger was reproduced in the other, i.e. movement was congruent for the two fingers. This illusion was induced in naïve human subjects ($n=20$) when intact and after a digital nerve block of the right index finger (lignocaine 1%, 3-5 ml). This removed input from skin and joint receptors but left intact the muscle receptors in the long flexor and extensor muscles of the fingers and the intrinsic hand muscles. We asked subjects to report the perceived vertical spacing between their left and right index fingers, and also the perceived height of each finger above the table.

After 3 mins of congruent movement, subjects reported a significantly smaller spacing of 1.5 [0.5, 4.0] cm (median [interquartile range]) between their left and right index finger when compared to a perceived spacing of 3.5 [1.0, 6.0] cm after 3 minutes of incongruent movement (Wilcoxon rank test, $p < 0.05$). Interestingly, the perceived height of the index fingers did not change. There was no effect of blocking the digital nerves. Furthermore, in a separate study we found that simply passively grasping the plastic finger ($n=10$) with the left index and thumb was enough to reduce the perceived spacing between the fingers from 8.0 [6.0, 10.0] cm to 4.0 [2.0, 8.0] cm (Wilcoxon rank test, $p < 0.01$).

There are three novel findings. First, in the absence of vision muscle receptors can generate a sense of body ownership. Second, holding a plastic finger with vision excluded is enough to alter perceived hand position. Finally, perceived finger position depends upon whether the fingers are considered separately or together.

Botvinick M & Cohen J. (1998). Rubber hands 'feel' touch that eyes see. *Nature* 391, 756.

Walsh LD, Moseley GL, Taylor JL & Gandevia SC. (2011). Proprioceptive signals contribute to the sense of body ownership. *J Physiol* 589, 3009-3021.

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C80

The role of resonance in physiological finger tremor

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Human postural physiological finger tremor has two main frequency components with variable preponderance. One is at a broad frequency of 20 – 25 Hz and is sometimes attributed to mechanical resonance. The other is at a lower frequency around 8-12 Hz and is usually attributed to a central oscillator. However, it has been known for some time that the resonant frequency of the finger cannot be expressed by a single number as it depends on the vigour with which it is oscillated and also on its history of movement (Lakie and Robson 1988). We

have investigated finger resonance using mechanical inputs and muscle stimulation so that no voluntary activity is required. The hand and forearm of fifteen comfortably seated subjects (24.73 ± 10.12 yr, 8 male) was fully supported at waist height. With ethical permission and informed consent, induced flexion-extension movements of the splinted middle finger were recorded by a miniature acceleromometer and a retro-reflective laser rangefinder. In one condition, mechanical inputs were applied to the finger by a miniature servomotor and titanium linkage. Any induced EMG was recorded from the extensor digitorum communis (EDC) muscle. In a second condition, percutaneous electrical stimulation was applied to the EDC muscle. In both cases the input was in the form of broad band random noise. The RMS size of the broad band noise was systematically altered from very small (producing a barely visible movement of the finger) to moderate (producing movements of the finger of several degrees). The acceleration and phase results showed that both forms of random excitation produced a clear resonance in finger acceleration. Also, although not identical, both forms of excitation revealed striking non-linearity in the resonance of the finger. With mechanical input the frequency peak was at 23 Hz for the smallest excitation and at 9 Hz for the largest (fig 1). We suggest that both components of finger tremor are likely to be due to resonance, with the high frequency predominating when the tremor is small and the low frequency predominating when the tremor is large. The change in resonance may result from a progressive change in a single resonant system or it may result from a switch from one mode of oscillation to a second mode.

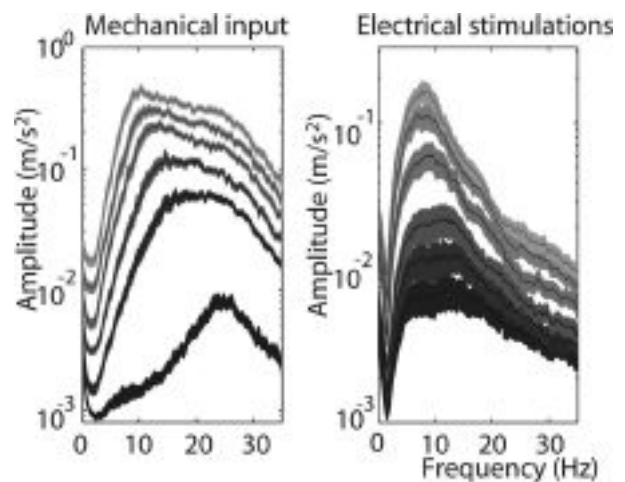


Fig 1. Frequency spectra of the acceleration. Left panel is obtained from a white noise mechanical input, right panel is obtained from a white noise electrical stimulation input. Lighter grey colours represent an increased RMS of the input, shaded areas represent the standard error

M. Lakie, L G. Robson (1988) Thixotropic changes in human muscle stiffness and the effects of fatigue. *Q J Exp Physiol* 73 487-500

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C81

Dimethylarginine dimethylaminohydrolase 2 regulates primary macrophage motility and phagocytosis

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Introduction:

Nitric oxide (NO) is a key regulator of endothelial cell function and has been shown to affect cell motility via Rho GTPases, key regulators of actin dynamics. However, its role in immune cells has not been studied in detail. Macrophages and oxy- low density lipoprotein are the predominant cause of atheroma, which leads to atherosclerosis. If NO controls macrophage motility as it does endothelial cell motility, then the development of atheroma may be modulated by the addition of specific NO inhibitors.

Methods:

Primary peritoneal macrophages were obtained from wild type (WT) and dimethylarginine dimethylaminohydrolase 2 macrophage specific knock-out (DDAH2 mac^{-/-}) mice. The knockout model was verified using flow cytometry for the macrophage specific marker F4-80. Their cell motility was tracked for 20 hours following incubation with; NO inhibitor asymmetric dimethylarginine (ADMA), non-NO inhibitor symmetric dimethylarginine (SDMA), NO donor in control conditions or with an inflammatory cytokine treatment. Results were analysed manually using an Image J (Licor) tracking program. Nitrite levels and cell phagocytosis were determined using the Griess assay and the measurement of an E-coli fluorescent Bio-particle ingested by macrophages. Immunohistochemistry of F-actin using a phalloidin stain was used to investigate the effect of the NO/ADMA pathway on GTPases in particular stress fibre formation which controls retraction of cell end and directional movement.

Results:

DDAH2 mac^{-/-} macrophages moved significantly slower and less far than WT macrophages in control conditions (0.252±0.0182 µm/min; 0.151±0.0290 µm/min, for WT and DDAH2 mac^{-/-} respectively where p<0.05 and n=45 cells from 3 mice). The addition of an NO donor significantly increased motility in DDAH2 mac^{-/-} (0.213±0.0198 µm/min where p<0.05 and n=45 cells from 3 mice). Inflammatory cocktail-treated macrophages from DDAH2 mac^{-/-} had significantly lower nitrite levels over a total period of 48 hours (42.63±4.79 µmol; 22.05±4.50 µmol for WT and DDAH2 mac^{-/-} respectively where p<0.05 and n=8 WT and n=9 DDAH2 mac^{-/-}) and less phagocytosis when treated with an inflammatory cocktail (5.346±2.723 au; 2.335±1.338 au, for WT and DDAH2 mac^{-/-} respectively where P=NS and n=4). F-actin staining revealed more stress fibres around the periphery of cells and the intracellular in DDAH2 mac^{-/-} animals when compared to WT.

Conclusion:

NO reduction in macrophages, via the deletion of DDAH2, leads to reduced motility, nitrite production and phagocytosis indicating that NO is critical for the function of these immune cells. Increased stress fibres with DDAH2 mac^{-/-} highlights that this effect is mediated by GTPases. The reduction in macrophage motility and phagocytosis capability suggests a possible therapeutic against the development of plaque formation in atherosclerosis.

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Differential effects of hypertension on KCNQ4-encoded voltage dependent potassium channel activity in mesenteric and middle cerebral arteries

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KCNQ4-encoded voltage-dependent potassium channels (Kv7.4) are important regulators of vascular tone and are severely compromised in models of hypertension (1, 2). Moreover, we have recently demonstrated that these ion channels underlie β-adrenoceptor-stimulated relaxation in rat renal arteries (2). The present study investigates the contribution of Kv7.4 channels to cAMP-mediated relaxation in mesenteric and middle cerebral arteries and their function in spontaneously hypertensive rats (SHRs). Third-order branches of the superior mesenteric artery and middle cerebral arteries were isolated from male Wistar rats or SHRs (200-225 g) in cold Krebs' solution. Arteries were mounted in a wire myograph and placed under a tension equivalent to that generated at 0.9 times the diameter of the vessel at 100 mmHg (mesenteric arteries) or 70 mmHg (middle cerebral arteries). Responses to isoprenaline or calcitonin gene-related peptide (CGRP) were determined in the absence and presence of the Kv7 channel blocker linopiridine. Some experiments were carried out in arteries depleted of KCNQ4 by transfecting them with selective siRNA using either reverse permeabilization or TransIT-LT transfection reagent (Mirus). Relaxation of mesenteric arteries by isoprenaline and middle cerebral arteries by CGRP was inhibited by 10 µM linopiridine (~30% and 70% attenuation in Emax, respectively). Mesenteric and middle cerebral arteries incubated with siRNA targeted against KCNQ4 were less responsive to the Kv7 activator S-1 compared to vessels incubated with scrambled siRNA. Relaxation in response to isoprenaline (mesenteric artery) and CGRP (middle cerebral artery) were significantly reduced (1-log unit rightward shift in pEC₅₀) in vessels depleted of KCNQ4. In mesenteric arteries from SHRs, the relaxation to S-1 was drastically impaired coinciding with a marked attenuation in responses to isoprenaline. Conversely, S-1 and CGRP induced relaxations were not different between middle cerebral arteries from SHRs and normotensive rats. This study demonstrates that Kv7.4 channels contribute to the relaxation of mesenteric and middle cerebral arteries associated with activation of receptors coupled to adenylyl cyclase (β-adrenoceptors and CGRP-like receptors, respectively). In addition, the present study indicates that whilst Kv7.4 channel activity is drastically impaired in mesenteric, aorta (1) and renal (2) arteries of SHRs, middle cerebral arteries are unaffected. Further studies are required to ascertain whether Kv7.4 channels are protected in the cerebral vasculature of SHRs or if other Kv7 subtypes compensate for their function.

Jepps TA et al. (2011). *Circulation* 124, 602-11

Chadha PS et al. (2012). *Hypertension* 59, 877-884

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Aging and reactivity of resistance coronary arteriesE. Mourmoura¹ and L. Demaison^{1,2}¹INSERM, Laboratoire de Bioénergétique Fondamentale et Appliquée, Grenoble cedex 09, France and ²INRA, Unité de Nutrition Humaine, Université d'Auvergne, Clermont-Ferrand, France

Aging is associated with vascular dysfunction, but its effects on the function of the vascular cells of coronary resistance arteries remain to be determined. This study aimed at evaluating the influence of aging (youth, young adulthood and middle age) on the activity of the endothelial (EC) and smooth muscle cells (SMC) of coronary arterioles. 3-, 6- and 11-month old male Wistar rats were anaesthetised deeply with sodium pentobarbital (30 mg/kg) and their heart were perfused according to the Langendorff procedure with a Krebs-Heinselett buffer containing glucose 11 mM at 37°C. The endothelial-dependent and -independent vasodilations were then determined in the beating heart with acetylcholine (injections of 4, 10, 20, 40, 60, 80 and 100 pmoles) and nitroprusside (injections of 100, 200, 400, 600, 800 and 1000 pmoles) after vasoconstriction through a constant infusion of a thromboxane A2 analogue (U46619, 30 nM). The mitochondrial oxidative stress was then evaluated either in the whole heart by measuring the aconitase to fumarase ratio or in the isolated cardiac mitochondria by estimating the Amplex red-related rate of H₂O₂ production. Values are means $\pm$ S.E.M., compared by ANOVA. Although aging modified only minimally the cardiac mechanical function, it decreased progressively the endothelial-dependent vasodilatation (22.6 ± 0.8 , 17.1 ± 1.5 and $13.4 \pm 1.9\%$ at 3, 6 and 11 months for the highest dose of acetylcholine, $p < 0.01$, $n = 8$). This was associated with specific age-associated modulation of EC and SMC functions. Compared to 3-month old rats, 6-month old animals had a higher endothelial vasodilatation capacity (553 ± 146 vs 108 ± 47 pmoles nitroprusside equivalents), but a lower SMC sensitivity to nitric oxide (28.6 ± 3.1 vs $53.6 \pm 5.4\%$). This was not associated with any change of the oxidative stress. However, at middle age, the endothelial vasodilatation capacities returned to a normal value (186 ± 42 pmoles nitroprusside equivalents) and the SMC sensitivity to nitric oxide remained low ($31.8 \pm 2.1\%$). The reduction of the endothelial vasodilatation capacities between adulthood and middle age was associated with the development of a mitochondrial oxidative stress observed at the level of the isolated organelles and in the whole heart (the aconitase to fumarase ratio equalled 0.287 ± 0.012 , 0.268 ± 0.023 and 0.157 ± 0.015 at 3, 6 and 11 months, respectively, $p < 0.001$, $n=8$).

In conclusion, the SMC seem to be the first vascular cells whose function is deteriorated with aging. In contrast, the activity of EC appears to be improved until young adulthood and decreases thereafter with the development of a mitochondrial oxidative stress.

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Microanatomy of pericytes in the rat ventricular myocardium

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Pericytes are contractile cells found on the abluminal surface of capillaries. In the CNS they bidirectionally regulate capillary diameter (Peppiatt et al., 2006) and constrict irreversibly in ischaemia resulting in a prolonged blood flow decrease even after a thrombus in a feeding artery has been cleared (Yesmisci et al., 2009). They also regulate the permeability of the blood-brain barrier and play a role in angiogenesis and formation of the glial scar after injury. Pericytes may, therefore, play a significant role in CNS health and disease. The heart, like the brain, can suffer a "no-reflow" phenomenon after ischaemia (Krug et al., 1966), raising the possibility of ischaemia-evoked capillary level constriction. We investigated the density and anatomical characteristics of myocardial pericytes in the rat to determine whether these suggested a potential role in controlling capillary diameter and blood flow.

P12 ($n=6$) and P30 ($n=5$) Sprague-Dawley rats were sacrificed by Schedule 1 methods, prior to removal and immersion fixation of their hearts in paraformaldehyde. Transverse ventricular sections (100 μ m thick) were prepared for immunohistochemistry using a vibratome. Ventricular pericytes were labelled for NG2 and/or PDGFR β , and capillaries were labelled with FITC-conjugated isolectin B₄. Laser scanning confocal microscopy was used to gather image stacks for pericyte quantification. Data were analysed offline using ImageJ software. In the left and right ventricle of P12 rats, pericytes were present at a density of $4.4 \times 10^7/\text{cm}^3$ and $4.8 \times 10^7/\text{cm}^3$ respectively (not significantly different, $p=0.17$, t-test). In P30 rat left ventricle, pericytes were on average separated by $35 \pm 3 \mu\text{m}$ along capillaries and were present at a density of $2.4 \times 10^7/\text{cm}^3$. The capillary density at P30 was $84,000/\text{cm}^2$. Pericytes extend NG2-positive processes along and around coronary capillaries, providing a potential anatomical substrate for control of capillary diameter. Some of these processes make repeated small ($\sim 1 \mu\text{m}$) diameter pads of contact onto the capillaries along their length, the function of which is unknown.

Pericytes are as abundant as cardiac myocytes in the ventricular tissue of young rats, and are present at a similar density in both ventricles. The high density of pericytes in the heart suggests a potential role in regulating myocardial blood flow or capillary permeability.

Krug A et al. (1966). *Circ Res* 19 (1): 57-62.

Peppiatt CM et al. (2006). *Nature* 443: 700-704.

Yemisci M et al. (2009). *Nat Med* 15: 1031-1037.

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Arteriovenous fistula stenosis: vascular remodelling, inflammation and the development of a rabbit modelM. MacAskill¹, R.M. Wadsworth¹, D. Kingsmore² and P. Coats¹¹Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK and ²Renal Unit, Western Infirmary, Glasgow, UK

An arteriovenous fistula (AVF) is a vein graft which is created to permit access to the bloodstream allowing haemodialysis. AVFs are considered the best option in clinical practice; in spite of a 50% failure rate at 6 months (Field et al., 2008). Failure is primarily due to proliferation, leading to neointimal formation and invasion of the lumen (Lee and Roy-Chaudhury, 2009). Inflammation is known to play a key role in the development of vascular hyperproliferative remodelling, although the exact mechanisms and triggers are not fully known in AVF stenosis. The aims of this study were to; 1) evaluate the pathological changes undergone in human failed AVF vs. non-stenosed controls, 2) investigate the inflammatory processes undergone in human failed AVF vs. non-stenosed controls, 3) develop a rabbit model of AVF creation to study potential mechanisms that may contribute to stenosis.

Histology of failed human AVF stained with H & E confirmed a significant increase in media: lumen ratio, from 2.2 ± 0.6 to 18.8 ± 6.9 in control and AVF vein sections respectively ($p < 0.005$, Control vs. AVF; $n=8$). The majority of these cells were positive for α -smooth muscle actin (α -SMA). The percentage of cells undergoing proliferation, measured by expression of PCNA, increased from $3.5 \pm 1.5\%$ to $33.6 \pm 4.9\%$ in control and AVF vein sections respectively ($p < 0.005$ AVF vs. Control; $n=5, 8$). The inflammatory profile of stenotic patients was measured by proteome array. Serum levels of pro-inflammatory molecules, including IL-6 and MCP-1 were significantly greater in Stenosed AVF patients vs. healthy control. Toluidine blue staining for mast cell infiltration highlighted a 5 fold increase in mast cells within AVF vein tissues ($p < 0.005$ Control vs. AVF; $n=9, 7$), and infiltration of lymphocytes was evident from H & E staining.

In our newly developed rabbit model, the animals were pre-medicated using hypnorm (IM, 0.3ml/Kg) 30 mins prior to surgery; and anaesthetised with a mixture of isoflurane (1.5%), oxygen (1L min⁻¹) and nitrous oxide (1L min⁻¹). An AVF was then created between the femoral artery and vein, with Rimadyl (SC, 4mg/Kg) given as analgesia. The vein vein was monitored by ultrasound for 28 days post-creation of a femoral AVF to confirm patency. Animals were then pre-medicated (hypnorm, IM, 0.3ml/Kg) and euthanised/euthanised (IV, euthatal, 1ml/Kg) and the vessels perfused fixed in situ with paraformaldehyde. H & E staining of the vein showed the development of neointima (positive for α -SMA by immunohistochemistry), as well as neoadventitia within the venous segment. There was evidence of healing and integration at the anastomosis site, and a degree of neointima within the artery. Throughout the fistula, inflammatory cell infiltrates were present.

These results highlight significant venous hypertrophic remodelling in human AVF-vein sections, largely attributed to vascular smooth muscle cell accumulation and inflammatory cell infiltrates. With successful development of the rabbit AVF model we are now in a good position to investigate the specific mechanisms leading to AVF stenosis.

Field, M. et al. (2008). J. Vasc. Access 9, 45-50.

Lee, T. & Roy-Chaudhury, P. (2009). Adv. Chronic Kidney Dis. 16, 329-338.

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Vascular smooth muscle cell response to calcium phosphate nanoparticlesD. Proudfoot¹, Y. Dautova¹, M. Eppele² and M. Bootman¹¹Babraham, Babraham, UK and ²University of Duisburg-Essen, Essen, Germany

Calcium deposits are commonly found in association with ageing and with several diseases including atherosclerosis, medial calcification (Mönckeberg's sclerosis), arthritis and cancer. In bone, nucleation of calcium crystals occurs in matrix vesicles which are nanoparticulate, membrane-bound structures released from osteoblasts and chondrocytes. Similar structures released from vascular smooth muscle cells (VSMCs) have been detected in human calcified arteries and from VSMCs in vitro. The amount of calcium phosphate (CaP) crystals deposited in arteries correlates positively with atherosclerotic plaque rupture and myocardial infarction but whether the crystals actively participate in driving the disease is unclear. The damaging effect of nano- and microparticulate CaP crystals in arthritic joints has been known for some time and recent studies in human atherosclerotic plaques suggest that very small CaP crystals occur in regions of stress and plaque rupture.

VSMCs have a role in protecting atherosclerotic plaques from rupture by forming a thick fibrous cap. To investigate whether CaP crystals could affect VSMC function, we added either synthetic crystals or calcified particles extracted from human atherosclerotic plaques to human aortic VSMCs in culture. Both types of crystals induced cell death in VSMCs, with the synthetic crystals being more potent than the plaque-extracted crystals. To investigate the mechanism of cell death, intracellular calcium ion levels were measured using video imaging of Fura-2-loaded cells. CaP crystals caused rapid rises in intracellular calcium ion concentration preceding cell death and these effects were inhibited when lysosomal acidification was blocked with bafilomycin A. This suggested that the crystals were endocytosed by VSMCs, dissolved in lysosomes and subsequent release of free calcium ions into the cytosol resulted in calcium overload and cell death. By imaging individual cells, we have observed that although CaP crystals induce cell death in VSMCs, the response is not uniform, with some cells responding to the toxic insult rapidly, while others are more resistant. This may be due to the heterogeneous nature of VSMCs. To investigate why plaque-extracted crystals are less potent than synthetic crystals, we tested the hypothesis that calcification-associated proteins such as fetuin could influence crystal toxicity. We found that fetuin inhibited rises in intracellular calcium and cell death induced by the CaP crystals, in a dose-dependent manner (in the range 0.1-10mM). Uncovering how CaP crystals can impact on VSMC survival and function may provide strategies to limit their damaging effects in the vessel wall.

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Angiotensin II type 1 receptor physically interacts with the BK channel to modify its properties and microlocalization

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Angiotensin II (Ang II) has a large impact in cardiovascular physiology and serves as a potent pharmacological target in the treatment of hypertension. In coronary arteries, Ang II has been reported to inhibit the activity of the large conductance calcium-activated potassium (BK) channel, a primary determinant of vascular tone. The present study aims to investigate: 1) whether Ang II regulates BK channels in the renal artery, as the kidney plays a pivotal role in maintaining normal systemic blood pressure; and 2) the underlying molecular mechanisms of the regulation. Male three month old Sprague Dawley rats were used. Animal protocols received institutional approval. We found in freshly isolated rat renal arterial smooth muscle cells (SMCs) that 1 μ M Ang II reduced the iberiotoxin-sensitive whole-cell BK currents by $44 \pm 8.7\%$. This inhibitory effect was fully prevented by 10 μ M losartan, an antagonist of Angiotensin II type 1 receptor (AT1R) but remained intact in the presence of 500 μ M GDP β S, an inhibitor of G-protein activation. Results from whole-cell patch clamp in HEK293T cells further demonstrated that the presence of AT1R is required for the inhibitory effect of Ang II on BK channel activity. Moreover, AT1R was found in physical association with BK channel α -subunit (BK α , Slo1) by co-immunoprecipitation analysis. Consistent with the biochemical results, we observed a substantial colocalization of AT1R and BK α at the plasma membrane using live labeling and confocal microscopy. In keeping with a tight coupling between AT1R and BK channels resulting in an overall inhibitory effect on BK channels, electrophysiological studies revealed that the sole expression of AT1R (in the absence of Ang II) shifted the half activation potential of BK channels as much as ~ 32 mV towards more positive membrane potentials. Finally, Ang II induced internalization of AT1R together with a dramatic increase of the cytoplasmic presentation of BK channels in HEK293T cells. These results strongly suggest that AT1R is the mediator of the Ang II effect on BK channels from coronary and renal arterial SMCs, and unravel a protein-protein interaction-based mechanism independent of G-protein activation by which AT1R modifies BK channel properties and microlocalization.

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PKC α and β activation by elevated extracellular glucose cause a marked inhibition of Kv currents in mesenteric arterial smooth muscleJ.L. Brignell², R.I. Norman¹ and R.D. Rainbow¹¹*Cardiovascular Sciences, University Of Leicester, Leicester, UK and*²*Cell Physiology and Pharmacology, University of Leicester, Leicester, UK*

Hyperglycaemia causes a marked vasoconstriction in resistance arteries. Protein Kinase C (PKC) activation has been demonstrated to be activated by an increase in extracellular glucose in a number of cell types including cardiac myocytes, renal tissue and vascular smooth muscle. We have previously identified that elevated extracellular glucose caused a marked inhibition of Kv currents in mesenteric arterial smooth muscle cells. Here we present data showing that increasing extracellular glucose concentration causes a marked concentration-dependent inhibition of Kv currents that can be reversed using selective PKC α and β inhibitors.

Rat mesenteric arterial smooth muscle was enzymatically isolated as previously described (Rainbow et al 2006). All animals were humanely culled in accordance with Home Office guidelines. Using patch clamp electrophysiology, a concentration response curve of the effects of extracellular glucose on Kv current density was constructed. A current voltage (IV) relationship was recorded from -40 to +60 mV using 400 ms pulses in 10 mV steps from a holding potential of -70 mV. This was recorded initially in a solution containing 0 mM glucose and was followed by 10 minutes of perfusion with either 2, 5, 8, 10, 12, 15, 18, 20 or 30 mM glucose (all osmotically balanced to 30 mM with mannitol) when a second IV curve was recorded and the mean current over the final 100 ms of the pulse to +60 mV was measured and compared to that in 0 mM glucose. At least 6 recordings were made for each concentration across a minimum of 4 animals. Data showed a marked inhibition of current best fitted with a biphasic curve giving a top half IC₅₀ value of 5.2 mM and a lower half of 14.2 mM. Using Western blotting we identified that PKC α , β , γ , δ and ϵ were present in mesenteric smooth muscle cell lysates (n=5 blots from 5 animals). In whole cell recording, inhibiting PKC α and β with either Go6976 or cell permeant Tat-peptide linked isoform-specific inhibitor peptides (Tat-PKC) attenuated the inhibition of Kv current by 20 mM glucose (fractional current 0.52 ± 0.04 in control, $0.95 \pm 0.07^{***}$ in Go6976, $0.78 \pm 0.06^*$ in Tat-PKC α , $0.85 \pm 0.09^{**}$ in Tat-PKC β and $0.94 \pm 0.03^{***}$ with both Tat-PKC α and Tat-PKC β inhibitor present, all n=6, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, (ANOVA, Bonferroni)). Selective inhibition of PKC γ , δ or ϵ did not significantly affect the glucose-induced Kv inhibition (0.51 ± 0.7 , 0.59 ± 0.8 and 0.61 ± 0.9 , all n=4, $P > 0.05$ (ANOVA, Bonferroni)).

These data suggest that PKC α and β are activated by elevated extracellular glucose and can act to inhibit Kv channels contributing to the glucose-induced depolarisation. Despite PKC ϵ having been shown to inhibit Kv current in other studies (Rainbow et al 2009), inhibition of PKC γ , δ or ϵ had no effect on the inhibition of Kv by elevated extracellular glucose.

Rainbow RD, Hardy MEL, Standen NB, & Davies NW (2006). Glucose reduces endothelin inhibition of voltage-gated potassium channels in rat arterial smooth muscle cells. *Journal of Physiology* 575, 833 – 844

Rainbow RD, Norman RI, Everitt DE, Brignell JL, Davies NW, & Standen NB (2009). Endothelin I and Angiotensin II inhibit arterial voltage-gated K⁺ channels through different PKC isoforms. *Cardiovascular Research* 83, 493-500

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C89

Ampicillin affects some the fatty acids and cholesterol values of rat heart tissues in non infections situation

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Ampicillin, a broad spectrum acid-resistant amino derivative of the penicillin group, is one of the most widely used anti-bacterial drugs in medical and veterinary medicine. In this study, we have investigated effect of ampicillin on fatty acid and cholesterol values that exist in the cell membranes and have physical significance in the Wistar albino rat heart tissues. Study were divided into two groups as control (serum physiologic) and ampicillin (25 mg/kg/day). After all experimental applications animals were anaesthetized 10 mg/kg xylazine (Rompun, Bayer, Turkey) and 70 mg/kg ketamine (Ketalar, Eczacibasi, Turkey) and animals viability were terminated by the method of taking blood from the heart under terminal anaesthesia. The fatty acids which were obtained from the heart tissues were analyzed by gas chromatography (GC) and cholesterol values were analyzed by high-performance liquid chromatography (HPLC). According to our calculations, total monounsaturated fatty acids (MUFA, such as 16:1, 18:1) values of heart tissues in the ampicillin groups were lower as statistically compared with control groups. Diametrically, total polyunsaturated fatty acids (PUFA, such as 18:2, 20:4, 22:5, 22:6) values in the same groups were higher than control group's PUFA values ($p < 0.05$). Besides, the cholesterol values in ampicillin groups were higher than compared with control groups. According to results, we suggest that ampicillin may affect molecular structure of fatty acids as a physical in the heart tissues and may affects some fatty acids synthetase and desaturase enzymes in the liver which is center of metabolism.

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Antimicrobial properties of *Momordica charantia* on *Salmonella typhi* and its effect on liver function in typhoid infected rats

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Typhoid fever is a disease prevalent in the tropics. In spite of the availability of various therapies, treatment of patients with the disease have been quite challenging in the face of resistance to used drugs and the quest for the development of more effective antimicrobial agents particularly from plant sources is being pursued relentlessly. *Momordica charantia* has been used in many Nigerian communities to treat typhoid. This study therefore investigated the antimicrobial effects of methanolic extract of *M. Charantia* leaves on strain of *Salmonella typhi* in male albino rats (*Sprague dawley*) and its effects on biomarkers of liver function. There were 5 groups of 10 rats each. 1ml aliquot of the 4th dilution of *S. Typhi* was administered orally to rats in four of the groups to infect with typhoid, while the last group served as the control. Infected groups were orally treated with 100 mg/kg, 200mg/kg of *M. Charantia* and 10mg/kg of Chloramphenicol respectively for seven days, while the fourth group was not treated after infection. Blood was collected by cardiac puncture and the effect of infection and subsequent treatment on parasitemia level, body weight and liver enzymes were thereafter investigated. Marked reduction in parasitemia level was observed in all treated rats. Rats treated with 200mg/kg of the plant extract had total clearance by the sixth day of treatment, while significantly higher reduction in parasitemia level ($p < 0.05$) was recorded in rats treated with the plant extract than those treated with chloramphenicol. Mean body weight of all treated rat groups increased during treatment. Concentrations of total and direct bilirbin, ALT, ALP, AST and GGT were higher ($p < 0.05$) in untreated rats than in treated rats. Result obtained showed that leave extract of *M. charantia* is a potent antimicrobial drug against *S. Typhi* with minimal pathology on the liver.

Key words: typhoid, *Momordica charantia*, liver, *Salmonella typhi*

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Cl⁻ not K⁺ controls the plasma membrane potential in rat white adipocytes

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The plasma membrane potential (Vm) drives many physiological processes; its dysregulation often underlies functional pathologies. K⁺ is widely believed to be the predominant ion that controls Vm for many cell types. Although, the plasma membrane of primary white fat adipocytes contains K⁺ channel conductances (Lee and Pappone 1997), whether they control its Vm is unknown. We have investigated the ionic species involved in the control of Vm of primary and differentiated 3T3-L1 adipocytes. Since insulin and β -adrenoceptors are key

regulators of adipocyte function we also explored their effect on adipocyte Vm.

Primary adipocytes were isolated via enzymatic dispersion of rat (250-340g) epididymal fat pads as described by (Rodbell 1964). 3T3-L1 adipocytes were prepared as described by (Mehra, Macdonald et al. 2007). We measured Vm with the perforated patch clamp technique at 32°C. Values are given as mean ± S.E.M. and are compared with Friedman's test or Wilcoxon sign test as appropriate.

The Vm of primary and 3T3-L1 adipocytes were -34.4 ± 1.5 mV (n=68) and -28.5 ± 1.2 mV (n=88) respectively. Elevation of extracellular K⁺ from 5.6 to 50 mM, by equimolar substitution of bath Na⁺, had no significant effect on the Vm of either type of adipocyte (n=8-11). As a positive methodological control, similar experiments performed on primary mouse beta cells demonstrated that the same change in extracellular K⁺ reversibly depolarised their Vm from -71.7 ± 1.8 to -20.8 ± 2.4 mV (n=8; P<0.001); a result similar to that reported by others (Atwater, Ribalet et al. 1978). We next investigated the effect of Cl⁻ on adipocyte Vm. Reduction of extracellular Cl⁻ from 138 to 5 mM, by equimolar substitution with gluconate, significantly depolarised the Vm of both primary and 3T3-L1 adipocytes to -9 ± 4 mV (n=9; P<0.005) and -9.6 ± 3.7 mV (n=12; P<0.005) respectively. Equimolar substitution of extracellular Na⁺ from 148 to 10 mM with N-methyl-D-glucamine, significantly hyperpolarised the Vm of both primary and 3T3-L1 adipocytes to -39 ± 5 mV (n=7; P<0.05) and -49 ± 4.7 mV (n=7; P<0.05) respectively.

Neither insulin (100 nM) or the β-adrenoceptor agonist isoprenaline (10 μM) significantly changed adipocyte Vm (n=3-12).

Our data indicates that the Vm of primary and differentiated 3T3-L1 adipocytes is predominantly controlled by Cl⁻, and not K⁺, with a minor role for Na⁺. Furthermore, we show that the functional effects of insulin and β-adrenoceptor stimulation on adipocytes are unlikely to be modulated by alterations in membrane potential.

Atwater, I., B. Ribalet and E. Rojas (1978). "Cyclic changes in potential and resistance of the beta-cell membrane induced by glucose in islets of Langerhans from mouse." *J Physiol* 278: 117-139.

Lee, S. C. and P. A. Pappone (1997). "Membrane responses to extracellular ATP in rat isolated white adipocytes." *Pflügers Arch* 434(4): 422-428.

Mehra, A., I. Macdonald and T. S. Pillay (2007). "Variability in 3T3-L1 adipocyte differentiation depending on cell culture dish." *Anal Biochem* 362(2): 281-283.

Rodbell, M. (1964). "METABOLISM OF ISOLATED FAT CELLS .I. EFFECTS OF HORMONES ON GLUCOSE METABOLISM + LIPOLYSIS." *Journal of Biological Chemistry* 239(2): 375-380.

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The hormonal profile of duodenal I-cells

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Enteroendocrine cells (EEC) secrete key gastrointestinal (GI) hormones such as gastric-inhibitory peptide (GIP), cholecystokinin (CCK) and ghrelin that are involved in nutrient sensing pathways and are often implicated in GI pathology. Traditionally, these cells have been classified by the 'Wiesbaden' system according to the specific hormone they contain. The enteroendocrine 'I-cell' is concentrated in the proximal duodenum and, according to this classification, secretes CCK, which acts to co-ordinate optimal digestion and absorption of nutrients and induce a satiety response to control food intake. The aim of this study was to determine the hormonal profile of duodenal I-cells.

I-cells were purified by fluorescence activated cell sorting (FACS) of duodenal cells isolated from (CCK_eGFP)BJ203G transgenic mice (CCK-eGFP mice). Total RNA was prepared from eGFP+ (I-cells) and eGFP- cells, and subjected to semi-quantitative RT-PCR. RT-PCR showed enrichment for CCK, ghrelin, peptide YY (PYY), GIP, neurotensin, secretin, somatostatin and glucagon-like peptide-1 (GLP-1).

Paraffin sections were prepared from the mid-duodenum segment of CCK-eGFP mice. Sections were subjected to immunostaining with antisera against proCCK, ghrelin, PYY, GIP and GLP-1.

Initial studies showed 89% (±1.25 n=19) eGFP cells labelled positively for CCK, confirming the validity of the mouse model. Significant numbers of eGFP cells were shown to co-express ghrelin (50% ±1.71 n=25) or PYY (45% ±2.76 n=9) or GIP (36% ±1.85 n=10) or GLP-1 (14% ±2.14 n=10). Co-expression was confirmed by dual immunostaining that showed proCCK to be expressed alongside ghrelin, PYY, GIP or glucagon. This showed that CCK I-cells contain a number of key GI hormones.

Analysis of high magnification micrographs revealed a complex pattern of vesicular staining. In dual labelling experiments some vesicles stained for only one hormone whereas others were positive for both hormones. Therefore, some hormones were co-packaged within a single vesicle, suggesting that I-cells have the ability to co-release CCK alongside other hormones.

It is clear that I-cells have a complex repertoire of key gut hormones; thus EEC's can no longer be simply classified as containing only one hormone and also suggests that further I-cell 'subsets' possibly exist. These data, combined with previous data showing the spectrum of nutrient-sensing receptors expressed in I-cells, suggest I-cells integrate several nutrient signals and have the ability to secrete a cocktail of hormones.

BBSRC

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Increases in nutrient-sensitive anabolic protein expression following a time-course of n-3 polyunsaturated fatty acid supplementation in human skeletal muscleC. McGlory¹, S.D. Galloway¹, L. Breen², C. McClintock¹ and K.D. Tipton¹¹*School of Sport, University of Stirling, Stirling, UK and* ²*Department of Kinesiology, McMaster University, McMaster, ON, Canada*

Age or injury-related skeletal muscle atrophy can often induce a host of metabolic diseases. Nutritional interventions have previously been examined in an attempt to identify strategies to minimize skeletal muscle atrophy (1). Recent work has shown that n-3 polyunsaturated fatty acid (n-3 PUFA) supplementation amplifies the anabolic response of human skeletal muscle to amino acid infusion (2). The molecular mechanisms detailing exactly how n-3 PUFA supplementation achieves an increase in the muscle protein synthetic response are yet to be elucidated. The aim of this study was to examine the expression of nutrient-sensitive anabolic signalling proteins at baseline and the time course of any changes over 4 weeks of n-3 PUFA supplementation. Healthy, male humans, (n=10), aged 21 ± 3yrs; body mass 76 ± 5kg (means + SD), consumed 5g.d⁻¹ of fish oil capsules (3500mg eicosapentaenoic acid [EPA]: 900 mg docosahexaenoic acid [DHA]) for 4 weeks. Muscle biopsies were obtained from the vastus lateralis in the fasted state at baseline (-2 and 0 weeks) then at 1, 2 and 4 weeks of n-3 PUFA supplementation. Western blot with zero replicates was performed for assessment of changes in expression of total mammalian target of rapamycin (mTOR), eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), focal adhesion kinase (FAK) and p70S6 kinase (p70S6K). Using a paired T-test it was identified that total mTOR, 4E-BP1, FAK and p70S6K were unchanged between -2 and 0 weeks. Using a single factor (week of supplementation) repeated-measures ANOVA we observed a significant main effect of week for fold change in mTOR (P<0.05), and FAK (P<0.05), but no effect for fold change in p70S6K or 4E-BP1. However, post-hoc comparisons using Bonferroni corrections were unable to detect individual differences between weeks for fold change in mTOR or FAK during the supplementation period. The peak fold change (mean ± SD) was 3.2 ± 2.8 for mTOR (0-2 weeks) and 3.9 ± 4.8 for FAK (0-4 weeks). These data indicate that consumption of 5g.d⁻¹ of fish-oil capsules for 4 weeks may promote an increase in the content of mTOR and FAK. Given that both mTOR and FAK are also known to be sensitive to muscle contraction (3), future research investigating the interaction between exercise, n-3 PUFA supplementation and muscle protein synthesis is warranted.

Tipton, K. D., Ferrando, A. A., Phillips, S. M., Doyle, D. Jr. & Wolfe, R. R. (1999). Postexercise net protein synthesis in human muscle from orally administered amino acids. *American Journal of Physiology Endocrinology and Metabolism*, 276, E628-34.

Smith, G., Atherton, P., Reeds, N. D., Mohammed, S., Rankin, D., Rennie, M. J. & Mittendorfer, B. (2011b). Dietary Omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults. A randomized, controlled study. *American Journal of Clinical Nutrition*, 93, 402-412.

Witard, O. C., Tieland, M., Beelen, M., Tipton, K. D., Van Loon, L. J. C. & Koopman, R. (2009). Resistance exercise increases postprandial muscle protein synthesis in humans. *Medicine and Science in Sports and Exercise*, 41, 144-54.

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Cbl/CAP signalling regulates lipid metabolism in muscle cellsS. Mora¹, C. Aye¹, E. Planet² and A. Zorzano²¹*Physiology, University of Liverpool, Liverpool, UK and* ²*Institute for Research in Biomedicine, Barcelona, Spain*

The Cbl family of proteins are multidomain proteins that have a dual function, as protein adaptors for tyrosine kinase receptors and as E3-ubiquitin conjugating enzymes. As such, they perform a variety of functions in different tissues and in response to various stimuli. In adipocytes, c-Cbl functions as an insulin receptor adaptor protein together with CAP (Cbl Associated Protein) and APS to regulate the insulin-stimulated translocation of the glucose transporter Glut4 to the plasma membrane. We have shown that in muscle, c-Cbl is also rapidly phosphorylated in tyrosine residues in response to insulin administration, however, this does not lead to the activation of the same signalling cascades that regulate glucose transport in adipocytes. To elucidate the function of Cbl and CAP in muscle cells we have constitutively knocked-down the expression of c-Cbl or CAP in C2C12 cells using lentiviral transduction of specific shRNAs and carried out gene expression profiling experiments to identify genes that are regulated by these proteins. Quantitative PCR and biochemical analyses were carried out to validate the DNA microarray data. We found that Cbl depleted cells display normal insulin-stimulated activation of phosphatidylinositol 3-kinase/Akt, and Mitogen Activated Kinases (p44/p42) in response to insulin. However, Cbl KD cells exhibit basal increased activation of AMPK-regulated kinase compared to non-infected control cells or cells expressing Non-targeting shRNAs. Microarray data analysis revealed that genes coding for proteins involved in lipid metabolism were altered in Cbl and CAP KD cell lines compared to control cells. Validation experiments confirmed reduced transcript levels for long chain fatty acid acylCoA synthases and carnitine palmitoyltransferase genes, suggesting reduced fatty acid activation and transport into the mitochondria. In vitro assays using palmitate as substrate showed that fatty acid acyl CoA synthase activity was reduced in Cbl and CAP KD cells. Cbl-deficient cells also displayed higher triglyceride levels than control cells. These data suggest that Cbl signalling contributes to regulate lipid metabolism and fatty acid utilization in muscle cells.

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Plasma ceramides are elevated in patients with type 2 diabetes and promote skeletal muscle insulin resistance and inflammation

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Obesity is associated with the development of insulin resistance, which is a central feature of the pathophysiology of type 2 diabetes. While the mechanisms responsible for the development of insulin resistance are not fully defined, there is compelling evidence for a causative role for defective lipid metabolism (1) and subclinical inflammation (2). It is widely accepted that the intracellular accumulation of ceramide causes inflammation and insulin resistance (3). Recent clinical data indicate that circulating ceramides correlate with systemic insulin resistance and inflammation (4); however, there is no evidence for a direct effect of circulating ceramide on peripheral insulin action and inflammation. Accordingly, we explored the role of circulating ceramide on the pathogenesis of insulin resistance. Plasma ceramides were examined in age-matched lean, insulin sensitive; obese, insulin sensitive and obese individuals with type 2 diabetes (T2DM). Almost all circulating ceramide was transported in lipoproteins. Ceramide transported in low density lipoproteins (LDL) accounted for ~40% of the plasma ceramide and LDL-ceramide levels in T2DM was increased by 51 and 72% compared with lean and obese subjects, respectively. LDL-ceramide correlated with insulin resistance ($r=0.43$, $P=0.01$, $n=22$).

To pursue the biology of circulating ceramides, we created a reconstituted LDL preparation, with or without the addition of ceramide (LDL 43 $\mu\text{g}/\text{ml}$; ceramide $2.1 \pm 0.3 \mu\text{mol}/\text{L}$). Reconstituting ceramide in LDL reduced insulin stimulated glucose uptake, Akt phosphorylation and GLUT4 translocation in cultured myotubes and this was associated with increased LDL-ceramide uptake. There was no evidence of inflammation in myotubes in vitro. Next, we demonstrated that LDL-ceramide induced a shift towards a pro-inflammatory profile in macrophages, which was due to both the uptake / metabolism of ceramide and activation of toll-like receptor signalling. To elucidate the physiological role of LDL-ceramide in vivo, we infused LDL-ceramide into the jugular vein of lean C57Bl/6 mice 24 h before assessing insulin-stimulated glucose transport using intravenous co-administration of 0.5U/kg insulin and 2-[1-³H] deoxyglucose (10 μCi). LDL-ceramide induced pro-inflammatory signalling and decreased insulin signalling in skeletal muscle, and reduced whole-body insulin stimulated glucose uptake. The impaired insulin action was specific to skeletal muscle.

In summary, these data support a role for plasma ceramides in the development of skeletal muscle insulin resistance and macrophage inflammation, findings that support the hypothesis that ceramide signals generated both from within skeletal muscle and those circulating in LDL contribute to the development of whole body insulin resistance.

Savage DB, Petersen KF, Shulman GI. Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiol Rev.* 2007 Apr;87(2):507-20.

Schenk S, Saberi M, Olefsky JM. Insulin sensitivity: modulation by nutrients and inflammation. *J Clin Invest.* 2008 Sep;118(9):2992-3002.

Holland WL, Brozinick JT, Wang LP, Hawkins ED, Sargent KM, Liu Y, et al. Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab.* 2007 Mar;5(3):167-79.

Haus JM, Kashyap SR, Kasumov T, Zhang R, Kelly KR, Defronzo RA, et al. Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes.* 2009 Feb;58(2):337-43.

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Retinal Growth Hormone (GH): Local regulation by GH-Releasing Hormone (GHRH)

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Growth hormone (GH), identical to that in the pituitary, is expressed in retinal ganglion cells (RGCs) in chickens, rats, mice and humans. Retinal GH is a neuroprotective factor, as it stimulates RGC survival and, in humans, the concentration of GH in vitreous fluid may be a marker for ocular disease. Indeed, vitreal GH concentrations are low in diabetic patients that have increased RGC death and glaucoma. Increasing GH expression in RGCs could thus have therapeutic potential in the treatment of glaucoma, which results from RGC death. The factors regulating retinal GH expression are, however, unknown. The possibility that GH-releasing hormone (GHRH) might be involved in the expression of retinal GH was therefore investigated, since hypothalamic GHRH is the primary stimulator of pituitary GH secretion. The possible presence of GHRH was determined by immunohistochemistry, using retinal sections from embryonic chicks and fixed QNR/D cells (a commercial neuroretinal cell line). The possible GH-releasing activity of GHRH was determined by the in vitro culture of QNRD cells with exogenous GHRH and by the measurement of GH mRNA, using of quantitative PCR. GHRH immunoreactivity was abundantly present in the cytoplasm of chick retinal RGCs and in QNR/D cells and in both cases was co-localized with GH immunoreactivity. After a 24h incubation in exogenous GHRH (at 10^{-6}M) GH mRNA in the QNR/D cells was significantly ($<P0.05$) increased. These results demonstrate, for the first time, that GHRH is present in retinal RGCs and that exogenous GHRH promotes retinal GH expression. The co-localization of GH and GHRH also suggests that retinal GH regulation occurs locally by autocrine or paracrine mechanisms.

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Single nucleotide polymorphisms rs3278 and rs3755652 enhance alternative transcription of the sodium/bicarbonate transporter *slc4a7* gene

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The sodium/bicarbonate transporter plays a role in intracellular pH regulation in the body. Genetic studies show that some single nucleotide polymorphisms of human *SLC4A7* gene are prevalent in alcohol abusers and control individuals in the European American population. In this study, we examined the physiological genomic effect of two SNPs rs3755652 (non-synonymous mutation) and rs3278 (synonymous mutation) on gene transcription and protein expression. These two sites are located upstream of exon 9 that is predicted to produce an N-terminally deleted transporter missing as much as one third of its amino acids. Analyzed by the transcription start site database and promoter prediction algorithm, multiple promoter sites were identified in intron 7. Deleting the promoter site located 0.25 kb upstream of exon 8 markedly decreased transcription activity, determined by luciferase reporter gene assays. The minor allele at rs3755652 increased luciferase activity by 80% compared to the major allele ($p < 0.05$; $n = 6$), while the minor allele at rs3278 increased luciferase activity by 20% ($p < 0.05$; $n = 6$). In transfected HEK 293 cells, the N-terminally deleted transporter caused by alternative transcription had reduced membrane expression compared to the non-deleted canonical transporter. The deleted transporter had reduced colocalization with the endosomal marker Rab5 determined by double-label immunocytochemistry. In functional studies, the deleted transporter failed to raise intracellular pH from a CO₂-induced acidification. The non-synonymous mutation E326K caused by rs3755652 produced negligible effect on the canonical transporter function. Our data suggest that rs3278 and rs3755652 enhance an alternative transcription in intron 7, leading to elevated production of the N-terminally deleted transporter with impaired expression and function.

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Orai3 regulates cell proliferation and survival in lung cancer cells

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Despite therapeutical progress, lung cancer is the leading cause of cancer-associated deaths worldwide, and non-small cell lung cancer (NSCLC) accounts for almost 80% of lung cancer deaths. Therefore, it is desirable to develop therapeutic agents that prevent cancer proliferation.

A number of studies have shown that Ca²⁺ influx, capacitative calcium entry (CCE) or store-operated Ca²⁺ entry (SOCE),

mediated by store-operated channels (SOCs) triggers several physiological processes including proliferation and apoptosis. Recently, Orai3 channels have been reported to constitute SOCs and to mediate CCE in breast cancer (1). Moreover, they are overexpressed in breast cancer and regulate cell proliferation and survival (2). However, their expression and role in lung cancer are still unknown. In the present study, we analyzed Orai3 expression in normal and cancerous lung tissue samples, and investigated their role in adenocarcinoma cell lines. We found that Orai3 and STIM1 are expressed in both A549 and NCI-H23 cell lines where they mediated a SOC entry. Down-regulation of Orai3 inhibited 60% of protein expression and reduced both NCI-H23 and A549 cell proliferation by 50% 72h post-transfection. In contrast, no significant effect was observed on cell mortality in both cell lines using trypan blue exclusion test. To elucidate the mechanism by which the inhibition of Orai3 reduced lung cancer cell proliferation, we performed flow cytometry to study the effect of Orai3 down-regulation on cell-cycle progression. The silencing of Orai3 led to NCI-H23 and A549 cell-cycle arrest with a significant accumulation of cells in the G0/G1 phase (13% for NCI-H23, and 20% for A549, $p < 0.001$), and to a concomitant decrease in the cell percentage in both S (13% for NCI-H23 and 16% for A549, $p < 0.001$) and G2/M (4% for NCI-H23, $p < 0.001$, and 3.5% for A549, $p < 0.05$) phases. This phenomenon is associated with a reduction in the expression of Cyclin D1, cdk4 and cdk2 (cyclin-dependent kinases) in siOrai-3 transfected NCI-H23 cells but not in A549 cells. Interestingly, in A549 cells, Orai3 silencing induced an increase of apoptosis (8%, $p < 0.05$) and a decrease of 50% of c-myc expression. Indeed, c-myc has been reported to be involved in lung survival (3). Finally, the expression of Orai3 was evaluated in 30 human lung adenocarcinoma specimens using Tissue Macro Array. We found that Orai3 was overexpressed in 70% of cases.

Our results provide evidence for a significant effect of Orai3 on lung cell proliferation and survival in vitro and show that this effect is associated with the induction of cell cycle and apoptosis resistance. Our study highlights the role of Orai-3 channel as an essential actor of pulmonary tumorigenesis, and supports its role as a potential prognostic or diagnostic marker in NSCLC.

Motiani RK, Abdullaev IF, Trebak M. A novel native store-operated calcium channel encoded by Orai3: Selective requirement of Orai3 versus Orai1 in estrogen receptor positive versus estrogen receptor negative breast cancer cells. *J Biol Chem* 2010. 285: 19173–19183.

Faouzi M, Hague F, Potier M, Ahidouch A, Sevestre H, Ouadid-Ahidouch H. Down-regulation of Orai3 arrests cell-cycle progression and induces apoptosis in breast cancer cells but not in normal breast epithelial cells. *J Cell Physiol*. 2011 Feb;226(2):542-51.

Zajac-Kaye M. Myc oncogene: a key component in cell cycle regulation and its implication for lung cancer. *Lung Cancer*. 2001 Dec;34 Suppl 2:S43-6.

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Differential contributions of Transient receptor potential melastatin type 2 channels in mediating hydrogen peroxide-induced calcium influx and cell death in macrophages

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Transient receptor potential melastatin type 2 (TRPM2) channels, a calcium permeable cation channel, which can be activated by intracellular ADP-ribose (ADPR) and reactive oxygen species, are involved in several physiological and pathological progresses, including cytokine production, insulin release and cell death. However, the expression of the TRPM2 channels and their functions in macrophage are not fully understood. This study aims to find whether TRPM2 channels are expressed and mediate calcium influx and cell death induced by H_2O_2 in primary peritoneal macrophages and two immortalized macrophage cell lines, RAW 264.7 and PMA-differentiated THP-1 cells. Immunofluorescent confocal microscope was used to detect protein expression of the TRPM2 channels. Single cell calcium imaging and XTT assay were used to investigate the changes in concentration of intracellular calcium ($[Ca^{2+}]_i$) and the number of cells in response to H_2O_2 , respectively. The result of confocal showed that the TRPM2 proteins are expressed in all the primary macrophages and macrophage cell lines. In the room temperature, H_2O_2 at 300 μM induced 0.18 ± 0.02 , 0.47 ± 0.03 and 0.32 ± 0.03 increases in $[Ca^{2+}]_i$ in primary peritoneal macrophages, RAW 264.7 cells and PMA-differentiated THP-1 cells respectively, mainly resulting from calcium influx. The inhibition of these increase in all primary peritoneal macrophages, RAW 264.7 cells and PMA-differentiated THP-1 cells by PJ34 and the enhance at body temperature suggested that the increase in $[Ca^{2+}]_i$ induced by H_2O_2 is mainly mediated by TRPM2 channels. Exposure to 300 μM H_2O_2 for 10-60 min caused significant cell death up to $55 \pm 1\%$, $50 \pm 1\%$ and $22 \pm 0.9\%$ in primary peritoneal macrophages, RAW 264.7 and PMA-differentiated THP-1 cells, respectively. The partially reduction of the cell death in primary macrophages, RAW 264.7 and PMA-differentiated THP-1 cells pre-treated with PJ34 and macrophages from TRPM2 knockout mice and implied the contribution of TRPM2 channel in H_2O_2 -induced cell death, whereas other factors are also involved in these cell death. These results taken together suggest that TRPM2 proteins are expressed in macrophages and all two macrophage cell lines examined, and potentially mediate H_2O_2 -induced calcium influx and also involve in cell death induced by H_2O_2 .

Perraud AL et al. (2001). Nature 411:595-599.

Wehage E et al. (2002). J Biol Chem 277:23150-23156

Campo B et al. (2003). J Immunol 170:1167-1173.

Togashi K. (2006). EMBO J 3;25(9):1804-1815.

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C100

17 β -estradiol rapidly induces KCNQ1 internalization and post-endocytic trafficking in HT29 colonic epithelial cells

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17 β -estradiol (E2) rapidly reduces cAMP-dependent intestinal Cl^- secretion by inhibiting K^+ recycling (1). KCNQ1:KCNJ3 is the rate-limiting K^+ channel involved in Cl^- secretion in the colon (2) and E2 rapidly inhibits KCNQ1 current in the female rat distal colon by a gender-specific mechanism (3). Regulation of KCNQ1 surface density has been shown to play a role in the control of KCNQ1 activity. The aim of this study was to determine if membrane trafficking plays a role in the E2 inhibition of KCNQ1 function in the human colonic cell line, HT29cl19A. Data are given as Mean $\pm$ S.E.M.

Using chamber experiments revealed that E2 (10 nM) reduced both forskolin induced Cl^- secretion ($30 \pm 6\%$ $n=6$ $p<0.05$) and KCNQ1 activity ($45 \pm 8\%$ $n=4$ $p<0.05$). Confocal microscopy showed that KCNQ1 is removed from the plasma membrane and internalized in cytosolic pools after 15 min E2 treatment ($n=5$). A biotin internalization assay confirmed this observation (internalized KCNQ1 after E2 treatment = $226 \pm 9.9\%$ of control, $n=5$, $p<0.001$). Our results suggested that KCNQ1 internalization is clathrin and dynamin dependent since chlorpromazine and dynasore reversed E2 mediated KCNQ1 internalization as shown by immunofluorescence ($n=3$). Fluorescence co-localization studies indicated that KCNQ1 rapidly co-localized with the clathrin adaptor AP2 $\mu 2$ (Adaptor Protein 2 sub-unit $\mu 2$) 10 min after E2 treatment (overlap coefficient (O.C.) = 0.28 ± 0.04 , control - 0.77 ± 0.01 E2, $n=4$). Following internalization, a subset of KCNQ1 appeared to accumulate in early endosomes (EE), (EE marker, EEA-1; O.C. with KCNQ1 = $0.30 \pm 0.07\%$ control, 0.60 ± 0.04 E2, $n=4$, $p<0.05$). Further experiments revealed that KCNQ1 is recycled to the membrane rather than degraded. Following E2 exposure KCNQ1 rapidly co-localized with Rab4 (O.C. = 0.31 ± 0.02 , control, 0.64 ± 0.02 E2 15 min), but Rab11 co-localization was only observable after 120 min (O.C. = 0.3 ± 0.03 control, 0.67 ± 0.01 , E2) suggesting that KCNQ1 is sorted from the EE to the recycling endosomes. After 240 min exposure to E2, $70 \pm 5\%$ ($n=6$, $p<0.001$) of internalized KCNQ1 was recycled back to the membrane as shown by a biotin recycling assay. Following E2 treatment, PKC δ ($287 \pm 51\%$ $n=4$, $p<0.05$) and AMPK ($232 \pm 24\%$, $n=5$, $p<0.05$) phosphorylation were increased within 2 min exposure to E2. Immuno-staining and biotinylation experiments revealed that E2 failed to induced KCNQ1 endocytosis in HT29cl19A cells when pre-treated with BIS1 a general PKC inhibitor, rottlerin a potent PKC δ inhibitor or Compound-C a AMPK inhibitor ($n=4$).

This study establishes a role for E2 in the regulation of KCNQ1 surface density. In conclusion, we propose that the internalisation of KCNQ1 is a rapid estrogen response in modulating intestinal secretion.

Condliffe SB. et al J Physiol. (2001) 1; 530(Pt 1):47-54.

Preston P. et al J Biol Chem. (2010); 5; 285(10):7165-75.

O'Mahony F. et al J. Biol. Chem. (2006); 282:24563-73.

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Regulation of large conductance voltage- and calcium-activated potassium channels by reversible protein S-acylation

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S-acylation, the only reversible post-translational lipid modification of proteins, is emerging as a major mechanism to control ion channel function and physiology. The most common form of S-acylation, palmitoylation, results from the addition of the C16 lipid palmitate via a labile thioester bond to reactive cysteines in proteins. However, as for most other palmitoylated proteins, the enzymes controlling palmitoylation of ion channels are largely unknown.

In this study we have developed an siRNA based screen and enzyme overexpression strategy in HEK293 cells to define the candidate acyl transferase (zDHHC) and acyl thioesterase (APT) enzymes that control palmitoylation of large conductance voltage- and calcium-activated potassium (BK) channels. Palmitoylation of a cluster of cysteine residues (C53:54:56) in the intracellular N-terminal S0-S1 loop of the pore-forming α -subunit was mediated by only two of the 23 zDHHs: zDHH22 and 23. siRNA-mediated knockdown of zDHH22 or 23 reduced 3H-palmitate incorporation into BK channel α -subunits by $67 \pm 4\%$ and $76 \pm 8\%$ of control respectively. These enzymes are distinct from the zDHHs that control palmitoylation of an alternatively spliced insert (STREX) in the C-terminus of the BK channel α -subunit. In contrast, the S0-S1 site is depalmitoylated by the cytosolic APT1 and APT1-like, but not APT2 or lysosomal PPT1, thioesterases. Overexpression of APT1 or APT1-like enzymes reduced channel palmitoylation by $68 \pm 2\%$ and $52 \pm 7\%$ of control respectively whereas the corresponding catalytically inactive mutants were without effect. Palmitoylation of the S0-S1 loop was required for efficient cell surface trafficking of the BK channel α -subunit. siRNA knockdown of zDHH22 or 23, or overexpression of catalytically active APT1 or APT1-like thioesterases, reduced plasma membrane surface expression of the BK channel α -subunit by $> 50\%$ compared to control. This reduction in surface expression was similar to that observed with the palmitoylation deficient BK channel α -subunit mutant C53:54:56A and channel depalmitoylation resulted in retardation of the α -subunit in the trans-golgi network (TGN).

This study has defined the repertoire of palmitoylating and depalmitoylating enzymes that control palmitoylation of the BK channel. Our studies show that both palmitoyl acyltransferases and acyl thioesterases display discrete substrate specificity for BK channels. Furthermore, as depalmitoylated BK channels are retarded in the TGN, reversible protein palmitoylation provides a critical check-point to regulate exit from the TGN and thus control BK channel cell surface expression.

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Inhibition of the proteasomal pathway increases epithelial sodium channel (ENaC) activity in mCCD_{d1} cultured mouse renal cortical collecting duct cells

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The epithelial sodium channel (ENaC) marks the tightly regulated rate-limiting step of sodium reabsorption in the aldosterone-sensitive distal nephron (ASDN). In the ASDN, stimulation of ENaC activity by the mineralocorticoid aldosterone and the antidiuretic hormone AVP is mediated via complex mechanisms which are still incompletely understood [1]. These include enhanced insertion of either recycled or newly synthesised channels into the plasma membrane and/or inhibition of channel retrieval via Nedd4-2 mediated ENaC ubiquitination and proteasomal degradation [2]. The aim of this study was to investigate the role of the proteasomal pathway in basal and hormonally-regulated ENaC activity.

Cultured mouse cortical collecting duct cells (mCCD_{d1}) which respond to physiological concentrations of aldosterone and AVP [3] were used and ENaC activity was quantified by recording the equivalent short circuit current (I_{SC}) in Ussing chambers. Biotinylation studies were performed to monitor membrane abundance of β -ENaC subunits. Values are means $\pm$ SEM, compared by Student's t-test. Addition of $1 \mu\text{M}$ MG132, a specific proteasomal inhibitor, maximally stimulated I_{SC} from 9 ± 1 to $23 \pm 2 \mu\text{A cm}^{-2}$ ($n=7$, $p<0.01$) after 3.5h. Application of 3 nM aldosterone increased I_{SC} from 10 ± 2 to $25 \pm 4 \mu\text{A cm}^{-2}$ ($n=7$, $p<0.05$) reaching a plateau after 2h. Subsequent application of MG132 further increased I_{SC} to $47 \pm 6 \mu\text{A cm}^{-2}$ ($n=7$, $p<0.01$). Increases in I_{SC} correlated with an increased abundance of β -ENaC at the plasma membrane. In contrast to the stimulatory effects of aldosterone and MG132, a slight run-down of I_{SC} $\sim 20\%$ was observed in matched control cells over the course of 7h. Treatment with 25 pM AVP rapidly increased I_{SC} by $13 \pm 1 \mu\text{A cm}^{-2}$ ($n=3$) and $13 \pm 0 \mu\text{A cm}^{-2}$ ($n=3$) in vehicle- and aldosterone-treated cells, respectively. This effect was significantly reduced in both groups in the presence of MG132, $\sim 65\%$ (vehicle-treated) and $\sim 70\%$ (aldosterone-treated). Application of $10 \mu\text{M}$ amiloride abolished I_{SC} in all experiments indicating that changes in I_{SC} can be attributed to altered ENaC activity. Our results demonstrate that inhibition of proteasomal degradation in mCCD_{d1} cells stimulates ENaC activity. This effect is preserved in cells pre-stimulated with aldosterone which suggests that the stimulatory effect of MG132 is additive to that of aldosterone. In contrast, the response to AVP is reduced following MG132 treatment. Interestingly, ENaC stimulation by AVP has been shown to involve channel recycling from a subapical pool to the plasma membrane. Thus, the inhibitory effect of MG132 on the AVP response may reflect altered channel recycling due to proteasomal inhibition.

Löffing J and Korbmayer C (2009). *Pflug Arch Eur J Phys* **458**, 111-135.

Rotin D and Staub O (2011). *Pflug Arch Eur J Phys* **461**, 1-21.

Gaeggeler HP et al. (2005). *J Am Soc Nephrol* **16**, 878-891.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C103

P2X4 null mice have a salt-sensitive blood pressure and impaired ENaC-mediated sodium reabsorption

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Extracellular nucleotides act via P2 receptors to modify epithelial Na channel (ENaC)-mediated Na reabsorption in the renal distal tubule (1). Pharmacological profiling and the use of genetically engineered mice suggest an inhibitory role for P2Y2 receptors on ENaC activity (2,3). An in vitro investigation using rat split-open CDs has provided evidence that apical P2X4 receptors may also modulate ENaC activity, and, at a Na concentration that mimics distal tubular fluid, activation of P2X4 by extracellular nucleotides can potentiate ENaC activity (4). There is also evidence that P2X4 may be involved in BP regulation as P2X4 null mice (P2X4^{-/-}) are hypertensive (5).

In the present study we investigated the possible role of the P2X4 receptor in the regulation of ENaC activity in P2X4^{-/-} and wildtype littermate controls (P2X4^{+/+}). P2X4^{+/+} and P2X4^{-/-} mice were maintained on either a standard Na diet (SSD; 0.3%) or a low Na diet (LSD; 0.03%) for 2 weeks. They were then anaesthetised (thiobutabarbital sodium (Inactin), 100mg/kg IP) and surgically prepared for renal clearance studies. After control collections of urine and plasma, mice were given benzamil (1 mg/kg bolus, IV) and further collections were made. Mean arterial (MA) BP was recorded during the experiments. Data are presented as mean±SEM; statistical comparisons were made using ANOVA and post-hoc Bonferroni.

MABP was significantly elevated in P2X4^{-/-} mice compared with P2X4^{+/+} mice on a SSD (95.04±2.3 vs 85.2±2.6mmHg; P<0.01; n=8 for both). On a LSD MABP was similar in the 2 genotypes: the reduction in dietary Na had no effect on MABP in P2X4^{+/+} mice but significantly reduced MABP in P2X4^{-/-} mice (87.4±3.2 vs 86.7±2.6mmHg; n=6 for both). On a SSD benzamil administration led to a significant increase in fractional Na excretion (ΔFENa) in both genotypes: values were similar for P2X4^{+/+} and P2X4^{-/-} mice (1.73±0.25 vs 1.91±0.17%; n=8 for both). On a LSD P2X4^{+/+} mice showed an increased excretory response to benzamil (expressed as ΔFENa) compared with their response while on a SSD, indicating an increase in ENaC-mediated Na reabsorption (3.72±0.69 vs 1.73±0.25%; P<0.01; n=6 and 8, respectively); however, P2X4^{-/-} mice did not significantly increase their ΔFENa on a LSD compared to a SSD (1.91±0.17 vs 1.74±0.44%; n=6 and 8 respectively), and had a significantly lower ΔFENa than P2X4^{+/+} mice on the LSD (1.74±0.44 vs 3.72±0.69%; P<0.01; n=6 for both).

The difference in MABP response to the LSD between the P2X4^{-/-} and P2X4^{+/+} mice suggests that the normally raised BP in P2X4^{-/-} mice is Na-dependent. Moreover, the inability of the P2X4^{-/-} mice to increase their ENaC-mediated Na reabsorption after LSD feeding, as indicated by their response to benzamil, is compatible with the earlier in vitro finding in CDs that at a distal tubule luminal fluid Na concentration P2X4 activation by extracellular nucleotides potentiates ENaC activity.

Bailey & Shirley 2009, Purinergic Signal, 5:473

Pochynyuk et al 2010, FASEB J 24: 2056

Toney et al, 2012, Curr Opin Nephrol Hypertens, 21:52

Wildman et al 2008, JASN 19: 731

Yamamoto et al 2005, Nat Med, 12:113.

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C104

Exosomal transmission of functional aquaporin 2 in kidney cortical collecting duct cells

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Exosomes are vesicles released following fusion of endosomes with the plasma membrane. Urine contains exosomes that are released from the entire length of the nephron and change in composition with kidney disease. Exosomes can shuttle information between non-renal cells via transfer of protein and RNA. In this study murine kidney collecting duct (mCCDC11) cells were used to demonstrate that exosomes can act as a signalling mechanism between cells. First, the release of exosomes by mCCDC11 cells was confirmed by multiple approaches. Following isopycnic centrifugation, exosomal proteins flotillin-1 and TSG101 were identified in fractions consistent with exosomes. Electron microscopy demonstrated structures consistent in size and shape with exosomes. Exposure of mCCDC11 cells to the synthetic vasopressin analogue, desmopressin, did not affect exosomal flotillin-1 or TSG101 but increased AQP2 in a dose- and time-dependent manner that was highly correlated with cellular AQP2 (exosomal AQP2 v cellular AQP2, Pearson correlation coefficient r=0.93). To test whether the ratio of exosomal AQP2/flotillin-1 is under physiological control in vivo, rats were treated with desmopressin. The ratio of AQP2/flotillin-1 in the urinary exosome was significantly increased. Inter-cellular signaling by exosomes was demonstrated: exosomes from desmopressin-treated cells stimulated both AQP2 expression and water transport in untreated mCCDC11 cells (water flow across cells: control exosome treatment 52.8 ± 11 μl/cm²; AQP2 containing exosomes 77.4 ± 4 μl/cm², p=0.05, n=4). In summary, the amount of AQP2 in exosomes released from collecting duct cells is physiologically regulated and exosomal AQP2 closely reflects cellular expression. Exosomes can transfer functional AQP2 between cells and this represents a novel physiological mechanism for cell-to-cell communication within the kidney.

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C105 & PC268

Enhancing student engagement and delivery of feedback in physiology laboratory classes

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With recent growth in class sizes and demands on staff time and resources, it is increasingly challenging to engage students in laboratory practical classes and give them constructive feedback on their work. We have developed an interactive web-based tool, eBiolabs, that supports students' preparation for practicals by providing introductory material in the form of

interactive animations, videos and quizzes. The tool also enables students to complete web-based, post-practical assessments on which they receive instant feedback on their knowledge and understanding.

In 2010-11, eBiolabs was used to support the 13 practical classes in our first year Physiological Science BSc unit, taken by ~200 undergraduates. 95% of these students reported that accessing the site for the first time was easy and 91% said they felt more prepared for their first practical than if asked to read handbook notes. The average submission rate across all post-practical assessments was 94%, with an average score per assessment of 85%. In 2011-12, eBiolabs was extended to the second year of the BSc programme and to all first and second year units on the professional programmes (medicine, dentistry and veterinary science) on which physiology is taught. Prior to the introduction of eBiolabs, 91% of ~200 second year medical students reported spending less than 20min preparing for each practical class (with 66% spending almost no time on this). After its introduction, 72% of the same students reported that they spent at least 20min preparing (with 25% spending more than 1hr). Whereas only 30% of medical students had felt well-prepared for practical classes before the use of eBiolabs, that figure rose to 48% after using eBiolabs for one term (for 5 physiology practicals). Furthermore, the percentage of medical students reporting that feedback received on their post-practical work was clear and timely rose from 6% to 72%. In agreement with these data, 87% of first year dental students agreed that eBiolabs was useful in helping them prepare for practical classes and 67% agreed that the assessment feedback was useful for their learning (n = 63).

The system has been well received on all courses and eBiolabs has brought other benefits to physiology practical teaching. Staff and demonstrators are able to access teaching material which allows better preparation for practicals, and student performance is easily accessible and clearly presented. With students being better prepared for practical classes they gain more from the laboratory experience and can engage fully with the physiological principles behind experiments rather than focusing on operational/technical issues. eBiolabs has become an integral part of physiology teaching at the University of Bristol as a vital tool that allows student engagement in practical classes and is accessible for staff and students alike.

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C106 & PC269

Potential roles for tablet devices in practical settings: evidence from two case studies with biomedical science students

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Technology enhanced learning is expanding rapidly within Higher Education, due to the increasing volume of evidence showing the benefits for learners in terms of engagement, convenience, attainment and enjoyment. Harnessing mobile devices to enhance learning has major potential, in particular during practical classes, field work and private study. However, there are few systematic studies evaluating the impact of tablet devices on students' learning. The aim of this study was to gather evidence of the effectiveness of a tablet device to enhance second year undergraduate biomedical science stu-

dents' learning within two practical scenarios: a neuroanatomy blended learning class and an electrophysiology mini-project. In the neuroanatomy class, students (n=178) used pre-configured Apple® iPads® alongside plastic brain models and printed resources. Following the practical, students (n=115) completed a questionnaire recording their use of the device. Students used the following apps during the 120 minute practical class: 3D Brain (16.2 ± 9.5 min, mean ± S.D.; n=112), Google (14 ± 9 min; n=86), HD Brain (9.5 ± 7 min; n=83) and Slyvius Brain Atlas (10 ± 8 min; n=30). The apps were used to look at images of brain structures (95% of students), look up definitions (61%), read text (52%) and write notes (11%). Overall 76.3% of students agreed that the iPads® were beneficial to their learning and 80.7% agreed that the devices made the experience more enjoyable.

During the 4 week electrophysiology class, students (n=36) were provided with an Apple® iPad® alongside the printed practical schedule. The tablet device contained an interactive eBook (generated using iBooks Author®) consisting of dissection videos, information about how to use the equipment and MCQs. Seventeen students completed a questionnaire about their use of the iPad® after the first week of the practical. All students (n=17) indicated that they had watched the dissection video, 70% had viewed the eBook and 63% had used the MCQs. All students that used the eBook (n=12) indicated that it was useful for enhancing their learning. However, students did not make much use of other tools on the device, including bibliographic databases and reference management tools, citing lack of time.

Data from these experiments indicate that tablet devices are beneficial in some practical settings, but illustrate that adequate time must be provided for students to make full use of the wide range of functionality that they offer. Students are increasingly using tablet devices and studies such as this are essential to help understand their potential for enhancing learning.

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C107 & PC270

Educational internships: Extra-curricular opportunities to enhance graduate employability

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The "Working towards your Future" report recommended that Universities provide opportunities, both within and outside of the curriculum, for students to develop employability skills (CBI & NUS, 2011). They should also have relevant work experience. Two-thirds of graduate recruiters recently warned that they would not employ graduates who had no such experience (High Fliers, 2011). One way for students to develop both employability skills and gain relevant work experience outside of the taught curriculum is through completion of internships. Within the Faculty, we have been offering research laboratory placements to Level 4 students for a number of years. However, the majority of our students do not go onto careers in scientific research and therefore we have developed two

programmes of internships for Level 4 and 5 students, educational research and curriculum development internships, which develop a different range of employability skills and more closely match the final career destinations of the majority of our graduates.

Students undertaking educational research internships contribute to ongoing programmes of educational research currently being undertaken by Teaching and Scholarship focused staff within the Faculty e.g. evaluation of young people's opinions of the use of research animals. Students undertaking curriculum development internships work either individually or in groups to develop and/or evaluate resources which will either enhance the student learning experience within the Faculty or externally. Examples of such internships include student evaluation of educational resources for IUPHAR and the development of a wiki of Open Educational Resources for teaching ethics in schools.

These internships are extremely popular, with 91 applications for the 28 internships offered in 2011-12. Students are required to regularly reflect on their internships and the skills gained, and to blog these reflections. They are also required to write a short case study on completion of the internship. In these blogs and case studies, they recognise the skills gained e.g. team working, project and time management, communication skills, and the benefit of these to their future employability; they also have a tangible outcome or resource they can show to prospective employers. Educational research and curriculum development internships are therefore a valuable means outside of the taught curriculum for students to develop employability skills and to gain work experience. They also provide significant benefits for the Faculty in enhancing the student learning experience, facilitating pedagogical and educational research and in the external promotion of its activities. CBI & NUS (2011).

http://www.cbi.org.uk/media/466204/60519E9E213D34268025788F003808EF__CBI_NUS_Employability%20report_May%202011.pdf

High Fliers (2011). <http://www.highfliers.co.uk/download/GMReport11.pdf>

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C108 & PC271

A Student Selected Component (SSC) for teaching medical students to generate their own reusable learning objects (quizzes or animations) in Flash

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INTRODUCTION. E-learning materials produced by one's students may lead to increases in instructor efficiencies given limited staff time. Among Student Selected Components (SSCs) in a medical school, one set of popular topics involves empowering the students to teach other students. Interactive reusable learning objects (such as those made using Flash) can improve learning by increasing end-user engagement. Narrative-based quizzes (e.g. those with a "learning companion" or where "you are the doctor") can increase engagement because they leverage emotions. Animated "movies" made with Flash can be valuable as teaching objects because: they can show how

objects move in 3 dimensions, they can highlight change over time, they can have sound, they are interactive, and they can provide feedback. **METHODS.** This poster outlines a short course (7-8 weekly one-hour sessions) run ten times so far, called "Using Flash to make didactic animated movies about (the cardiovascular system / the digestive system)". The complete teaching materials for instructors to teach this SSC are provided online at www.harrywitchel.com/elearning. In this SSC students are introduced to Flash, they are empowered immediately to make very simple (but rewarding) animations on their own, they are taught visual elements to define "regions of interest", "storyboarding" (telling a story in pictures, including "shot choice"), and very simple Flash interactions (e.g. buttons, scoring systems and gotoAndPlay statements). **RESULTS.** This course develops attitudes among the SSC participants concerning how pedagogy (and memory and attention) work, and makes students think how "physiology is function in motion". This course ends with students clearly learning a skill, as well as learning some physiology. The SSC feedback for "how interesting did you find doing this project" (where 1 = "not at all" and 5 = "extremely interesting") was 4.75 ± 0.13 (mean $\pm$ SEM, $n = 41$). For "How difficult or easy was it for you to learn to make interesting things with Flash?" (where 1 = "extremely difficult", 3 = "challenging" and 5 = "quite easy"), the feedback was 3.23 ± 0.31 ($n = 40$). In an open question about what they liked, many students mentioned the creativity, the freedom, and learning about the physiology (which usually required the students to research material beyond their lectures). In an open question about what they disliked, students repeatedly mentioned that actionscript was frustrating and that there was not enough time. One student summarised what they disliked succinctly: "addictive and time-consuming." **IMPLICATIONS.** As electronic learning materials become more widespread, student-developed e-learning materials may have a role in focusing teaching to what students see as challenging material to learn.

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C109 & PC272

The use of music in physiology classes to build learning communities and foster active learning

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It is well established that for learning to be effective, it must be a process in which the student participates actively. With such active learning, any knowledge gained is more likely to be retained by students who are better able to apply it to different contexts (Svinicki, 1998; Michael, 2006), an important characteristic of the Medicine, Science and Nursing students who populate basic Physiology classes. Cognitive theory posits that effective learning takes place when students present their own mental models for challenge, so that new information can be incorporated into the pre-existing network of associations that the learner already has (Svinicki, 1998). It is incumbent upon us to provide a safe space in which students will risk putting forward these mental models for challenge, without fear of being wrong or appearing foolish. Modell et al (2008) have proposed that we build learning communities within our classes and has detailed the use of music in the Medical Phys-

iology classroom in fostering this sense of community and identity.

Following the Modell et al (2008) model, I have used up-tempo (ska, rock, pop) music at the start of classes to energise students and raise attention levels, and baroque music during periods when they have been set class tasks. I have also used music to teach rhythmic concepts in Physiology (eg. illustrating systolic and diastolic murmurs using “on the beat” rock music and “off the beat” ska music). Student attitudes to such use of music were assessed by questionnaires distributed to 71 first year Biomedical Science students during a practical class. Questions focused on whether students felt afraid of questions in lectures, what they thought of the music played and the musical activities during lectures. A 5 point Likert scale was used to evaluate the student response to the questions with 5 indicating strong agreement with a statement and 1 strong disagreement. In addition, there were 3 open-ended questions focusing on the ways in which students might feel safer in answering questions, the use of music in achieving this and whether or not they would participate in a class singalong. For Likert scores, ratings are given as mean mark out of 5 $\pm$ S.E.M, n = 55.

55 of the 71 questionnaires were returned. 28 of the 55 students who responded, agreed or strongly agreed that they were afraid to look foolish answering questions in lectures, indicating a challenge that exists for us in creating the atmosphere for active learning. The pre lecture music was seen as energising for learning (Likert score of 4.3 ± 0.1) and creative of a friendly atmosphere (4.7 ± 0.1). Only 2 of the 55 respondents found the music annoying and disruptive.

This preliminary study indicates that the use of music in class may prove useful in establishing the tone for collaborative, student led learning in the modern Physiology classroom.

Michael, J. (2006). Where’s the evidence that active learning works? *Advances in Physiology Education* 30 159-167

Modell, H. I., DeMiero, F. G. & Rose, L. (2009). In pursuit of a holistic learning environment: the impact of music in the medical physiology classroom *Advances in Physiology Education* 33 37-45

Svinicki, M. D. (1998). A theoretical foundation for discovery learning *Advances in Physiology Education* 20 (1) S4-S7

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PC1

Crosstalk between TLR4-MyD88 and SCAP-SREBP2 pathways mediates macrophage foam cell formationL. Li^{1,2}, Z. Varghese¹, J.F. Moorhead¹ and X. Ruan¹¹Centre for Nephrology, Royal Free hospital, UCL, London, UK and²Nephrology Division, Chang-Gung Memorial hospital, Kaohsiung, Taiwan

Atherosclerosis, the leading cause of mortality worldwide, is characterized by chronic inflammation of vascular wall with accumulation of lipid and macrophage-derived foam cells in the subendothelial space. It has been evident that Toll-like receptors (TLRs), especially TLR4, contribute to the progression of atherosclerosis¹. Myeloid differentiation factor 88 (MyD88) plays a crucial role as an adaptor molecule in signal transduction of TLR superfamily in human macrophage. The feedback regulations of LDL receptor (LDLr) and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCoAR) mediated by sterol regulatory element binding proteins (SREBPs) cleavage-activating protein (SCAP)-SREBP2 pathway are considered to be key regulatory elements for cholesterol homeostasis in human cells². Studies³ have shown that a reduction of atherosclerosis in MyD88^{-/-} mice but the mechanism remains unclear. This study was designed to investigate the crosstalk between TLR4-MyD88 and SCAP-SREBP2 pathways in macrophage foam cell formation under stimulation with lipopolysaccharide (LPS). Phorbol, 12-myristate, 13-acetate (PMA) activated-human monocyte cell line (THP-1) macrophages were transfected with negative control or MyD88 siRNA, and incubated with LPS in the absence or presence of LDL. Oil Red O staining and a quantitative assay were used to assess the intracellular cholesterol content. The mRNA and protein expression of LDLr, HMGCoAR, SCAP and SREBP2 were examined by real-time quantitative RT-PCR and Western blotting. The translocation of SCAP from the endoplasmic reticulum (ER) to the Golgi was detected by immunofluorescent staining. Values are expressed as mean $\pm$ S.D., compared by ANOVA. We demonstrated that LPS induced intracellular cholesterol ester accumulation and foam cell formation in the absence (LPS vs. Control : 215.0 $\pm$ 16.3 vs. 151.9 $\pm$ 11.6 μ g/mg protein, $P < 0.01$, $n = 4$) or presence of LDL (LPS+LDL vs. LDL : 284.1 $\pm$ 26.1 vs. 205.1 $\pm$ 12.2 μ g/mg protein, $P < 0.01$, $n = 4$), while knocking down MyD88 attenuated the above effects by LPS (siMyD88+LPS vs LPS : 163.9 $\pm$ 12.5 vs. 215.0 $\pm$ 16.3 μ g/mg protein ; siMyD88+LPS+LDL vs. LPS+LDL : 185.4 $\pm$ 16.0 vs. 284.1 $\pm$ 26.1 μ g/mg protein, $P < 0.01$, $n = 4$). LPS increased the gene and protein expression of LDLr (2X, $P < 0.01$, $n = 4$) and HMGCoAR (1.8X, $P < 0.01$, $n = 4$) which can be ameliorated by knocking down MyD88, suggesting the involvement of MyD88 in cholesterol homeostasis. LPS also increased SCAP gene and protein expression (1.9X, $p < 0.01$, $n = 4$). Furthermore, immunofluorescence using antibodies to SCAP and Golgi demonstrated that LPS caused abnormal translocation of SCAP from the ER to the Golgi. Both effects were blocked by knocking down MyD88. These results suggest that MyD88 plays an important role in the regulation of cholesterol homeostasis and that inhibition of MyD88 might be a potential approach in preventing macrophage foam cell formation.

Lundberg AM & Hasson GK. (2010). *Clin Immunol* 134(1), 5-24.Rawson RB. (2003). *Nat Rev Mol Cell Biol* 4(8), 631-640.Björkbacka H et al. (2004). *Nat Med* 10(4), 416-421.

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PC2

The effect of gender and high-fat diet on expression of uncoupling protein 3 in apolipoprotein E knockout mouse heartJ. Hawi², H. Lin¹ and M. Suleiman¹¹University of Bristol, Bristol, UK and ²University of Balamand, Balamand, Lebanon

High-fat diet has been shown to increase expression of uncoupling protein 3 (UCP3) in genetic and non-genetic obesity and has been implicated in the decreased cardiac efficiency (1). Apolipoprotein E knockout (apoE^{-/-}) mice fed high-fat for 6 months, are not obese and develop atherosclerotic lesions (2). In contrast to their male counterparts, female apoE^{-/-} mice on high-fat diet do not show evidence of occlusive coronary lesions or myocardial infarction (3). The aim of this work was to investigate whether high-fat diet alters cardiac UCP3 expression in this model and whether there is a difference between male and female mice. Female and male apoE^{-/-} mice (mixed background C57Bl/6 and 129) at 8 weeks old were either switched onto high fat, Western-type diet (21% fat; 0.15% cholesterol) or were maintained on normal rodent diet for approximately 6 months. Myocardial tissue was collected from male ($n = 6$) and female ($n = 6$) mice and proteins were extracted as described previously (3). Western Blotting was used to estimate UCP3 expression in cardiac tissue using a polyclonal rabbit anti-UCP3 antibody (Abcam plc) and data expressed as a ratio of UCP3 band intensity to GAPDH using Image J. Data are presented as mean $\pm$ SE and differences determined using unpaired t-test ($n = 3$ for each group). High-fat diet was found to significantly increase UCP3 expression in male heart (1.11 $\pm$ 0.19 vs. 2.04 $\pm$ 0.15, $p < 0.05$) but not in female heart (0.66 $\pm$ 0.15 to 0.81 $\pm$ 0.16). There was no significant difference in UCP3 expression between male and female heart on normal diet (1.11 $\pm$ 0.19 vs. 0.66 $\pm$ 0.15). This work demonstrates that high-fat feeding increases UCP3 expression independent of obesity. Furthermore, the increase of UCP3 expression in heart appears to be associated with coronary disease as female hearts on high-fat diet without evidence of coronary disease do not show significant increase in UCP3 expression.

Basic Res Cardiol, 2011; 106(3): 447-457

Arterioscler Thromb Vasc Biol, 2002; 22(5): 788-92

Crit Care Med, 2007; 35(10): 2344-51

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PC3

Identification of a miR-212 target site in K_{ir}2.1 3' UTR by a novel dual-fluorescence microRNA targeting assay

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MicroRNAs (miRNAs) are increasingly recognised as important down-regulators of gene expression in health and disease. Luciferase-based transfection assays are commonly used for

identifying miRNA targets but these assays have limitations. We report miRNA target identification using a novel functional miRNA targeting assay. The 3' UTR of the human inward rectifier K⁺ channel K_{ir}2.1 was inserted downstream of the mCherry red fluorescent protein coding sequence in a mammalian expression plasmid. MiRNA or non-targeting control (SCR) sequences were inserted into the pSM30 miRNA expression vector. Expression of enhanced green fluorescent protein is an indicator of miRNA expression in this system [1]. HEK293 cells were co-transfected with the mCherry-K_{ir}2.1 3' UTR plasmid and a pSM30-based plasmid with miRNA insert. Alternatively the pSM30-based plasmids were transfected into cells stably expressing the mCherry-K_{ir}2.1 3' UTR construct. The principle of the assay is that functional targeting of the 3' UTR by the miRNA results in a decrease in the cell red/green fluorescence intensity ratio as determined by automated image analysis (Volocity or CellProfiler). The method was validated with miR-1, a known down-regulator of K_{ir}2.1 expression, and used to investigate targeting of the K_{ir}2.1 3' UTR by miR-212, as predicted by the TargetScan and MiRanda algorithms. MiR-212 is markedly up-regulated in heart failure [2] and chronic alcoholism [3], both of which feature down-regulation of inward rectifier K⁺ channel activity in their pathophysiology [4, 5]; and the affected tissues are known to express K_{ir}2.1. Red/green ratio was significantly lower in miR-1-expressing and miR-212-expressing cells compared to non-targeting controls (miR-1 0.50 ± 0.012 (mean $\pm$ sem), $n = 776$; miR-212 0.72 ± 0.024 , $n = 550$; SCR 1.21 ± 0.025 , $n = 731$; $p < .001$ for miR-1 vs SCR and miR-212 vs SCR, one-way ANOVA on log-transformed data). The effect of miR-212 was attenuated by mutating the predicted target site (% inhibition 58.0 ± 14.51 , $n = 3$ wild-type; 22.7 ± 1.25 , $n = 3$ mutant; $p < .05$, t-test on log-transformed data). This novel assay has several advantages over traditional luciferase-based assays including larger sample size, amenability to time course studies; and adaptability to high-throughput screening.

Wu J, Bonsra AN, Du G (2009). *Methods Mol Biol* 487:205-19.

Thum T, Galuppo P, Wolf C, Fiedler J, Kneitz S, van Laake LW, Doevendans PA, Mummery CL, Borlak J, Haverich A, Gross C, Engelhardt S, Ertl G, Bauersachs J. (2007). *Circulation* 116:258-67.

Tang Y, Banan A, Forsyth CB, Fields JZ, Lau CK, Zhang LJ, Keshavarzian A. (2008). *Alcohol Clin Exp Res* 32:355-64.

Beuckelmann DJ, Nabauer M, Erdmann E (1993). *Circ Res* 73:379-385.

Sun H, Fang Q, Mayhan WG (2004). *Alcohol Clin Exp Res* 28:1557-61.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC4

Matrix Metalloproteinase inhibition is cardioprotective against reperfusion injury by a mechanism that is both independent and additive to mitochondrial permeability transition pore inhibition

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Introduction - Ischaemic Heart Disease is the leading cause of death worldwide¹. Gold standard therapy is reperfusion of the tissue at risk. However, the act of reperfusion is associated with lethal reperfusion injury – death of cells only reversibly injured by the preceding ischaemic insult².

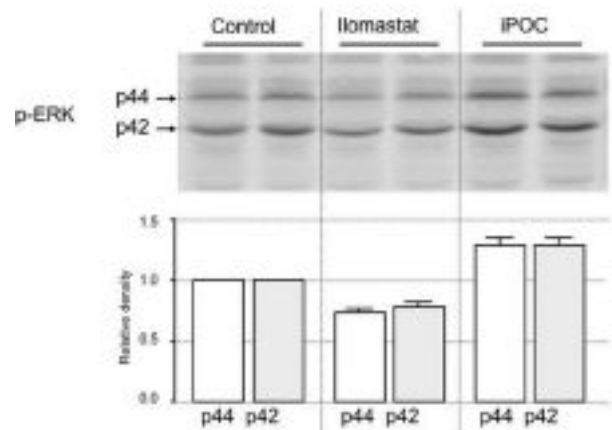
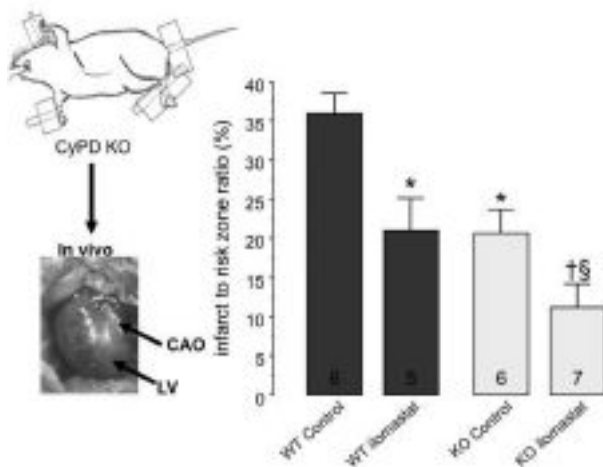
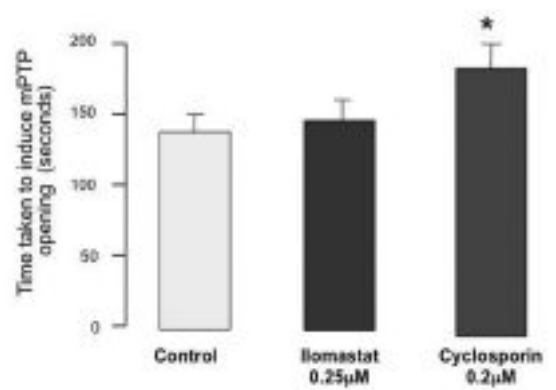
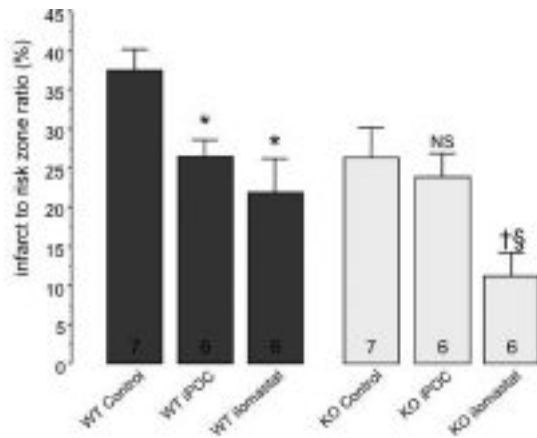
Current cardioprotective measures, ischaemic pre and post-conditioning (IPC & iPOC), reduce opening of the mitochondrial permeability transition pore (mPTP) following reperfusion – a key mediator of both necrotic and apoptotic cell death³. Matrix Metalloproteinase-2 and -9 are located in the mammalian heart⁴, and inhibition of these MMPs reduces infarct size following ischaemia-reperfusion⁵. The mechanism of cardioprotection by MMP inhibition is not known.

Aims - This investigation aimed to identify whether ilomastat, an MMP inhibitor, works by a mechanism other than inhibition of mPTP opening. We hypothesized that cardioprotection is independent of mPTP inhibition, the end-effector of iPOC.

Methods & Results - Experiments using animals were performed in accordance with the UK Home Office Guide on the Operation of Animal Act. The mice were anaesthetized by intraperitoneal injection with a combination of ketamine, xylazine and atropine (0.01 ml/g). Efficacy was determined by pedal pain withdrawal reflex before thoracotomy was undertaken. In ex-vivo and in-vivo mouse hearts, we investigated whether the MMP inhibitor, ilomastat (0.25 mmol/L), at reperfusion, could engender protection in the absence of cyclophilin-D (CyPD), an initiator of mPTP opening, against injurious ischaemia/reperfusion. Ilomastat attenuated infarct size in wild type (WT) animals (37 ± 2.8 to $22 \pm 4.3\%$, equivalent to iPOC, $27 \pm 2.1\%$, $p < 0.05$). CyPD knockout (KO) hearts had smaller infarcts compared to their WT brethren ($28 \pm 4.2\%$) and iPOC failed to protect, indicative of a pre-protected phenotype, yet ilomastat significantly attenuated infarct size in these hearts ($11 \pm 3.0\%$, $p < 0.001$). Furthermore, ilomastat was additive to the protection seen following iPOC in WT, restoring protection even after prolonged 50min ischaemia (49 ± 7.8 to $31 \pm 2.8\%$, $p < 0.05$). Moreover, ilomastat had no inducing effect on the reperfusion injury salvage kinase signalling (RISK) cascade (requisite for IPC and iPOC protection) and, unlike cyclosporine, had no impact upon mPTP opening, indicating no interaction with CyPD/mPTP in isolated cardiomyocytes.

Conclusions

1. MMP inhibition with ilomastat attenuates infarct size, independently of the mPTP and the RISK cascade
2. Ilomastat protection is additive to mPTP inhibition (and also to ischaemic postconditioning – [data not shown])
3. MMP inhibition remains protective even after more prolonged durations of ischaemia
4. Cardioprotection independent of mPTP manipulation suggests potential to further limit infarct size when ilomastat is combined with mPTP inhibition setting of acute coronary syndrome



Iloprost reduces infarct size in Cyp-D knockout mice, both ex-vivo and in-vivo

Iloprost shows no ability to slow mPTP opening in a cell based model, and does not induce RISK expression

- 1 WHO. The Global Burden of Disease: 2004 Update.
- 2 Piper HM et al (1998). Cardiovasc Res. 38,291-300.
- 3 Yellon D & Hausenloy D. (2007). N Engl J Med 357, 1121-1135.
- 4 Tyagi SC et al (1993). Mol Cell Biochem. 126, 49-59.
- 5 D'Annunzio V et al (2009). J Cardiovasc Pharmacol. 53, 137-44.

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PC5

2-Photon excitation fluorescence microscopy enables transmural voltage and Ca^{2+} recordings in Langendorff-perfused mammalian hearts

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Monitoring action potential (AP) characteristics in intact tissue gives an insight into the electrical behaviour of cardiomyocytes in situ. Combining these measurements with intracellular Ca^{2+} ($[Ca^{2+}]_i$) measurements gives a better understanding of the relationship between voltage changes and Ca^{2+} flux. Previous studies have described voltage and Ca^{2+} measurements in intact hearts using widefield epifluorescence techniques. However, this does not provide the depth resolu-

tion required for investigating transmural heterogeneities in whole heart preparations. Two photon laser scanning microscopy (2PLSM) enables measurements to be made in multicellular preparations with subcellular depth resolution. This study describes the feasibility of measuring voltage and $[Ca^{2+}]_i$ in whole hearts using 2PLSM for transmural investigations. Rabbit, rat and mouse hearts were Langendorff perfused and contraction inhibited with 10mM butanedione monoxime and 10 μ M blebbistatin. Hearts were electrically paced and positioned horizontally for imaging of the left ventricle (LV). Epicardial membrane potential recordings were made with sharp microelectrode (ME), resistance 50M Ω with 1M KCl for comparison with 2-photon (2P) measurements to validate the technique. Ratiometric voltage signals were measured using di-4-ANEPPS (excitation 920nm) and Ca^{2+} signals using fura-2 AM (excitation 760nm). Values are means $\pm$ S.E.M, compared by paired t-test. Using 2PLSM, sequential voltage and Ca^{2+} recordings were initiated 50 μ m below the LV surface and continued to a maximum depth of approximately 500 μ m. Poor signal:noise prevented deeper measurements. Comparison of AP duration at 90% repolarisation (APD_{90}) and upstroke rise time (TRise) obtained by ME and 2P methods revealed no difference in APD_{90} (134.4 $\pm$ 4.7ms vs. 133.4 $\pm$ 7.1ms, n=4 rabbit; 49.7 $\pm$ 6.0ms vs. 51.6 $\pm$ 6.8ms, n=3 rat and 50.7 $\pm$ 4.1ms vs. 55.3 $\pm$ 7.2ms, n=4 mouse). No difference in TRise was observed in rabbit APs (2.54 $\pm$ 0.24ms ME vs. 2.88 $\pm$ 0.30ms 2P). However, in rat and mouse hearts, TRise values obtained from ME measurements were faster than those obtained by 2P methods (0.50 $\pm$ 0.05ms vs. 0.92 $\pm$ 0.13ms in rat, p<0.05 and 0.65 $\pm$ 0.06ms vs. 1.18 $\pm$ 0.23ms in mouse, p=0.065). Subsampling of ME data from 28kHz to 2.8kHz to more closely match 2P sampling frequencies of 2.6kHz reduced the difference in TRise values (new rat ME TRise 0.70 $\pm$ 0.02ms, new mouse ME TRise 0.78 $\pm$ 0.03ms). 10-90% rise times of Ca^{2+} transients were slower (no faster than 5ms in all species), and could thus be accurately sampled with 2P measurements. In conclusion, voltage and Ca^{2+} sensitive dyes can be used with 2PLSM to study transmural differences in AP and Ca^{2+} signalling characteristics. This technique is best suited for use in rabbit hearts, where upstroke velocity is sufficiently slow to be faithfully recorded with 2P sampling frequencies.

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PC6

Neuronal nitric oxide synthase is up-regulated by angiotensin II and attenuates NADPH oxidase activity and facilitates relaxation in murine left ventricular myocytes

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Angiotensin II (Ang II) is critical in myocardial pathogenesis, mostly via stimulation of NADPH oxidase (Ref 1, Ref 2). Neuronal nitric oxide synthase (nNOS) has recently been shown to play important roles in modulating myocardial oxidative stress and contractility (Ref 3, Ref 4). Here, we examine whether nNOS is regulated by Ang II and affects NADPH oxidase production of intracellular reactive oxygen species (ROSi) and contractile function in left ventricular (LV) myocytes.

Our results showed that Ang II-induced biphasic effects on ROSi and LV myocyte relaxation (TR50) without affecting the amplitude of sarcomere shortening and L-type Ca^{2+} current density: TR50 was prolonged at 30 min (P<0.001, n=141 with Ang II vs. n=201 in controls) but was shortened after 3hrs (P<0.001, n=106, or after Ang II in vivo treatment, P<0.0001, n=153 compare to that in sham). Correspondingly, ROSi was increased (by 2 fold, P<0.0001, n=14 with Ang II vs. controls, n=16), followed by a reduction to control level (P=0.1, n=33 with Ang II vs. n=35 in controls). Quantitative RT-PCR and immunoblotting experiments showed that Ang II (3hrs) increased the mRNA and protein expression of nNOS (P=0.0002, n=10 for mRNA and P=0.01, n=9 for protein) and increased NO production (nitrite assay, P<0.0001, n=8) in LV myocyte homogenates, suggesting that nNOS activity may be enhanced and involved in mediating the effects of Ang II. Indeed, n(omega)-nitro-L-arginine methyl ester (L-NAME) or a selective nNOS inhibitor, S-methyl-L-thiocitrulline (SMTC) increased NADPH oxidase production of superoxide/ROSi (P<0.0001 between Ang II+SMTC and SMTC only, n=22 and n=27, respectively) and abolished faster myocyte relaxation induced by Ang II (P=0.1 between SMTC and Ang II + SMTC, n=57 and n=90). The positive lusitropic effect of Ang II was not mediated by PKA-, CaMKII-dependent signaling or peroxynitrite. Conversely, inhibition of cGMP/PKG pathway abolished the Ang II-induced faster relaxation (P=0.9 between ODQ and ODQ+Ang II, n=43 & n=32; P=0.8 between KT 5823 & KT 5823+Ang II, n=46 & n=56) by reducing phospholamban (PLN) Ser16 phosphorylation PLN-p/PLN ratio: from P=0.003 between control and Ang II, n=18 to P=0.9 between KT 5823 and KT 5823+Ang II, n=8 or P=0.8 between ODQ and ODQ + Ang II, n=8). Taken together, these results clearly demonstrate that myocardial nNOS is up-regulated by Ang II and functions as an early adaptive mechanism to attenuate NADPH oxidase activity and facilitate myocardial relaxation. Rats (of 8 weeks old) were anesthetized with isoflurane (2.5 %). An osmotic minipump (Alzet model 2004) containing Ang II (200l, 6 mM, infusion rate 125 ng/min/kg) was implanted in the midscapular region under sterile condition. Sham-operated animals underwent the same surgical procedure, except for no pump insertion.

Varagic J, Frohlich ED. Local cardiac renin-angiotensin system: hypertension and cardiac failure. J Mol Cell Cardiol. 2002; 34(11):1435-42.

Nediani C, Raimondi L, Borchi E, Cerbai E. Nitric oxide/reactive oxygen species generation and nitroso/redox imbalance in heart failure: from molecular mechanisms to therapeutic implications. Antioxid Redox Signal 2011;14:289-331.

Sears CE, Bryant SM, Ashley EA, et al. Cardiac neuronal nitric oxide synthase isoform regulates myocardial contraction and calcium handling. Circ Res 2003;92:52e-59.

Zhang YH, Dingle L, Hall R, Casadei B. The role of nitric oxide and reactive oxygen species in the positive inotropic response to mechanical stretch in the mammalian myocardium. Biochim Biophys Acta 2009; 1787:811-7.

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PC7

Acid and alkali gating of Connexin-43 gap-junctional channels

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H⁺ ions, common end-products of metabolism, are potent modulators of cardiac function. H⁺ ions permeate at high flux through ventricular gap-junctions, providing one means for spatially equilibrating pH_i within the myocardium. Gap-junctional H⁺-permeability & electrical-conductance are pH-gated, being acutely reduced by intracellular acid or alkaline-overload[1], a feature that regulates spatial H⁺_i dispersion. We have now investigated the pH-sensitivity of connexin-43 gap-junctional channels, the dominant ventricular Cx-channel, stably transfected (rCx43, from rat) into HeLa and N2A cell-pairs, under conditions where surface membrane Na⁺/H⁺ exchange is inhibited with 30μM cariporide. Data are mean±SEM. Raising pH_i from 7.0 to ~7.5 in 180s (20mM trimethylamine superfusion, TMA; pH_i confocally imaged with SNARF-fluorophore) reversibly reduced cell-to-cell conductance (G_j; dual-cell voltage-clamp, -10mV hyperpolarising-step applied sequentially to each cell) by 53±7% in N2A pairs (n=5). Reducing pH_i to <6.7 in 180s (80mM acetate superfusion) again reduced G_j (by 42±3%, n=6). Wild type (WT) cell-pairs lacking Cx43 exhibited no significant cell-to-cell conductance. Single channel conductance measurements (open conductance in 2 mM Halothane), were 122±10 pS, n=250, similar to previous values at normal and low pH_i[2-3], suggesting that G_j-changes were caused by alterations in channel-gating kinetics. A pH-dependent G_j decrease was absent in N2A cell-pairs transfected with functional Cx43m275 mutant channel (Cx protein lacking much of original C-terminus, n=3), suggesting that the Cx cytoplasmic tail is necessary for both acid and alkali-gating. In other experiments, photolytic H⁺-uncaging from intracellular nitrobenzaldehyde (1mM)[4], within one cell of an rCx43 HeLa pair, promoted a pH_i-fall (confocally imaged) in both cells, indicating junctional H⁺ permeation (again absent in WT cells lacking Cx43). Cell-to-cell H⁺ flux was large (up to 7.5mM/min for a cell-to-cell H⁺ gradient of <80nM; n=15). In some experiments, resting pH_i was pre-adjusted (with TMA or acetate) before H⁺-uncaging. Apparent junctional H⁺-permeability estimated from the initial time course of H⁺-permeation, was reduced (by 88±12% and 93±5% n=4 & 6), when pH_i in the junctional region was either increased or decreased (to 7.38 and 6.86 pH_i respectively). Cell-to-cell H⁺ permeation was blocked by β-glycyl-L-histidine (60μM, selective Cx channel inhibitor; n=3), and was doubled (n=15) when 5%CO₂/22mM HCO₃⁻ (plus 200μM DIDS) instead of 20mM Hepes-buffered superfusates were used.

We conclude that Cx43 channels display characteristics of both high and low pH block, as previously identified for the ventricular gap junction. They are thus likely candidates for mediating spatial cell-to-cell pH_i control in the myocardium.

Swietach, P., et al., H⁺ ion activation and inactivation of the ventricular gap junction: a basis for spatial regulation of intracellular pH. *Circ Res*, 2007. 100(7): p. 1045-54.

Moreno, A.P., et al., Role of the carboxyl terminal of connexin43 in transjunctional fast voltage gating. *Circ Res*, 2002. 90(4): p. 450-7.

Bukauskas, F.F. and C. Peracchia, Two distinct gating mechanisms in gap junction channels: CO₂-sensitive and voltage-sensitive. *Biophys J*, 1997. 72(5): p. 2137-42.

Swietach, P., K.W. Spitzer, and R.D. Vaughan-Jones, pH-Dependence of extrinsic and intrinsic H⁺-ion mobility in the rat ventricular myocyte, investigated using flash photolysis of a caged-H⁺ compound. *Biophys J*, 2007. 92(2): p. 641-53.

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PC8

Role of cytoplasmic buffers in spatial H⁺-Ca²⁺ interactions in ventricular myocytes

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Cytoplasmic Ca²⁺ is modulated by H⁺-ions. We investigated the relationship between intracellular pH (pH_i) and diastolic or resting Ca²⁺ ([Ca²⁺]_{dia}) in confocally-imaged rat myocytes loaded with Fluo-3 (Ca²⁺) or cSNARF-1 (pH_i). Intracellular release of H⁺-ions from a membrane-permeant weak-acid (80mM acetate; pH_i decreases from 7.2 to 6.6) produced a 0.6±0.03μM (mean±SEM) rise in [Ca²⁺]_{dia} (N>50). Intracellular H⁺-release from a photo-labile caged H⁺-compound (2-nitrobenzaldehyde; 0.5-2mM) also produced a rise in [Ca²⁺]_{dia}. The rise in [Ca²⁺]_{dia} was restricted spatially to the acid-microdomain formed by H⁺-release (N>50). The resulting cytoplasmic Ca²⁺-gradient was maintained for the lifetime of the underlying pH_i-microdomain, but could be dissipated by AM-loaded BAPTA (100μM; a Ca²⁺-buffer; N=20). The spatial H⁺-Ca²⁺ interaction was not abolished by removal of extracellular Na⁺ or Ca²⁺, or by exposure to thapsigargin (10μM), caffeine (10mM), bafilomycin (5μM), glycyl-L-phenylalanine 2-naphthylamide (100μM) or ruthenium-360 (10μM) (N=10-50). However, inhibition of mitochondrial respiration with FCCP (1-5μM), rotenone (10μM), myxothiazole (10μM), or of glycolysis with deoxyglucose (2mM) (N=10-20), reduced the H⁺-evoked [Ca²⁺]_{dia}-rise in a manner that correlated with the decline of [ATP] measured by luciferase assay (pH_i-[Ca²⁺]_{dia} relationships shifted left by 0.3 pH_i units as [ATP]_i is halved). We conclude that the H⁺-evoked Ca²⁺-rise is not due to extracellular Ca²⁺-influx, or Ca²⁺-release from organelles. It most likely arises from competitive Ca²⁺/H⁺-binding to cytoplasmic buffers, some of which are diffusible and therefore engage in spatial Ca²⁺/H⁺ exchange, resulting in recruitment of Ca²⁺ to acidic microdomains. We therefore investigated the in vitro pH-sensitivity of Ca²⁺-binding to a range of known cytoplasmic buffers, using H⁺-uncaging in agar-set mixtures. We find that histidyl-dipeptides (e.g. carnosine) and ATP are the myocyte's principal pH-sensitive mobile Ca²⁺-buffers (physiological 7.5 mM ATP and 10 mM carnosine release 45±2μM and 30±3μM μM Ca²⁺ per pH unit decrease, respectively, N=9). Involvement of Ca²⁺-bound ATP confers metabolic sensitivity to the cytoplasmic H⁺-Ca²⁺ interaction. Results indicate that

cytoplasmic pH-microdomains, formed during membrane H⁺- or weak-acid transport will generate Ca²⁺-microdomains. These may help to compensate for the reactive effects of H⁺-ions on Ca²⁺-dependent protein function.

Vaughan-Jones RD et al. (2009) *J Mol Cell Cardiol* 46(3): 318-31

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PC9

Assessment of cardiac fibrosis in hypertrophic hearts using second harmonic generation

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Chronic pressure overload leads not only to hypertrophy but can also result in fibrosis of the myocardium, ultimately leading to diastolic dysfunction. Fibrosis results from increased cardiac fibroblast (CF) proliferation and collagen synthesis, both of which can be quantitatively assessed in normal and diseased myocardium. The excess collagen produced by CFs in fibrosis is a birefringent material. Using a suitable laser source, it is possible to generate a harmonic wavelength from collagen and to collect this signal as a contrast mechanism in order to study collagen density within a three-dimensional specimen. This approach may be superior and more selective than previously used methods, such as histological staining, as no disruptive chemical labels are required. Here, we optimise the use of second harmonic generation (SHG) microscopy for imaging collagen in left ventricular (LV) tissue sections from normal and hypertrophic hearts. A minimally invasive transverse aortic banding (MTAB) mouse model was used to induce LV hypertrophy. Mice (C57, 25-30g) were anaesthetised with 3% Isoflurane in oxygen, maintained with 1.5% Isoflurane in oxygen, and were given analgesic (60µg/kg Buprenorphine) intramuscularly. Resulting hypertrophy and contractile dysfunction were assessed by increased heart weight:body weight ratios (9.4±0.6 cf. 5.1±0.3, n=9, p<0.0001, MTAB vs sham) and decreased % fractional shortening (22.6±2.2 cf. 42.7±2.1, n=9, p<0.0001, MTAB vs sham). Importantly, a hyperproliferative phenotype was evident in CFs isolated from MTAB hearts in response to 1µM Angiotensin II stimulation (59.6±2.5 cf. 41.8±2.3 (mean cell count per field measured), n=3, p=0.006, MTAB vs sham). Collagen deposition was assessed in LV sections using a multi-photon laser scanning microscope, capturing both two-photon excited autofluorescence (TPEF) and SHG images simultaneously in two channels. The femtosecond-pulsed laser was tuned to 900nm for excitation of LV sections, and the two channels recorded emission in the wavelength range of 480-700nm and 450±2.5nm for TPEF and SHG signals, respectively. Collagen deposition was significantly increased in MTAB hearts (121.5±28.6 cf. 32.9±4.7 (relative intensity, AU), n=5, p=0.0157, MTAB vs sham). Verification of the suitability of this novel approach was confirmed using parallel traditional picrosirius red staining, which was markedly increased (6.31±1.2 cf. 1.45±0.2 (% area of staining in field measured), n=7, p=0.0014, MTAB vs sham). In comparison with traditional histological staining, SHG/TPEF appears a sensitive tool for collagen characterisation in heart tissue. This novel and non-destructive imaging method will allow more

accurate assessment of the extent of fibrosis occurring during disease progression. Crucially it will provide an invaluable screening tool for testing novel anti-fibrotic treatments.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC10

Calcium-independent shortening of resting sarcomere length in rat failing right ventricular myocytes

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Though there is intense interest in left ventricular (LV) heart failure, less is known about the etiology of diastolic dysfunction in right ventricular (RV) failure caused by pulmonary arterial hypertension. Diastolic dysfunction can be caused by increased fibrosis but also by dysregulation of myofilament interactions. In this study we are investigating the diastolic properties of myocytes from a rodent model of RV failure induced by pulmonary arterial hypertension.

Male Wistar rats (200g) were injected with monocrotaline (60mg/kg i.p.) to induce RV failure (FAIL, n=16) or a saline equivalent (CON, n=14). Within 4 weeks FAIL animals developed RV hypertrophy and symptoms of heart failure. Rats were humanely killed by stunning and cervical dislocation then single LV and RV myocytes were obtained by enzymatic dissociation. Sarcomere length was measured online by fast Fourier transform of the video image of cells using an inverted microscope. Experiments were carried out at 22 and 37°C in a physiological HEPES buffered solution. Data are presented as mean ± SEM.

Compared to CON, resting SL was significantly shorter in the RV of FAIL animals (FAIL, 1.77±0.01 µm; CON, 1.91±0.01 µm; p<0.001) but not in the LV (FAIL, 1.89±0.01 µm; CON, 1.89±0.01 µm) (n=18-22; 2 way ANOVA). There was no difference in resting [Ca²⁺] between CON and FAIL myocytes loaded with the Ca²⁺-indicator Fura-2AM (p>0.05, n=14-18). Myofilament Ca²⁺-sensitivity was estimated from the SL-Ca²⁺ relationship in intact myocytes loaded with Fura-2AM and exposed to the SERCA inhibitor thapsigargin (1µM, 15min) during relaxation from 10s field stimulation at 10Hz. Myofilament Ca²⁺ sensitivity (indexed by the slope of the SL-Ca²⁺ relationship) was significantly decreased in FAIL RV and LV cells compared to CON (RV, FAIL -1.20±0.09, CON -1.70±0.08 µm/ratio unit, p<0.001; LV, FAIL -0.98±0.10, CON -1.43±0.1 µm/ratio unit, p=0.002; n=16-26).

Incubating intact myocytes with the reactive oxygen species (ROS) scavenger n-acetylcysteine (20mM for at least 1h) did not lengthen SL in RV FAIL myocytes (p>0.05 vs. untreated FAIL, n=53-61). Chemically skinning myocytes by exposure to 0.01 mg/ml saponin did not lengthen SL in RV FAIL myocytes (p>0.05 vs. untreated FAIL, n=16-27).

Decreased resting SL is likely to contribute to diastolic dysfunction in failing hearts. We conclude that the shorter resting SL in failing RV myocytes is not caused by changes in either resting Ca²⁺ levels or myofilament Ca²⁺ sensitivity. Even though ROS levels are reported to be enhanced in MCT hearts (Redout et al, 2007) acute antioxidant treatment was ineffective and the lack of effect of skinning suggests alteration of the intracellular milieu is not responsible for the shortened SL. Mechanisms associated with local regulation of cross-bridges are under investigation.

Redout EM et al. (2007) Cardiovasc. Res. 75, 770-81

Supported by the MRC

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PC11

High speed acquisition of sarcomere length in Langendorff-perfused hearts using arbitrary plane 2-photon microscopy

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Introduction: Cardiac myocytes exhibit cross striations which are formed by alternating segments of thick (A bands) and thin (I bands) protein filaments. Quantitative imaging of the A-I band spacing (i.e., sarcomere length, SL) can provide important information about the relation of interfilament overlap and mechanical contractile performance. SL cannot be measured directly from confocal images as the orientation of cells in the tissue relative to the imaging plane is unknown (Bub et al., 2010). To overcome this limitation, we introduce a novel two-photon microscopy approach, where imaging planes with user defined angles can be scanned at high speed (Botcherby et al. 2008).

Methods: Hearts, isolated from Sprague-Dawley rats (~250g), killed by Schedule 1 in accordance to UK Home Office regulations, were swiftly mounted to a Langendorff system for coronary perfusion with normal tyrode, and loaded by coronary perfusion for 5 min with 5 μ M di-4-ANEPPS. Hearts were then perfused with zero calcium Tyrode (In mM: 140 NaCl, 5.4 KCl, 1.0 MgSO₄, 5.0 HEPES, 1.0 Glucose, pH 7.4, 37°C) to reduce contractile activity, placed on an organ-tailored cradle, stabilized using nylon mesh, and imaged.

The tissue orientation information can be obtained by imaging two perpendicular planes if their intersection line is predominantly along the primary (long) axis of the cell (Fig. 1). The cell axis in each of these planes can then be identified from a spatial frequency vector (K) evaluated in three dimensions. Two orthogonal sub-images can be captured within 500 msec allowing unambiguous determination of the angle of the surrounding tissue.

Results: Preliminary results demonstrate that cells oriented at a sharp angle relative to the confocal image plane display longer apparent SLs. By correcting for tissue angle, we can reduce measurement variance by as much as 15%.

Discussion: This method improves our ability to detect subtle changes in cell structure in diseased models where there is dysregulation in the excitation-contraction coupling mechanism (Li et al., 2012).

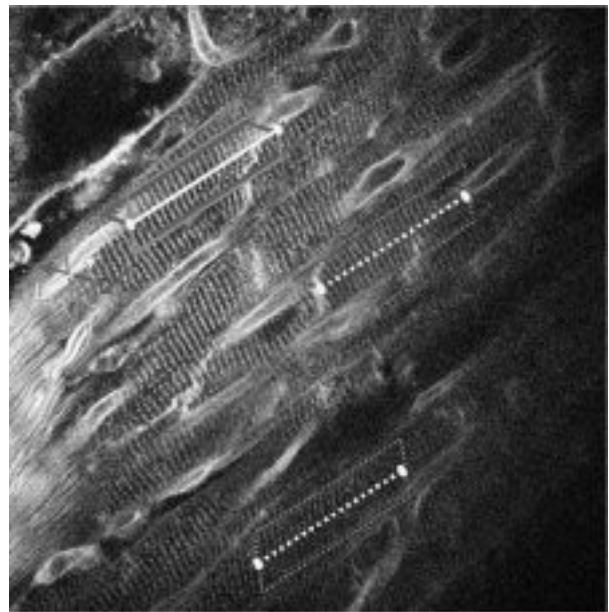


Figure 1: Capturing orientation information by imaging two perpendicular planes (Q-, Q+) along the cell axis for 3 cells in a field of view using a custom built arbitrary plane 2-photon microscope.

1. Bub G, et al. (2010). Am J Physiol 298, H1616-25.
2. Botcherby EJ, et al. (2008). Optics Commun 281, 880-7.
3. Li D, et al. (2012). Hypertension 59, 642-9.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC12

In the presence of β -stimulation, chronic myocardial infarction promotes atrial fibrillation and decreases atrial L-Type Ca^{2+} current

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In humans, heart failure (HF), as a result of ventricular myocardial infarction (MI), predisposes the heart to atrial fibrillation (AF). This is caused by the associated structural and functional remodeling of the atria although the precise mechanisms are unknown. Previous studies indicate reduced L-type Ca^{2+} current (I_{CaL}) in HF, in the presence of β -adrenergic (β -AR) stimulation (1). The aims of this study were to determine: 1) the effects of chronic MI in rabbits on AF threshold (AFT) in the presence of β -AR stimulation, and 2) the sensitivity of AFT to I_{CaL} blockade to mimic the lowered I_{CaL} seen in HF. **Methods:** MI was produced by ligation of the left descending coronary artery of adult male New Zealand White rabbits (~2.5kg). Animals were anaesthetised prior to thoracotomy (intravenous midazolam (0.2-0.5mg/kg, and ventilation with $\text{N}_2\text{O}/\text{O}_2/1\%$ halothane), and analgesia given post operatively (Vetergesic (0.04mg/kg)). Animals were sacrificed 8wks later with intravenous sodium pentobarbitone (100mg/kg). Sham operated animals were used as control (CON). Whole hearts were perfused in Langendorff mode with bicarbonate buffered Tyrode's

solution in the presence of isoproterenol (ISO 1 μ M). AFT was measured as the median of 5 AF induction attempts using a train of constant current pulses (100Hz for 1s, 5-100mA in 5mA increments). Single myocytes from the left atria were enzymatically isolated and superfused with a HEPES buffered Krebs-Henseleit solution in the presence of the ISO. In a subset of experiments I_{CaL} was blocked with nifedipine (NIF 2M). All experiments were carried out at 37°C. Data are expressed as mean $\pm$ SEM and compared using either unpaired t-test or repeated measures ANOVA, significant where $P < 0.05$. Results: In isolated hearts, HF resulted in a $\sim 60\%$ reduction in mean median AFT; from 50.8 ± 7.9 mA to 19.6 ± 4.4 mA ($n=17$ CON, 11HF, $P < 0.01$), indicating an increased susceptibility to AF. In isolated cells, HF resulted in a $\sim 35\%$ reduction in average I_{CaL} density compared to CON. A NIF dose-response curve for I_{CaL} was constructed, in the presence of ISO, and 2 μ M NIF found to reduce I_{CaL} in CON myocytes by a similar degree ($\sim 40\%$) to that resulting from HF. In whole hearts (CON), 2 μ M NIF reduced AFT by $\sim 80\%$, from 49.4 ± 9.2 mA to 10.0 ± 2.1 mA ($n=16$, $P < 0.001$). In the absence of β -AR stimulation, HF did not change AFT ($n=7$ CON, 7HF, $P > 0.05$) or I_{CaL} density ($n=13$ CON, 12HF, $P > 0.05$). Conclusion: These data suggest that there is an association between the reduction of atrial I_{CaL} by HF and the increased susceptibility to AF in HF, in the presence of β -AR stimulation. This study also highlights that, in HF-remodelled atria, Ca^{2+} channel blockade increases susceptibility to AF. This may have implications for the therapeutic use of Ca^{2+} channel blockers, since they are currently used for rate-control therapy in patients with AF.

Kettlewell, S., Smith, G.L., Workman, A.J. (2010) Left atrial cellular hypertrophy accompanying a left ventricular infarction is accompanied by altered intracellular Ca^{2+} handling in the presence of adrenergic stimulation. *Cardiovascular Research*, 87 (Suppl 1):S63.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC13

Histological characterisation and functional effects of ganglionic plexus activation in the rabbit heart

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Introduction

Discrete regions in the heart called ganglionic plexuses (GP), primarily parasympathetic in nature, represent a network of integration centres that constitute the hearts 'little brain' [1]. Recent data suggest that GP are involved in the pathogenesis arrhythmias such as atrial fibrillation but the precise mechanisms are not well understood [2]. Our aims were to 1) assess the presence of parasympathetic axons and putative ganglia and 2) investigate the functional role of the right atrial GP (RAGP) in controlling heart rate (HR).

Methods

The decentralised isolated innervated rabbit heart was used ($n=10$). After sedation (ketamine, 10mg/kg; medetomidine hydrochloride, 0.2mg/kg; butorphanol, 0.05mg/kg; im) anaesthesia was achieved with propofol (1%w/v ad libitum, iv) during which the innervated heart model was prepared [3]. Animals were killed with pentobarbitone (160mg/kg, iv) and the preparation was perfused via the aorta in constant flow.

Aim 1: 3 hearts were cut into regions, fixed and stained for acetylcholinesterase. A qualitative scoring system assessed axonal and putative ganglia levels.

Aim 2: (n=7) a) RAGP was stimulated using 0.1-0.4mg nicotine (0.1ml), b) in the presence of 0.1 μ M Atropine (AT) or 1.8 μ M Metoprolol (Met), c) The right vagus nerve was stimulated (VNS, 7H10V) before and after RAGP stimulation and d) VNS before and following coronary artery occlusion (CAO). Data are mean $\pm$ SEM, repeated measures ANOVA or Students t-test, * $P < 0.05$ vs control.

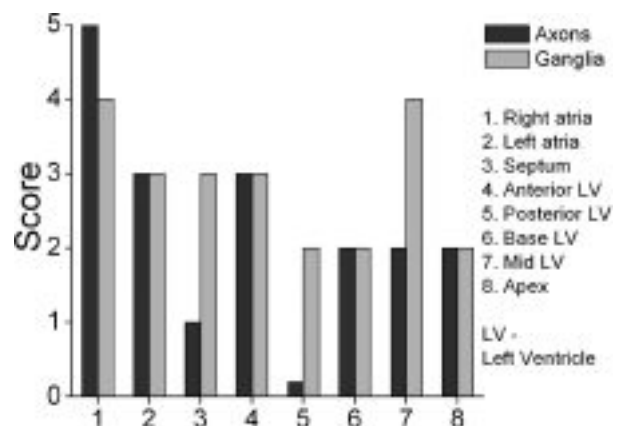
Results

Aim 1) See figure. Parasympathetic axons and putative ganglia were seen in all regions studied with the highest levels seen in the right atria. There was significant levels of axons and ganglia present in the left ventricle.

Aim 2) i) RAGP stimulation elicited an initial bradycardia (145 ± 9 to 109 ± 3 bpm*) and secondary tachycardia (226 ± 12 bpm*, $n=5$) ii) AT abolished the bradycardia (139 ± 8 to 138 ± 10 bpm), but not the tachycardia (247 ± 9 bpm*). Met did not affect the bradycardia (135 ± 7 to 106 ± 4 *) but abolished the tachycardia (128 ± 6 bpm, $N=5$). iii) VNS-bradycardia was augmented after RAGP activation (-81 ± 8 vs -142 ± 9 bpm*, $n=3$). iv) CAO increased VNS-bradycardia (-45 ± 6 vs -68 ± 8 bpm*, $n=4$).

Conclusion

These are the first data of the presence and functional effects of GP in the rabbit. Structurally, axonal and putative GP abundance differs throughout the heart being highest at the atria but also prominent in the LV. Functionally, RAGP activation elicits a mixed autonomic HR response, via vagal and sympathetic activation that enhanced VNS-bradycardia. In addition, novel data shows that acute ventricular ischemia augments the HR effect of cervical VNS confirming that the RAGP is important in controlling HR in this species.



Armour JA. *Exp Physiol* 2008;93(2):165-176.

Shen MJ et al. *Nat Rev* 2012;9:30-39.

Ng GA et al. *Exp Physiol* 2001;86(3):319-329.

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PC14

Marked remodelling of the sinoatrial node underlies bradycardia in rabbits with congestive heart failure

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Many heart failure patients die of bradyarrhythmias, presumably from dysfunction of the cardiac conduction system - insight into this has the potential to improve the outcome of patients affected. Here we studied the cardiac conduction system in rabbits with congestive heart failure (CHF). Male New Zealand White rabbits (2500-3000g) were anaesthetised with ketamine and isoflurane. Aortic incompetence was induced by inserting a catheter through the valve leaflets to cause LV volume overload. Then pressure overload was introduced by placing a silver clip (internal diameter 2.44 mm) just above right renal artery, 3 weeks later. The control group underwent sham procedures. The study was conducted in accordance with the Guide for the Care and Use for Laboratory Animals and approved by the local animal research ethics committee. By the end of 8 weeks, the rabbits showed significant LV hypertrophy, diminished LV fractional shortening and increased LV internal dimension. ECG recording after autonomic blockade showed that the intrinsic heart rate was slower and the RR interval was inversely related to fractional shortening ($R^2=0.680$; $P<0.05$), indicating dysfunction of the sinoatrial node in the CHF group. Additionally, we found significant increases in the PR interval (by 22%; $P=0.001$) and QRS duration (by 26%; $P=0.02$), which evidenced dysfunction of the atrioventricular node and His-Purkinje system.

Expression of ion channels (and related molecules studied) was studied at the mRNA and protein levels using quantitative PCR and immunohistochemistry. mRNA expression of 48%, 80% and 33% of 33 ion channels etc. studied was significantly decreased in the sinoatrial node (SAN), left Purkinje fibres and right Purkinje fibres, respectively. mRNA expression in the working myocardium, however, was less affected and only 21%, 3% and 6% of ion channels etc. changed in the LV muscle, right ventricular muscle and right atrial muscle. We observed marked reductions in the SAN in the expression of mRNA for funny channels (HCN1 and HCN4 by 36 and 87%), Ca^{2+} channels (Cav1.2 and Cav1.3 by 51% and 56%) and K^{+} channels (Kv1.5, Kir2.1, Kir2.2, ERG and KvLQT1 by 50-86%). Furthermore, the SAN had significantly less mRNA for HCN4 (45%), Ca^{2+} -handling proteins (NCX1 by 37% and RYR2 by 34%) and gap junction protein (Cx40 by 42%). At the protein level, HCN4 was reduced by 45%, NCX1 by 33%, RYR2 by 34% and Cx40 by 43%. Many of these changes could contribute to the slowing of the pacemaker activity of the SAN. In conclusion, our study shows that CHF causes widespread remodelling of ion channels in the cardiac conduction system and, in particular, the SAN, which may contribute to bradyarrhythmic death in heart failure patients.

Opthof, T. and R. Coronel (2000). The normal range and determinants of the intrinsic heart rate in man. *Cardiovasc Res* 45: 175-176.

Sanders, P., J. B. Morton, et al. (2004). Electrophysiological and electroanatomic characterization of the atria in sinus node disease: evidence of diffuse atrial remodelling. *Circulation* 109: 1514-1522.

Baartscheer, A., M. Hardziyenka, et al. (2008). Chronic inhibition of the Na^{+}/H^{+} -exchanger causes regression of hypertrophy, heart failure, and ionic and electrophysiological remodelling. *Br J Pharmacol* 154: 1266-1275.

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PC15

Measurement and interpretation of electrocardiographic QT intervals in murine hearts

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Alterations in ECG QT intervals correlate with the risk of potentially fatal arrhythmias, for which transgenic mouse hearts are becoming increasingly useful experimental models. However, QT intervals are poorly defined in murine ECGs owing to the short cardiac action potential (AP) waveforms. Previous studies have determined the QT interval as the times from the beginning of the QRS complex to (1) 95% recovery of the ECG deflection to baseline [1]; (2) the point at which recovery of the T wave deviates from the tangent to the steepest slope of its recovery [2]; (3) the return of the QT segment to the isoelectric baseline [3]; or (4) the point of convergence of T waves and their first derivative on a signal-averaged ECG recording [4] (Fig. 1). The present work develops a consistent measure of murine QT interval that correlates with changes in the duration of ventricular myocyte APs. All procedures were performed in accordance with the UK Animals (Scientific Procedures) Act (1986). Volume-conducted ECGs were compared with simultaneously-recorded APs obtained using floating intracellular microelectrodes in Langendorff-perfused mouse hearts. QT intervals were measured from the onset of the QRS complex. The interval, Q-APR90, measured to the time at 90% AP recovery was compared with two measures of QT interval. QT1 was measured to the recovery of the ECG trace to the isoelectric baseline for entirely positive T-waves, or to the trough of any negative T-wave undershoot. QT2, extensively used in previous studies, was measured to the return of any ECG trough to the isoelectric baseline. QT1, but not QT2, closely correlated with changes in Q-APR90 (Fig. 2). These findings were confirmed in *Scn5a*^{+/ΔKPQ} hearts used to model human long QT syndrome. Application of this method in whole, avertin anaesthetized mice (2.4 mg/10g body weight intraperitoneal) similarly demonstrated a prolonged corrected QT interval, QTc, in *Scn5a*^{+/ΔKPQ} hearts, and normal values in *RyR2*^{+/S} and *RyR2S/S* hearts containing the *RyR*-P2328S modification. Thus, we achieve a simple approach for the determination of QT and QTc intervals that correlates with the duration of ventricular myocyte action potentials in murine hearts.

Fig. 1

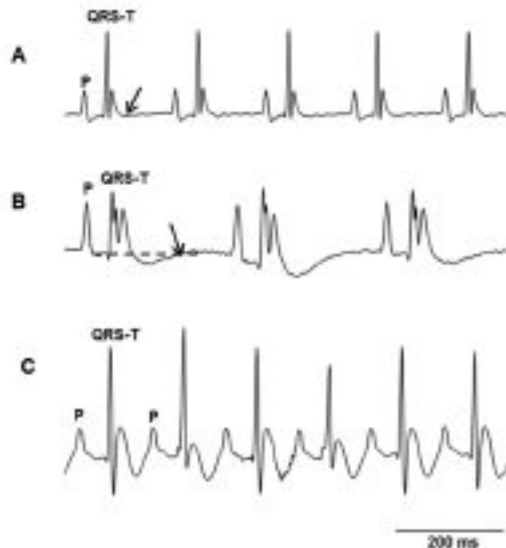


Fig. 1 ECG patterns from anaesthetised mice show marked variability.

In (A) the QRS-T complex ends with an upright T wave that then returns to the isoelectric baseline (arrow). In (B) the positive part of the T wave is followed by a negative undershoot before returning to the isoelectric baseline (arrow). In (C) the T waves show negative undershoot components not fully separable from the succeeding P waves.

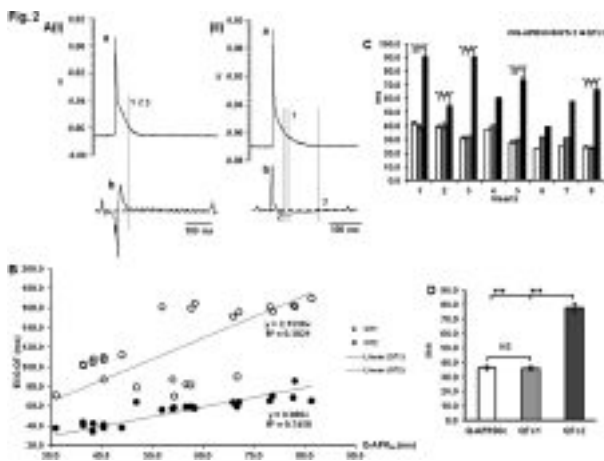


Fig. 2 Relationships between intracellular AP duration and volume ECG QT intervals in WT hearts.

Panels A(i) and (ii) show (a) APs and (b) the corresponding ECG recordings. Cursor 1 indicates the AP at 90% recovery (Q-APR90). Cursor 2 indicates a minimum value of a late negative undershoot (QT1). Cursor 3 indicates the point where the undershoot component regains the isoelectric baseline (QT2). (B) plots QT1 and QT2 intervals against Q-APR90 values observed and their respective regression lines. (C) summarizes Q-APR90c, QTc1 and QTc2 results from 8 individual hearts. (D) summarizes mean ($\pm$ SEM) Q-APR90c and QTc1 and QTc2 values.

London B et al. (2001). *Circ Res* 88, 940-946.

Lande G. et al. (2001). *Cardiovasc Res* 50, 328-334.

Le Bouter, S et al. (2003). *Circ Res* 92, 73-79.

Saito T. (2009). *Circ Res*, 2009. 105:343-352.

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PC16

Low Na⁺ solution increases gap junction resistivity and connexin 43 phosphorylation at serine 368 in guinea pig atrial myocardium: a role for calcineurin

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Cardiac arrhythmias are significantly initiated by abnormal myocardial conduction, in part caused by raised intracellular resistivity, R_i . In turn, R_i is a function of sarcoplasmic resistivity, R_c , and gap junction (GJ) resistivity, R_j . GJs are composed of connexin (Cx) proteins and in many cardiac arrhythmias, such as atrial fibrillation, changes in GJ electrical properties can be linked to alteration in the phosphorylation state of Cx and/or raised $[Ca^{2+}]_i$. A key intracellular signalling pathway affected by raised $[Ca^{2+}]_i$ is the activation of Ca^{2+} /calmodulin dependent, serine/threonine phosphatase; calcineurin (CaN). The objectives of this study were to: 1) determine if raised $[Ca^{2+}]_i$ in atrial myocardium altered R_j and if this was reversible with the CaN inhibitor cyclosporin A (CsA); and 2) measure the effect of increased $[Ca^{2+}]_i$ on the phosphorylation state of Cx protein, Cx43.

Male, Dunkin-Hartley guinea-pigs were sacrificed, hearts removed and transferred to Tyrode's solution (total $[Na^+] = 147.4$ mM; 24 mM $NaHCO_3$, 5% CO_2 , 37°C, pH 7.4). Using an oil-gap technique, tissue impedance (Z) was measured to derive R_j and R_c from Z values over a range of frequencies (0.02-100 kHz). These values were calculated from atrial appendage strips bathed in control and low Na^+ Tyrode's solutions (29.4 mM Na^+ , NaCl replaced by TRIS HCl, pH 7.4; used to increase $[Ca^{2+}]_i$ in the presence and absence of 5 μ M CsA. The protein expression of total Cx43 (T-Cx43) and (de)phosphorylation of the serine 368 residue of Cx43 (dephos-S368 and pS368) of tissue homogenates were determined using western blotting. Dephos-S368 and pS368 were normalised to corresponding T-Cx43. Data are means $\pm$ SEM, compared by Mann Whitney or ANOVA, the null hypothesis rejected at $p < 0.05$.

R_j was significantly increased by low Na^+ ($383 \pm 40 \Omega \cdot cm$) when compared to control ($209 \pm 26 \Omega \cdot cm$; $n=6$; $p < 0.0001$). This effect was reversed in the presence of 5 μ M CsA ($250 \pm 27 \Omega \cdot cm$; $n=6$; $p < 0.0001$), which alone had no effect ($181 \pm 17 \Omega \cdot cm$). R_c did not change significantly during any intervention. Western blots did not show any difference in T-Cx43 protein expression between control and low Na^+ . However, there was a significant increase in the normalised pS368 in low Na^+ tissue compared to control tissue (pS368; Control, 59.6 ± 25.2 , low Na^+ 115.1 ± 17.1 ; $n=3$; $p < 0.05$).

Raised $[Ca^{2+}]_i$ in atrial myocardium is associated with an increased R_j , as well as greater phosphorylation of the S368 residue of Cx43; the increase of R_j was ameliorated by the CaN inhibitor CsA. Phosphorylation of S368 of Cx43 is associated with a lower conductance state of gap junctions. We propose that CaN increases GJ resistivity by enhancing the phosphorylation of the S368 residue of Cx43 and decreasing GJ conductance.

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PC17

Effects of heart failure on phosphodiesterase expression in the sheep ventricle

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Heart failure (HF) is a major cause of premature mortality and increased morbidity. One of the hallmarks of HF is a decrease in responsiveness to beta-adrenergic (β -AR) stimulation. β -AR dysfunction is characterised by alterations to key components of excitation contraction coupling (ECC), such as reduced L-type calcium current and attenuated rises in calcium transient amplitude. As HF patients have elevated levels of circulating catecholamines, it is possible that changes to β -AR signalling are caused by desensitisation of the β -adrenoreceptors. The β -AR signalling pathway influences ECC through phosphorylation by downstream effector protein kinases (PKA and PKG), which are activated by cyclic nucleotide second messengers (cAMP and cGMP). Therefore, the bioavailability of these second messengers directly influences the efficacy of β -AR signalling. A key group of enzymes, phosphodiesterases (PDE), hydrolyse cyclic nucleotides and strictly control their bioavailability in the cytosol. Thus, the current study sought to elucidate whether changes in PDE expression in HF contributes to the observed β -AR dysfunction.

We induced heart failure in sheep using tachypacing. Animals were anaesthetised for pacemaker implantation (isoflurane, 1-3% in oxygen) and perioperative analgesia provided (meloxicam, 0.5 mg/kg). After 7 days recovery, RVP (210-220 bpm) was applied until clinical symptoms of HF were evident. Animals were sacrificed by I.V. injection of pentobarbitone (200mg/kg) and left ventricular tissue homogenised for analysis of PDE expression using Western blotting. PDE isoforms blotted for included the cAMP-hydrolysing PDE isoforms: PDE3A, PDE4B and PDE4D; and the cGMP-hydrolysing isoform: PDE5A. PDE expression was quantified by measurement of band intensity normalised to an internal control and data expressed as an average of three repeats.

Protein expression analysis revealed that the cGMP-inhibited, cAMP-hydrolysing PDE3A was significantly reduced in HF (58% decrease control (n=7) vs failure (n=6), $P=0.005$). A reduced expression in failing tissue was also observed for the two isoforms of PDE4 (PDE4B, 45% decrease control (n=7) vs failure (n=6), $P=0.05$; PDE4D, 36% decrease control (n=7) vs failure (n=6), $P=0.001$). Furthermore, the cGMP-hydrolysing PDE5A showed a tendency for reduction in HF ventricular tissue (24% decrease control (n=7) vs failure (n=6), $P=0.08$). Statistical significance compared by student's t-test.

This study shows that in a large animal model of HF in which β -AR signalling is disrupted, PDEs are down regulated. This suggests a reduced control over cyclic nucleotide bioavailability in failing cardiac myocytes. In turn this may reveal a mechanism, which may contribute to the changes in calcium homeostasis observed in failing cardiac myocytes.

Experiments were carried out in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986.

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PC18

Measurement of intracellular sodium following a change of pacing frequency in sheep ventricular myocytes

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Changes in action potential duration (APD) occur following a change in heart rate. The time course of these changes has two distinct initial phases; i) a rapid component occurring immediately upon a change of rate and, ii) a slower component occurring over a period of tens to hundreds of seconds. We have previously reported a hysteresis between the change of intracellular calcium ($[Ca^{2+}]_i$) and APD¹, which suggests that the slow change of APD is not dependent on $[Ca^{2+}]_i$. Our aim here therefore, was to determine if the slow change in APD would correlate with changes in intracellular sodium ($[Na^+]_i$). Sheep were killed in accordance with The Home Office Animal (Scientific Procedures) Act 1986 for enzymatic isolation of left ventricular mid myocardial myocytes. Myocytes loaded with the sodium indicator SBFI were current clamped via perforated patch and paced at 0.25 Hz, 1 Hz and 0.25 Hz sequentially, to steady state APD and SBFI fluorescence values.

On increasing pacing frequency from 0.25 to 1 Hz the rate constant of APD₉₀ change was $0.062 \pm 0.024 \text{ s}^{-1}$ whilst the rate constant of change of APD₉₀ on returning to 0.25 Hz was $0.032 \pm 0.011 \text{ s}^{-1}$. These values agree with those we reported previously¹. However, these changes in pacing frequency did not result in any consistent change in SBFI ratio. Furthermore, a more abrupt frequency change from 0.25 to 4 Hz also failed to produce a detectable change of SBFI ratio. To demonstrate that we were able to detect changes in $[Na^+]_i$ we adopted two approaches; i) in un-stimulated cells application of ouabain (1-5 mM) and nickel (5 mM) was used to block both the sodium-potassium pump and sodium-calcium exchanger and, ii) we stimulated cells in the presence of ouabain. Both approaches evoked a detectable increase of SBFI ratio.

From these data, and those observed previously¹, we suggest that the change in APD upon change of pacing frequency is neither dependent on a change in $[Ca^{2+}]_i$ or a change of bulk $[Na^+]_i$.

Greensmith DJ, Trafford AW, Dibb KM and Eisner DA (2011). *Proceedings of the Physiological Society* 23, PC149.

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PC19

Scavenging RCS is more efficacious than scavenging ROS

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Oxidative stress is an underlying cause of cardiovascular complications including heart failure in individuals with diabetes mellitus. Two classes of oxidants are upregulated during diabetes, reactive oxygen species (ROS) and reactive carbonyl species (RCS). ROS-scavenging based therapies only minimally improved cardiovascular outcomes in clinical studies.

This study uses a rat model of type 1 diabetes (streptozotocin 45 mg/kg i.p. in citrate buffer pH 4.5) in conjunction with echocardiography, high speed video edge detection, confocal microscopy, Western blot, [3H]ryanodine binding, Ca²⁺ uptake assays and treatments with Tempol (TP, a superoxide dismutase mimetic), pyridoxamine (Py, a scavenger of RCS) and aminoguanidine (Ag, a mixed ROS/RCS scavenger) to test the hypothesis that RCS is the primary cause for heart failure development during diabetes. All data are given at mean $\pm$ S.E.M. Eight weeks of diabetes reduced cardiac and myocyte fractional shortening by $16.1 \pm 1.2\%$ and $19.3 \pm 1.1\%$. Rates of evoked Ca²⁺ release and reuptake from myocyte sarcoplasmic reticulum (SR) were also slowed by $36.3 \pm 6.4\%$ and $176.1 \pm 8.2\%$. Expression of ryanodine receptors (RyR2) and sarcoplasmic reticulum Ca²⁺-ATPase (SERCA2) did not change, but their activities were $40.3 \pm 6.1\%$ and $38.8 \pm 5.4\%$, respectively. Six weeks of treatment with TP starting two weeks after the onset of diabetes, did not blunt cardiac and myocyte fractional shortening ($18.1 \pm 2.0\%$ and $16.5 \pm 2.2\%$), slowing in SR Ca²⁺ release ($27.8 \pm 5.1\%$), RyR2 activity loss ($30.2 \pm 4.4\%$) and SERCA2 activity loss ($34.5 \pm 2.4\%$). However, it blunted the slowing SR Ca²⁺ reuptake rate ($75.6 \pm 7.2\%$, $P < 0.05$). Interestingly, Py and Ag treatments blunted reductions in cardiac and myocyte fractional shortening ($3.6 \pm 1.1\%$ and $9.7 \pm 0.6\%$, and $4.2 \pm 0.8\%$, $8.9 \pm 1.2\%$, $P < 0.05$), the slowing in rate of Ca²⁺ release from the SR ($13.2 \pm 2.3\% \pm 15.1 \pm 1.8\%$, $P < 0.05$), and SR Ca²⁺ uptake rate ($100.5 \pm 7.2\%$ and $90.2 \pm 9.1\%$, $P < 0.05$). Tp, Py and Ag treatments did not lower blood glucose and steady state levels of RyR2 and SERCA2 proteins. TP was more efficacious than Py and Ag in reducing serum MDA levels (an index of ROS), but Py and Ag were more efficacious in reducing activity of serum semicarbazide amine oxidase (an index of RCS). Using membrane vesicle preparations, Py, Ag, and Tp were also able to reduce carbonyl adducts on select proteins. From these data we conclude elevation in RCS rather than ROS is the leading cause for heart failure development during diabetes.

Shao CH et al.(2011). Diabetes 60(3):947-59.

Shao CH et al. (2010). Biochemical Pharmacology 80(2):205-17.

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PC20

Short dosing regimens of Amiodarone are more protective against arrhythmia than chronic doses in a computational model of heart failure

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Amiodarone (AMI) is a potent anti-arrhythmic agent and is of therapeutic benefit in acute doses in the incidence of ventricular fibrillation or tachycardia. It is also used in chronic doses in patients with heart failure (HF) as arrhythmia is recognised as a leading cause of sudden death in this patient group (Doval 1994). The doses of amiodarone given in each instance differ and are shown to affect different ionic currents (Kamiya et al 2001).

Several substrates for arrhythmia exist, both at the cellular level where initiation (triggered activity) occurs and at a tissue level where this activity can be propagated. We examined the effects of HF and AMI on these arrhythmia substrates using computational models of ventricular cells and tissue. A modified version of the Ten Tusscher Human Virtual Ventricle (Ten Tusscher & Panfilov 2006) was created for single cell models. Propagation of arrhythmia was examined in a 1D heterogeneous tissue strand model (Benson et al 2007).

Results from the single cell models confirm HF to increase arrhythmia susceptibility; action potential duration (APD) is increased (from 308 ms to 323 ms) alongside a significant decrease in the calcium transient amplitude (factor 2.4) and an increase in its decay time (by 205 ms). Despite this, only endocardial cells in HF showed APD alternans in our model, with a smaller APD difference (68 ms control vs 14 ms HF).

Simulation of acute AMI suppressed APD alternans in control and HF epicardial cell models, decreased delayed afterdepolarisation (DAD) amplitude and increased the threshold for triggered activity (TA) propagation (173% control, 128% HF). In contrast, simulation of chronic AMI increased DAD magnitude and decreased TA threshold (by 4 ms in control, 6 ms in HF). Doses of chronic AMI are therefore more arrhythmogenic at the cellular level.

It was shown that chronic AMI can prevent propagation; it decreased the conduction velocity (CV) restitution curve slope in HF (0.0024 vs 0.002) and transmural dispersion of repolarisation (TDR) along the strand modelled (by 9.7ms control, 3.5ms HF). Both provide an explanation for clinical observations of the anti-arrhythmic benefits of chronic AMI (Sign 2007). Acute AMI afforded a larger decrease in TDR (5 ms HF vs 3.5 ms control) and in the CV restitution curve slope. Again this shows acute AMI to be of superior benefit in reducing likelihood of arrhythmia propagation.

Few studies have examined the contrasting effects of dosing regimens on substrates for arrhythmia. These results, showing that acute AMI suppresses cellular initiation and reduces propagation at the tissue level, suggest acute AMI may be more beneficial than chronic doses in HF patients; decreased exposure may improve tolerability.

Benson AP, Halley G, Li P, Tong WC and Holden AV. (2007) Virtual cell and tissue dynamics of ectopic activation of the ventricles. Chaos 17, 015105

Doval HC et al (1994). Randomised trial of low-dose amiodarone in severe congestive heart failure. The Lancet. 344(8921); 493 - 498

Kamiya K, Nishiyama A, Yasui K, Hojo M, Sanguinetti MC, Kodama I (2001)

Short- and Long-Term Effects of Amiodarone on the Two Components of Cardiac Delayed Rectifier K⁺ Current. Circulation 103: 1317 - 1324
SIGN (2007). Management of Chronic Heart Failure. Retrieved February 2011 from: <http://www.sign.ac.uk/pdf/sign95.pdf>

Ten Tusscher KHWJ & Panfilov AV (2006) Alternans and spiral breakup in a human

ventricular tissue model. American Journal of Physiology - Heart and Circulatory

Physiology 291, H1088-H1100

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PC21

Effects of chronic myocardial infarction on atrial electrophysiology in intact hearts, examined using a voltage sensitive dye

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Myocardial infarction (MI) is associated with an increased risk of atrial fibrillation (AF). We have shown that MI in rabbits decreases atrial L-type Ca^{2+} current (I_{CaL}) in the presence of β -adrenergic (β -AR) stimulation (1). However, how MI or I_{CaL} reduction may affect atrial action potentials (AP) in the intact heart is unclear. This study aims to examine effects of MI and pharmacological inhibition of I_{CaL} on atrial electrophysiology in intact rabbit hearts using a voltage sensitive fluorescent dye excited by a light guide system. **Methods:** MI was induced in New Zealand White rabbits (~2.5 kg, n=6): rabbits were anaesthetised prior to thoracotomy (intravenous midazolam (0.2-0.5mg/kg), and ventilated with N2O/O2/1% isoflurane) with post operative analgesia given (Vetergesic (0.04mg/kg)). After 8 weeks, rabbits were sacrificed by intravenous administration of sodium pentobarbitone (100 mg/kg). Stock animals served as controls (CON, n=10). Hearts were removed and perfused in Langerdorff-mode with Tyrode's solution in the presence of the mechanical uncoupler blebbistatin (10 μM). Hearts were loaded with the voltage-sensitive dye Di-4-ANEPPS. APs were recorded in the absence and presence of the β -AR agonist isoproterenol (ISO, 1 μM), or with ISO plus the Ca^{2+} channel blocker nifedipine (NIF, 0.02 and 2 μM). Hearts were either unpaced, or paced (5Hz) with bipolar electrodes. Data are expressed as mean $\pm$ SEM, and compared by ANOVA or unpaired t-tests. $P<0.05$ was regarded as significant. **Results:** In unpaced hearts (mean rate=2.8Hz), MI increased atrial AP duration at 50% repolarisation (APD_{50}), by 40% (66 ± 4 vs 47 ± 4 ms, $P<0.01$), and APD_{90} by 41% (117 ± 7 vs 83 ± 5 ms, $P<0.01$), respectively, vs CON. In paced hearts, MI increased APD_{50} , by 22% (67 ± 6 vs 55 ± 3 ms, $P=0.07$), and APD_{90} by 23% (114 ± 3 vs 93 ± 5 ms, $P<0.01$), respectively, vs CON. In unpaced CON hearts, ISO also significantly increased APD_{90} , by 22% (101 ± 4 vs 83 ± 5 ms, $P<0.05$ (mean rate=4.3Hz)), but ISO had no significant effect on APD_{90} in MI hearts (mean rate=-ISO 2.7Hz, +ISO 4.2Hz). In paced hearts, ISO had no significant effect on APD_{50} or APD_{90} in either CON or MI hearts. In the presence of ISO, APD_{50} or APD_{90} were not significantly altered by MI in either unpaced or paced hearts. To mimic the reduction in atrial I_{CaL} by MI, in the presence of β -AR stimulation, i.e. 30-40% (1), CON hearts were perfused with NIF at 0.02 μM and 2 μM . NIF significantly decreased APD_{50} and APD_{90} (0.02 μM : ~30%; 2 μM : ~50% in unpaced and paced hearts, $P<0.01$), in CON but not MI. **Conclusion:** Chronic MI in rabbits increases atrial APD in the absence, but not in the presence of β -adrenergic stimulation. Our data indicate that the atrial I_{CaL} reduction seen in HF is not associated with altered APD.

(1) Kettlewell, S., Smith, G.L., Workman, A.J. (2010) Left atrial cellular hypertrophy accompanying a left ventricular infarction is accompanied by altered intracellular Ca^{2+} handling in the presence of adrenergic stimulation. *Cardiovascular Research*, 87 (Suppl 1):S63.

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PC22

The effects of a non-obesogenic high-fat diet on mitochondrial protein expression

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An unhealthy "Western Style" high-fat diet, lack of exercise and smoking all contribute to increase cardiovascular diseases. This type of high-fat diet can cause cardiomyopathy directly by altering cardiac metabolism or indirectly due to other factors (e.g. obesity). In addition to cardiomyopathies, "Western Style" high-fat diet predisposes hearts to the damaging effects of cardiac insults. We have recently shown that high-fat diet alters mitochondria morphology, consistent with increased fission. Whether such a change is due to changes in mitochondrial proteins associated with fission/fusion is not known. The aim of this study was to determine whether a chronic "Western Style" high-fat diet, without obesity and associated co-morbidities, alters mitochondrial protein expression. Male C57/BL 6 mice aged 6 weeks were fed either normal chow diet (13% calories from fat) or a high-fat diet (45% calories from fat) for 20 weeks. In this model high-fat diet hearts and cardiomyocytes are more vulnerable to ischemia-reperfusion injury. The mice do not show signs of obesity, diabetes or hypertrophy. Hearts from high-fat diet and control were used to detect the relative expression of mitofusin-1 (Mfn-1), mitofusin-2 (Mfn-2) and FIS1 using western blotting. Mfn-1 and Mfn-2 are thought to control mitochondrial fusion and FIS1 is a regulator of mitochondrial fission. Data are presented as mean $\pm$ SEM and were analysed using students t-test. A P-value of <0.05 was assumed to be significant. n = 6 hearts/group. In the high-fat diet group there was no change in the protein expression of Mfn-1 when compared to the normal diet. However, there was an increase in Mfn-2 (0.929 ± 0.046 vs. 0.653 ± 0.025 relative protein expression, $P<0.001$) in the high-fat group compared to the normal diet group. There was a reduction in FIS1 protein expression in the high-fat group versus the normal diet (0.684 ± 0.039 vs. 0.812 ± 0.033 relative protein expression, $P<0.05$). These data suggest that in the high-fat diet group there is an up regulation of mitochondrial fusion and a decrease in mitochondrial fission. This information does not correlate with our mitochondrial morphology measurements where we see a decrease in mitochondrial area ($P<0.01$) and length (fewer mitochondria $>2.1\mu\text{m}$ ($P<0.05$)) in the high-fat group, suggesting more fission. Interestingly, recent work using an Mfn-2 knockout mouse model (1) also reported longer and larger mitochondria as well as increased resistance of cardiomyocytes to insults when Mfn-2 was lower. It is evident therefore that alterations in mitochondrial proteins expression are not simple indicators of fission and fusion. Our work indicates that the effects of high-fat diet on mitochondrial morphology and the associated reduced resistance of cardiomyocytes to cardiac insults could be largely due to an increase in Mfn-2.

Papanicolaou, K. N.; Khairallah, R. J.; Ngoh, G. A.; Chikando, A.; Lupat, I.; O'Shea, K. M.; Riley, D. D.; Lugus, J. J.; Colucci, W. S.; Lederer, W. J.; Stanley, W. C.; Walsh, K., Mitofusin-2 maintains mitochondrial structure and contributes to stress-induced permeability transition in cardiac myocytes. *Molecular and cellular biology* 2011, 31 (6), 1309-28.

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PC23

Differential changes in pro and anti apoptotic protein expression in mice fed high-fat diet

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Apolipoprotein E knockout (apoE^{-/-}) mice fed a high-fat, Western-style diet for 6 months, are grossly hypercholesterolaemic and develop atherosclerotic lesions (1). In contrast to their male counterparts, female apoE^{-/-} mice on high fat diet do not show evidence of occlusive coronary lesions or myocardial infarction (2). The aim of this work was to investigate whether high-fat diet is associated with increased apoptotic signalling (BCL2 and BAX protein expression) and whether gender is important. Female and male apoE^{-/-} mice at 8 weeks old were either switched onto high fat, Western-type diet (21% fat; 0.15% cholesterol) or were maintained on normal rodent diet for approximately 6 months. Proteins were extracted from myocardial tissue collected from male (n=6) and female (n=6) mice. Half of the mice were fed high-fat diet. Western blotting was used to estimate protein expression and data expressed as ratio to GAPDH (Chemicon Int.). Antibodies for BCL2 and BAX were purchased (Cell Signalling Technology) and used according to the suppliers instructions. Image J was used to estimate band intensity. Data are presented as mean±SE and differences determined using unpaired t-test (n=3 for each group). There was no difference in the ratio of BAX expression between male and female heart on normal diet (0.73±0.07 vs. 0.57±0.01). High-fat diet significantly decreased BAX expression in male heart (0.74±0.07 vs. 0.35±0.03, p<0.05) but causing an increase in female heart (0.57±0.01 to 0.99±0.08, p<0.05). BCL2 expression was higher in male compared to female heart on normal diet (0.84±0.13 vs. 0.23±0.14, p<0.05). High-fat diet decreased BCL2 expression in male heart (0.84±0.13 vs. 0.08±0.03, p<0.05) but increased the expression in female heart (0.23±0.14 to 0.89±0.09, p<0.05). This work indicates that high-fat diet and gender alter apoptotic signalling in hearts of apoE^{-/-} mice. The changes appear to be associated with the incidence of coronary disease.

Arterioscler Thromb Vasc Biol, 2002; 22(5): 788-92

Crit Care Med, 2007; 35(10): 2344-51

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PC24

Spontaneous calcium release from the sarcoplasmic reticulum mediated by the low affinity β 1-adrenoceptor in rat atrial myocytes does not occur via inositol 1, 4, 5-triphosphate receptors

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Some β -adrenoceptor blockers, such as pindolol, cause cardiostimulation at high concentrations. Freestone *et al* (1999) have shown that CGP12177, a structurally similar molecule to pindolol, is 40 times more potent than isoprenaline (ISO) in causing arrhythmias in mouse ventricular myocytes. Pro-arrhythmic effect of CGP12177 occurs despite that it increases intracellular calcium levels by 30% of that caused by ISO. Furthermore, in ferret ventricle it has been shown that CGP12177 causes an increase in the plateau phase of the action potential whilst shortening the overall action potential duration more potently than noradrenaline acting on conventional β 1-adrenoceptors (Lowe *et al*, 1998). This led to designation of a new receptor – the low affinity β 1-adrenoceptor (β 1L). The mechanism for arrhythmogenic effects mediated by this receptor is currently unknown. Rat atrial myocyte arrhythmias have previously been observed with endothelin due to calcium release via inositol 1, 4, 5-triphosphate receptors (IP3R) located on the SR (Li *et al*, 2005). In this study we used CGP12177 and a blocker of IP3R-mediated calcium release, 2-APB, in quiescent rat atrial cells to investigate effect of CGP12177 on intracellular calcium release. Atrial cells from WKY rats were loaded with Fluo 4-AM (5 μ M). Images of calcium events within quiescent cells were obtained using LSM510 Meta confocal microscope every 3ms. Cells were perfused with 2-APB (5 μ M) in the presence of propranolol (200nM) followed by 2-APB and propranolol in the presence of CGP12177 (1 μ M). 2-APB and propranolol perfusion caused 0.4 ± 0.2 large but spatially restricted calcium release events (wavelets) per second and 30.4 ± 4.3 calcium sparks per second (n=7 cells from 3 animals). CGP12177 with 2-APB and propranolol increased the incidence of wavelets to 1.9 ± 0.4 wavelets per second (p < 0.005) but did not alter frequency of calcium sparks. CGP12177 in the presence of propranolol increased the incidence of wavelets to 0.86 ± 0.17 wavelets per second compared to 0.4 ± 0.1 wavelets per second (p < 0.01) in the presence of propranolol alone (n = 12 cells, 6 animals). In cells which did not exhibit calcium waves or wavelets, CGP12177 (with propranolol) increased the incidence (p < 0.01) of calcium sparks from 42 ± 5.3 to 62 ± 6.1 per second (n = 6 cells from 6 animals). As shown previously for mouse ventricular myocytes, CGP12177 is associated with potent arrhythmogenic effects in rat atrial cells. This effect is not affected by addition of 2-APB which has been suggested to be effective in controlling spontaneous calcium release which might contribute to calcium-mediated atrial arrhythmias (Zima and Blatter, 2004). It can be concluded that arrhythmic events mediated by β 1L are not the result of calcium release via IP3R.

Freestone NS *et al*. (1999). *Naunyn-Schmiedeberg's Arch Pharmacol*. **360**, 445-456.

Lowe MD *et al*. (1998). *Br.J.Pharmacol*. **124**, 1341-1344.

Li X *et al*. (2005). *Circ.Res*. **96**, 1274-1281.

Freestone NS *et al*. (2000). *Pflugers Arch*. **441**, 78-87.

Zima AV & Blatter LA. (2004). *J.Physiol*. **555**, 607-615.

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PC25

Gender differences and effects of long- and short-term estrogen treatment on the outcome of pacing postconditioning protection to the heart

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Motivation: We previously showed a protective role for the intermittent dyssynchrony (pacing postconditioning (PPC)), induced by ventricular pacing immediately at the beginning of reperfusion. The aim of this study was to determine possible gender differences and the effects of estrogen (E2) on ischemia reperfusion injury (I/R) and the outcome of PPC.

Methods: Hearts isolated from Wistar rats anesthetized with intraperitoneal injection of sodium pentobarbital (60 mg/kg) and anticoagulated with heparin (1000U/Kg body weight) through the femoral vein were used for this study. The hearts (n=8 per group) were cannulated to a modified Langendorff system. Controls were subjected to 30 minutes unprotected ischemia. PPC protocol was 3 cycles of 30 seconds left ventricular (LV) pacing alternated with 30 seconds right atrial (RA) pacing at the beginning of reperfusion. To study the short-term effect of E2, the hearts were perfused with 0.7 ng/ml E2 for 15 minutes at the beginning of reperfusion. For E2 long-term treatment female rats were anesthetized with intraperitoneal injection of sodium pentobarbital (60 mg/kg), ovariectomized and substituted with 60 days E2 or placebo releasing pellets. Hearts isolated from these animals were subjected to ischemia and PPC. All groups were allowed a period of 30 minutes reperfusion. Hemodynamics were computed by a data acquisition program. Infarct size was determined by triphenyltetrazolium chloride (TTC) staining.

Results: Unprotected I/R resulted in poor recovery of hemodynamics (left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (Pmax), Maximal derivative of left ventricular pressure (dP/dtmax), coronary flow (CF), and coronary vascular resistance (CVR)) compared to baseline data ($p < 0.02$). PPC significantly decrease ($P < 0.05$) the infarct size and improved hemodynamics in the male and female hearts compared to controls. Both short- and long-term E2 treatments did not protect the heart against I/R injury. However, PPC did not show any protection in hearts treated with E2 for 6 weeks. Significant ($P < 0.003$) decrease in infarct size and improvement in hemodynamics were seen in 6 weeks placebo treated hearts compared to E2 treated hearts and controls.

Conclusions: There were no gender differences in PPC protection. However Long-term E2 treatments abrogated PPC protection, short-term E2 treatment did not protect the heart.

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PC26

Beatbox: A novel multi-function cardiac simulation environment

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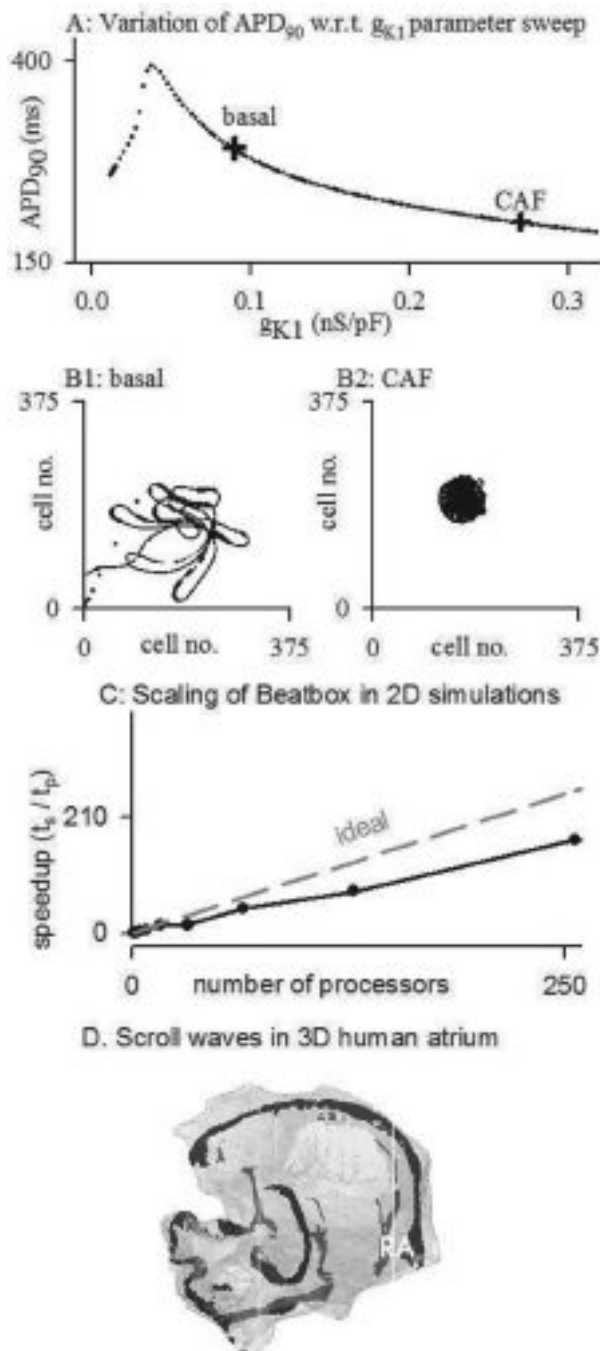
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This study demonstrates the functionality of a novel simulation environment, Beatbox [1], in a study of the effects of altered inward rectifier potassium current (I_{K1}).

Beatbox provides model repositories of cardiac cells as well as tissue and anatomies. The human atrial cell model by Courtemanche et al. [2] (CRN) was used in this study. The conductance of the I_{K1} current (g_{K1}) was altered in a graded manner to simulate atrial fibrillation (AF) or drug effects. The effects on action potential (AP), $[Ca^{2+}]_i$, and AP duration (APD_{90}) restitution were quantified using established pacing protocols [3]. Beatbox offers a user friendly computational mechanism for constructing spatially extended tissue models. It was used to construct 1D strands and 2D sheets of atrial tissue. The tissue level simulations were conducted using Beatbox's parallel domain decomposition algorithms. Inter-cellular coupling was chosen to give a conduction velocity (CV) of 0.33 mm/ms in solitary planar waves [3]. Using the 1D strands, CV restitution and vulnerability window (VW) were quantified at various values of g_{K1} . The 2D atrial sheets were taken to be 37.5 mm x 37.5 mm. Re-entrant waves were efficiently initiated by the phase distribution method [4] and optimised using the on screen run-time graphical functions. Re-entrant waves were allowed to evolve for 10 s. The stability of re-entrant waves was quantified by means of tip meander. Scroll waves in a 3D anatomical model were also simulated in the basal case. In all simulations, the time step for the forward Euler time solutions was taken to be 5 μ s [1] and a space step of 0.1 mm [2].

The basal AP duration (APD_{90}) in the CRN model is 312.1 ms. Upon increasing g_{K1} by 35% (simulation of paroxysmal AF), APD_{90} reduced to 260.3 ms, while reducing it by the same amount increased APD_{90} to 344.3 ms (effects of β -blockers). Chronic AF (CAF), simulated as a 200% increase of g_{K1} , gave an APD_{90} of 200.3 ms. APD_{90} in the physiological range of conductance values is shown in Fig 1A. In 1D strand models, CV restitution shows that an increase of g_{K1} increases the propensity of atrial tissue to sustain electrical propagation at high pacing rates. In the 2D simulations, re-entrant waves became stable with an increase of g_{K1} , and became hyper-meandering when g_{K1} was at or below the basal value of 0.09 nS/pF. The re-entrant wave tip trajectories under Control and CAF conditions are shown in Fig 1B. Scaling of these 2D simulations was quantified by running in serial and parallel mode. The scaling of Beatbox is shown in Fig 1C. The capability of Beatbox to simulate 3D phenomena is illustrated in Fig. 1D.

These simulations confirm our previous findings [3] obtained using alternative simulation environments. Beatbox was seen to be more flexible, and gave better performance on local and national HPC facilities.



Multi-scale simulations using Beatbox [1]. A: APD₉₀ at various values of g_{K1} . B: Re-entrant wave tip meander under Control (B1) and CAF (B2) conditions. C: Beatbox's computational scaling. D: Representative frame from 3D simulation.

McFarlane R and Biktasheva IV. BCS – Visions of Computer Science. 2008: 99-109.

Courtemanche M et al. Am J Physiol. 1998. 275:H301-21.

Kharche S et al. Prog Biophys Mol Biol. 2008 98:186-97.

Biktashev VN and Holden AV. Chaos. 1998 8(1): 48-56.

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Correlating serum potassium levels to ECG: A simulation study

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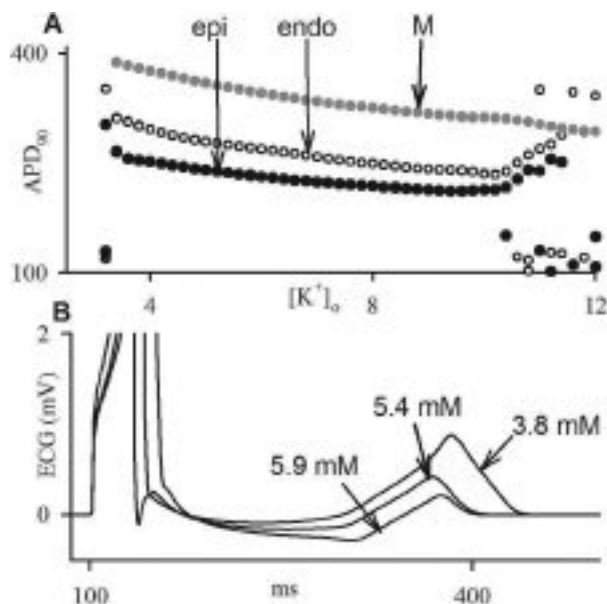
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Alterations in serum electrolyte concentrations as caused by renal failure are associated with cardiac disease. Altered serum or extracellular potassium ($[K^+]_o$) affects cardiac electrical activity and clinical ECG. Complementary to ongoing clinical studies, this modelling study quantifies the arrhythmogenic effects of abnormal $[K^+]_o$ levels on electrical properties of human ventricular cells, tissue and simulated ECG.

The O'Hara et al. [1] (ORD) family of epicardial (epi), midmyocardial (M) and endocardial (endo) cell models were used in this study. The ordinary differential equations were solved using in house developed robust solvers based on backward difference formulae and Newton iterations. Steady APs were elicited by pacing for 100 beats at 1 Hz after which the AP characteristics were computed for $[K^+]_o$ between 2 mM and 9 mM. Dynamic AP duration (APD₉₀) restitution was computed under basal, hypokalemic ($[K^+]_o = 3.8$ mM) and hyperkalemic ($[K^+]_o = 5.8$ mM) conditions. Regions of stable, aperiodic and alternans APs were identified. The cell models were incorporated into a 1D bidomain strand consisting of 25 endo cells, 25 M cells, and 115 epi cells [2]. The inter-cellular distance was assumed to be 0.1 mm. Implicit methods as implemented in Beatbox software were used to solve the 1D partial differential equations efficiently. A standard surface to volume ratio of 14 units was used in the simulations, while the intracellular and extracellular conductivities were optimised to give a conduction velocity (CV) of 0.44 mm/ms in a solitary wave propagating through this heterogeneous 1D strand. CV of steady waves (after 20 beats) as a function of $[K^+]_o$ was computed. Further, dynamic CV restitution was computed for high and low values of $[K^+]_o$. Pseudo-ECG and APD₉₀ dispersion were computed as a function of $[K^+]_o$. Further, the alterations of the simulated T-wave peak as well as T-wave repolarisation slope were computed and correlated to the $[K^+]_o$.

The basal APD₉₀ of endo cell model was found to be 272 ms, in M to be 332 ms, and epi to be 230 ms. APD was seen to reduce with increasing $[K^+]_o$ as shown in Figure 1A. APD restitution shows the M cell type to support high amplitude alternans. An alteration of $[K^+]_o$ increases the propensity of all cell types to sustain alternans at high (~ 220 to 300 ms pacing cycle length) pacing rates. In the 1D simulations, CV has a biphasic dependence on $[K^+]_o$ with a maximum value at $[K^+]_o = 4$ mM. Pseudo-ECG shows a reducing T wave amplitude with increasing K_o (Fig 1B). APD dispersion also increases with an increase of $[K^+]_o$. The repolarisation slope reduced with increasing $[K^+]_o$.

The effects of $[K^+]_o$ on ECG were confirmed in this simulation study.



A: Dependence of APD₉₀ on [K⁺]_o in the 3 cell types. B: Dependence of the simulated ECG T-wave amplitude on [K⁺]_o.

O'Hara T et al PLoS Comput Biol. 2011; 7(5) e1002061

Glukhov A et al. Circ. Res. 2010; 106(5): 981-9.

Keener JP and Bogar K. Chaos. 1998; 8(1): 234-241.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC28

Left ventricle structural remodelling and cardiomyocyte contractile function in type 1 diabetic rats

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Heart failure is a major cause of premature morbidity and mortality in diabetes mellitus (DM) but the underlying mechanisms are elusive and treatment remains empirical. Previously, we reported that type 2 DM was associated with structural remodelling and associated changes in inflammatory markers of the diabetic heart (D'Souza et al, 2011). The study now investigated the role of hyperglycaemia on cardiomyocyte contractile function and histopathological changes in the left ventricle (LV) of streptozotocin (STZ)-treated type 1 diabetic male Wistar rats compared to age-matched controls, 6-7 weeks after STZ-administration (60 mg/kg body weight) via single intraperitoneal injection, according to Home Office regulation. Contraction and intracellular calcium transients [Ca²⁺]_i were measured in electrically stimulated ventricular myocytes by a video edge detection system and fluorescent method, respectively. Histological and immunohistochemical studies were conducted in small portions of LV tissues stained with Haematoxylin and Eosin, labelled with FITC-conjugated Lectin and the Masson's trichrome stain for determination of myocyte size and quantitative assessment of fibrosis and caspase-3.

STZ-treated rats had significantly (Student's t-test; $p < 0.01$) higher blood glucose values and reduced heart to body mass ratio relative to controls. Blood glucose levels were 305 ± 7.48 mg/dl and 89.51 ± 3.56 mg/dl and heart to body mass ratios were 0.24 ± 0.11 and 0.31 ± 0.02 for diabetic ($n=8$) compared to control ($n=8$) rats. When electrically stimulated at 1 Hz, contractility (% of resting length) was depressed in myocytes from diabetic rats ($3.86 \pm 0.23\%$, $n=33$) compared to controls ($5.82 \pm 0.34\%$, $n=33$). Myocytes exhibited prolonged time(s) for contraction (129 ± 39 ms ($n=19$) vs 112.47 ± 5.01 ms ($n=16$)) and relaxation (48.47 ± 2.90 ms ($n=19$) vs 40.71 ± 2.09 ms ($n=16$)) ($p < 0.05$) in DM compared to controls. Alterations in [Ca²⁺]_i manifested as significant ($p < 0.05$) prolonged time to peak (89.53 ± 2.54 ms, ($n=21$) vs 77.16 ± 3.5 ms ($n=23$)) and prolonged rate of decay (0.75 ± 0.02 ms ($n=23$) vs 0.57 ± 0.02 ms ($n=21$)) of the Ca²⁺ transient in diabetic myocytes compared to controls. LV morphology was severely altered by DM with significant ($p < 0.05$) increments in fibrous tissue proliferation ($3.51 \pm 0.44\%$ vs $5.05 \pm 0.44\%$), and smaller myocyte transverse diameter (9.93 ± 0.26 μ m vs 9.11 ± 0.25 μ m) in diabetic compared to control LV muscle. In STZ-treated LV, the pathology manifested as focal scarring, myofibrillar loss, vacuolisation and large clusters of cells showing histological signs of apoptosis. Activity of cleaved caspase-3 (positive cells/mm²) was also significantly ($p < 0.01$) increased in the STZ-treated group (8.56 ± 0.79) compared to control (1.86 ± 1.30). The results indicate that STZ-induced DM can result in marked histopathological changes that are associated with functional abnormalities of the heart.

D'Souza, A, Wood, N.M., Boyett, M, Adeghate, E., Howarth, C.F., Bidasee, K.A. and Singh, J (2011). Left Ventricle Structural Remodelling in the Prediabetic Goto-Kakizaki Rat- Potential role of Transforming Growth Factor β 1. Experimental Physiology, 96.9. 875-888

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PC29

Early opening of sarcolemmal KATP channels does not contribute to PKC-mediated cardioprotection

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ATP-sensitive potassium (KATP) channels have been implicated in the mechanism of cardioprotection imparted by brief periods of non-lethal ischaemia prior to a prolonged insult. Such ischaemic preconditioning (IPC) has been demonstrated to markedly reduce infarct size so improving the recovery after myocardial infarction. After cardioprotective stimuli, sarcolemmal KATP (SarcoKATP) channels are hypothesised to open early during ischaemia to shorten the cardiac action potential (AP), limit calcium entry and so preserve ATP. We present data showing that sarcoKATP channel opening, and contractile failure, is actually delayed after IPC, direct PKC activation or adenosine pre-treatment.

Cardiomyocytes were enzymatically isolated from adult male Wistar rats culled in accordance with Home Office regulations. IPC cardiomyocytes were isolated from hearts after an IPC protocol of 3 cycles of 5 minutes of stopped perfusion. Cardioprotection was assessed using a contractile function protocol. Briefly, cardiomyocytes were perfused with normal Tyrode (NT) solution at $32 \pm 2^\circ\text{C}$ with 1 Hz electric field stimulation to trigger contractions. Solution was exchanged for a metabolic inhibition (MI) solution (2 mM cyanide and 1 mM iodoacetic acid

in substrate free Tyrode solution) for 7 mins, followed by 10 mins of simulated reperfusion with NT solution. The times to contractile failure and the percentage of cells showing contractile function and hypercontracted cells (recorded as a measure of reperfusion injury) were recorded in control, IPC and cells treated with either 1 μ M PMA or 3 μ M adenosine for 5 mins prior to the contractile function protocol.

Cell attached patch recording was used to assess the time to opening of SarcoKATP channels in the presence of MI-Tyrode solution. The opening of SarcoKATP channels was measured as the time to the first burst greater than 500 ms in duration. Using this assessment, SarcoKATP channel opening was significantly delayed in IPC ($182 \pm 11s^{***}$), and PMA ($162 \pm 6s^{*}$) and adenosine ($197 \pm 8s^{***}$) pre-treated cardiomyocytes compared to control ($127 \pm 6s$) (n=8, 6, 8 and 12 respectively, * $P < 0.05$, *** $P < 0.001$, Students t-test).

These data demonstrate that IPC, PMA or adenosine impart a marked cardioprotection to isolated cardiomyocytes. However, the opening of SarcoKATP channels is markedly delayed after cardioprotective stimuli. These findings suggest that early opening of SarcoKATP channels is not directly involved in the cardioprotection afforded by IPC or PKC activation. This does not exclude a role for mitochondrial KATP channel

Contractile function protocol results

	Control	IPC	PMA	Adenosine
Time to Contractile Failure	151 $\pm$ 7s	210 $\pm$ 7s ^{***}	190 $\pm$ 4s ^{***}	224 $\pm$ 5s ^{***}
Percentage contractile recovery	31 $\pm$ 3%	76 $\pm$ 5% ^{***}	68 $\pm$ 3% ^{***}	63 $\pm$ 4% ^{***}
Percentage Hypercontractant	29 $\pm$ 3%	7 $\pm$ 1% ^{***}	17 $\pm$ 1% ^{***}	1 $\pm$ 1% ^{***}
N (cells) Experiments	128(14)	105(16)	118(14)	113(14)

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PC30

PKC-mediated toxic effects of acute elevations of extracellular glucose concentration on cardiomyocyte contractile function

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In patients hospitalized with acute myocardial infarction (MI), acute hyperglycaemia is common and is associated with adverse outcome(1,2). In this setting, mortality risk increases in proportion to blood glucose concentration at admission irrespective of a prior diagnosis of diabetes mellitus. Hyperglycaemia may adversely influence cardioprotective stimuli, such as ischaemic preconditioning (IPC), thought to be mediated by PKC ϵ activation, and also causes perturbation of cardiac action potential (APD) repolarisation. While attenuation of IPC by hyperglycaemia has been demonstrated in vivo, the mechanisms remain unclear. It has been shown that elevated extracellular glucose can cause activation of PKC and so we used rat isolated cardiomyocytes to investigate the role of PKC in the deleterious effects of hyperglycaemia on cardiomyocyte function.

Cardiomyocytes were enzymatically isolated from adult male Wistar rats culled in accordance with home office regulations. IPC cardiomyocytes were isolated from hearts after an IPC protocol of 3 cycles of 5 minutes of stopped perfusion. Cardioprotection was assessed using a contractile function protocol(3). Using 1 Hz electric field stimulation (EFS) the effects of glucose on cardiomyocyte contraction was investigated when exchanging the solution from 5 to 20 mM glucose. In control experiments the number of contractile cardiomyocytes was

reduced by $40 \pm 4\%$ in 20 mM glucose which occurs concurrently with an increase in the number of asynchronous contractions (additional to the EFS). Inhibition of PKC α ($21 \pm 1\%^{***}$) and β ($16 \pm 3\%^{***}$), but not β II ($35 \pm 5\%$), γ ($54 \pm 1\%$), δ ($45 \pm 4\%$) or ϵ (100%) using isoform-specific Tat-linked PKC inhibitor peptides attenuated this decrease in contractile cardiomyocytes (** $P < 0.001$, n=6 experiments >100 cells for each experiment). The mean increase in APD in 20 mM glucose was also attenuated by inhibition of PKC α and β ($139 \pm 33ms$ to $12 \pm 2ms$ respectively, $P < 0.001$, n=8 for each).

Finally, these inhibitors were used to reverse the attenuation of IPC in 20 mM glucose. Contractile recovery in IPC cells was $72 \pm 3\%$ in 5 mM and $30 \pm 4\%$ in 20 mM glucose abolishing the beneficial effects of IPC. Inhibition of PKC α and β in 20 mM glucose prior to the contractile function protocol reversed the attenuation of contractile recovery ($58 \pm 5\%$) which was not significantly different to IPC cardiomyocytes in 5 mM glucose ($P > 0.05$, n=6 experiment, >100 cells each).

In summary, our findings provide a direct mechanistic link between hyperglycaemia at the time of MI and subsequent adverse prognosis via PKC α and β -dependent mechanisms. Our data suggest specific PKCs to be potential therapeutic targets in the treatment of MI, and strengthen the case for the active management of elevated glucose in MI in man.

Kosiborod M, Inzucchi SE, Krumholz HM, Xiao L, Jones PG, Fiske S, Masoudi FA, Marso SP, Spertus JA: Glucometrics in patients hospitalized with acute myocardial infarction: defining the optimal outcomes-based measure of risk. *Circulation* 2008;117:1018-1027

Squire IB, Nelson CP, Ng LL, Jones DR, Woods KL, Lambert PC: Prognostic value of admission blood glucose concentration and diabetes diagnosis on survival after acute myocardial infarction: results from 4702 index cases in routine practice. *Clinical Science* 2010;118:527-535

Hudman D, Standen NB: Protection from the effects of metabolic inhibition and reperfusion in contracting isolated ventricular myocytes via protein kinase C activation. *J Mol Cell Cardiol* 2004;37:579-591

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PC31

Differential expression of the cardiac slow delayed rectifier complex in the human heart

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In-vitro the voltage-gated potassium channel member Q1 (KCNQ1) associates with the accessory subunit KCNE1 to form an outward current with kinetics closely resembling that of the cardiac slow delayed rectifier current (IKs) (Sanguinetti et al, 1996). The IKs current is involved in the repolarisation phase of the cardiac action potential. Defects in KCNQ1 are the cause of several inherited diseases that can cause disturbances in heart rhythm such as long QT syndrome (LQT1). The IKs channel complex is also a known cardiovascular liability for drugs that prolong the cardiac action potential, suggesting a role in acquired long QT-like disorders (Towart et al, 2009). The aim of this study was to characterise the IKs current in a transfected human cell line and to determine the distribution of KCNQ1 and KCNE1 in healthy human hearts.

To characterise the IKs current whole-cell patch clamp recordings were obtained from Human Embryonic Kidney (HEK) 293 cells that were transiently transfected with plasmids (pCEP4)

containing KCNQ1 (n=3) or KCNQ1 and KCNE1 (n=10) at a ratio of 1:1 (Fig. 1). Total RNA was extracted from 16 different areas (including atria, ventricles, aorta, pulmonary vein and septum) of non-diseased female human hearts (n=2) from organ donors that were technically unsuitable for transplantation and expression of KCNQ1 and KCNE1 mRNA characterised using end-point PCR.

KCNQ1 only transfection produced a current with rapid activation kinetics whereas KCNQ1 and KCNE1 co-transfection produced a current that had much slower activation kinetics. PCR revealed that KCNQ1 and KCNE1 are differentially expressed in various regions of the heart. Both KCNQ1 and KCNE1 mRNA were expressed in most of the 16 areas examined, with the exception of the aorta and pulmonary vein which showed little or no expression. KCNQ1 expression was highest in the septum and AV (atrioventricular) node, with KCNE1 expression highest in septum, AV node and various regions in the left ventricle.

As KCNQ1 and KCNE1 co-expression produces a current that closely resembles that of the cardiac IKs current, this demonstrates that KCNQ1 and KCNE1 together are vital in producing the kinetics required for the channel complex to be involved in cardiac repolarisation. The co-localisation of KCNQ1 and KCNE1 mRNA in the heart suggests a possible functional complex between these proteins. High levels of expression between KCNQ1 and KCNE1 in the left ventricle were expected, however, in the AV node and septum high levels of expression suggests an alternative role for the IKs channel complex, possibly in signal generation and/or transduction.

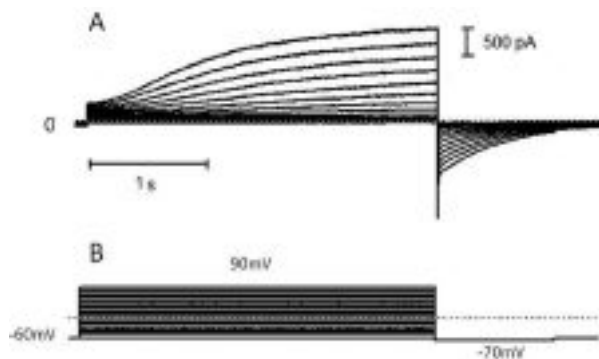


Figure 1. IKs current activation. A. HEK293 cells were transiently transfected with KCNQ1 and KCNE1 to produce a slowly activating current. B. Voltage protocol, IKs current was elicited by applying depolarizing potentials ranging from -60mV to +90mV in 10mV increments, from a holding potential of -60mV.

Sanguinetti, MC et al (1996). *Nature* 384(6604), 80-83.

Towart, R et al (2009) *J Pharmacol Toxicol Methods* 60(1), 1-10.

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PC32

PKC alpha as a potential target for cardioprotection in patients with congenital heart defect undergoing corrective surgery

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Congenital heart defects affect 1% of born babies. Although corrective surgery is generally successful, there is an increased risk of morbidity and mortality in cyanotic children compared with acyanotic. PKC alpha is a member of the PKC family that is required for maintaining cell survival and cardiac function in the postnatal animal heart. Our previous data showed that PKC α mRNA expression is altered in cyanotic patients. However, little is known about human PKC α expression in response to chronic hypoxia, and its potential role in survival and resistance to reperfusion injury remains unexplored. We first investigated the effects of cyanosis on protein levels of PKC α and its cell survival targets eNOS and Bcl-2 in myocardium samples from paediatric patients suffering from Tetralogy of Fallot. Our findings showed an upregulation of PKC α protein expression by cyanosis that was associated with increased phosphorylated Bcl2 and eNOS levels in the myocardium. We then used the H9c2 myoblast cell line to test whether over-expressing PKC α results in increased levels of phosphorylated Bcl2 and eNOS. Infecting H9c2 cells with an adenovirus expressing a wild type form of PKC α resulted in an upregulation of phosphorylated-Bcl2 but not eNOS suggesting that PKC α survival effect is mediated through the anti-apoptotic protein Bcl2. A dominant negative form of PKC α did not alter levels of these two proteins. A potent PKC modulator, Bryostatin-1, that has been used in Alzheimer's disease and cancer trials upregulated the phosphorylated PKC α protein levels within minutes to an hour in our cell model suggesting that this drug could be used in a cardiac surgery setting to activate PKC α . We finally tested the effect of this drug on a rat whole heart ischaemia/reperfusion injury model. Bryostatin-1 increased the functional recovery and reduced the infarct size in rat heart subjected to ischaemia/reperfusion.

In conclusion, our findings suggest that modulating PKC α provides a strategy for cardioprotection in patients with congenital heart defect undergoing corrective surgery.

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PC33

The Wilms' tumour transcription factor Wt1 is involved in the regulation of the innate immune system

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The Wilms' tumour suppressor gene WT1 encodes a zinc finger transcription factor originally identified as a tumour sup-

pressor, which has recently been shown to be critical for embryonic development and to be involved in adult tissue maintenance and regeneration [1]. WT1 is expressed in haematopoietic progenitors, and is used as a prognostic marker in acute myeloid leukaemia. However, (a) WT1 expression by mature phagocytes and (b) a potential role of WT1 in the regulation of the immune system have not been described yet. We have thus been investigating this relationship *in vitro* and *in vivo*. Interestingly, we detected WT1 transcripts in mature monocytes from healthy volunteers as well as in different monocytic cell lines. Macrophagocytic differentiation and/or stimulation with bacterial lipopolysaccharide changed cellular WT1 mRNA and protein levels in a context-specific manner. The immunosuppressant Interleukin-10 (IL-10), a candidate downstream target of WT1, was coexpressed with WT1 in monocytic cells. siRNA-mediated Wt1 knockdown reduced IL-10 transcript levels by 90%. IL-10 expression was increased > 15-fold upon forced expression of Wt1 in transient as well as in stably transfected UB27 cells [Wt1(-KTS)]. Interestingly, the Wt1(+KTS) protein, which had previously been associated mainly with posttranscriptional regulation, was almost as efficient in stimulating the IL-10 promoter. IL-10 promoter analysis revealed a Wt1 binding motif, which could be verified *in vitro* and *in vivo* (chromatin immunoprecipitation, electrophoretic mobility shift assays) in murine macrophagocytic cells. Targeted mutagenesis of the Wt1-binding cis-element abrogated inflammatory activation of the IL-10 promoter [2]. Currently, we are looking into the functional relevance of WT1 expression in immune cells and its interaction with immunoregulatory cytokines. So far, we detected significantly decreased IL-10 levels in the livers of Wt1-/- C57/BL6 embryonic mice at day E11.5. Our results suggest a novel immune regulatory function of Wt1. Miller-Hodges E, Hohenstein P. WT1 in disease: shifting the epithelial-mesenchymal balance. *J Pathol.* 2012 Jan;226(2):229-40

Sciesielski LK, Kirschner KM, Scholz H, Persson AB. Wilms' tumor protein Wt1 regulates the Interleukin-10 (IL-10) gene. *FEBS Lett.* 2010 Nov 19;584(22):4665-71

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PC34

Does domestic cooking environment influence risk of respiratory morbidities in rural Indian women?

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Whether continued use of biomass fuels along with other fuels in presence of poor domestic cooking conditions is as detrimental to respiratory health as the exclusive use of biomass fuels is unknown. The present study is an attempt to assess the risks associated with indoor air pollution in the context of adverse domestic cooking environmental conditions in rural women of central India. Hence we conducted a community based, cross-sectional study in 760 non-smoking, rural women of central India – 265 used non-biomass fuels (Group A), 243 biomass and other fuels (Group B) while 252 exclusively biomass fuels (Group C). Exposure to domestic smoke was estimated according to the average time per day spent near the fireplace (exposure index). Abnormal pulmonary function of the study subjects was assessed by the measurement of peak expiratory flow rate (PEFR) according to standards recommended by American thoracic society. PEFR less than 80% of

the predicted was considered as abnormal pulmonary function. It was observed that robust multivariate analyses which adjusted for height, illiteracy, physical activity, environmental exposure to tobacco smoke (ETS), mud house, overcrowding, inadequate ventilation and respiratory morbidity revealed that illiteracy [Odds ratio (OR) 2.48, 95 % Confidence interval (CI) 1.04-5.87]; physical activity (OR 3.93, 95 % CI 1.52-10.14); inadequate cross ventilation (OR 2.43, 95 % CI 1.23-4.77) and respiratory morbidity (OR 2.65, 95 % CI 1.38-5.08) were significant predictors of low PEFR for group C ($P < 0.05$). Whereas none of the predictors were found to be significantly associated with group B. Since women using partial biomass fuels showed no association of low PEFR with domestic cooking environment and respiratory morbidity even after robustly adjusting for confounding variables, we can conclude that even partial abolition of biomass use may be beneficial in improving the lung function of rural, non-smoking women in spite of having inadequate domestic cooking environment.

21. Bruce N, Perez-Padilla R, Albalak R. Indoor air pollution in developing countries: a major environmental and public health challenge. *Bulletin of the World Health Organisation* 2000;78(9):1078-92.

2. Perez-Padilla R, Schilman A, Riojas-Rodriguez H. Respiratory health effects of indoor air pollution. *Int J Tuberc Lung Dis* 2010;4(9):1079-86.

14. Riboli E, Preston-Martin S, Saracci R, Halay NJ, Trichopoulos D, Becher H, et al. Exposure of nonsmoking women to environmental tobacco smoke: a country collaborative study. *Cancer Causes Control* 1990;1243-52.

6. Aggarwal A.N, Gupta D, Sharma C.P, Jindal S.K. Effect of household exposure to environmental tobacco smoke on airflow mechanics in asymptomatic healthy women. *Indian J Med Res* 2004;119:18-23.

13. Behera D, Jindal SK, Malhotra HS. Ventilatory function in non smoking rural Indian women using different cooking fuels. *Respiration* 1994;61(2):89-92.

none

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PC35

Oxygen carrying capacity of blood in the three trimesters of nulliparous and multiparous pregnant women in Southern Nigeria

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Oxygen carrying capacity of blood is a function of hemoglobin concentration. Pregnancy being a condition with enormous physiologic, biochemical and anatomic adaptations require normal levels of hemoglobin for optimal pregnancy outcome. However, each trimester and parity may have effect on the hemoglobin concentration. Studies have however shown a physiologic anemia in the second trimester of pregnancy. This adversely affects the oxygen carrying capacity of blood, resulting in gestational anemic hypoxia. We designed this study to assess the oxygen carrying capacity of blood in various groups of parous women as pregnancy advances. We used a multi-stage sampling technique to group pregnant women attending antenatal clinic into seven groups: primigravida, para1, para2, para3, para4, para5 and >para5. We randomly selected thirty-eight subjects from the various groups. We selected forty control subjects from a group of women attending family planning clinic. Subjects gave informed consent and the

Ethical Committee of the hospital gave ethical clearance for the study. 2mls of blood was collected into an EDTA bottle for hemoglobin concentration measurement using standard method. We multiplied hemoglobin concentration by 1.34ml to get the oxygen carrying capacity of hemoglobin. We adopted 95% hemoglobin saturation for all subjects. Since hemoglobin concentration less than 10g% is anemia, subjects with values less than 12.73ml% were regarded as having gestational anemic hypoxia. Para2 subjects had the highest percentage (31.5%, n=12) of second trimester gestational anemic hypoxia. This was followed by para3 with a percentage (26.32%, n=10). The difference in these values was not statistically significant ($p>0.05$). Para1 and para4 had percentages 13.16%; n=5 and 10.53%; n=4 respectively. The primigravida, para5 and >para5 had no records of second trimester gestational anemic hypoxia. The oxygen carrying capacity of $13.14 \pm 2.10\text{ml}\%$ and $13.21 \pm 2.07\text{ml}\%$ for para2 and para3 for second trimesters respectively, were significantly ($P<0.05$) lower than that of control ($15.70 \pm 2.10\text{ml}\%$). Para2 and para3 had more pronounced tendency of causing gestational anemic hypoxia. This may be due to inadequate residual iron stores in these parities. Routine antenatal hematinics should be increased in these parities during the second trimester to forestall possible effects of gestational anemic hypoxia.

The management and staff of St Philomena Catholic hospital, Benin city, Edo state, Nigeria.

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PC36

Atrioventricular node adaptation to heart failure in a rabbit model

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Heart failure (HF) affects 1 million people in the UK with a similar number being undiagnosed. It carries a 50% mortality in 5 years and costs the NHS 2.5% of its annual budget (National Institute for Health and Clinical Excellence 2010). Atrioventricular node (AVN) conduction disturbance is common in HF and is associated with worse prognosis (Gervais et al. 2009; Olshansky et al. 2012).

Three-month old male New Zealand rabbits (n=9, 2500-3000g) were anaesthetised with ketamine intramuscular injection and isoflurane inhalation. They underwent destruction of the aortic valve leaflets by repeated insertion of a catheter through the aortic valve (week 0) resulting in volume overload. Three weeks later banding of the abdominal aorta was performed by placing a silver clip (inside diameter 2.44mm) just above the right renal artery using the same anaesthetic schedule. This resulted in pressure overload. Echocardiography confirmed reduction of ejection fraction, and signs of HF were present prior to termination (week 8). Control animals underwent sham procedures. In vivo ECG recording showed significant prolongation of the PR interval, which remained significant after autonomic blockade. Interval immunofluorescence and Masson's trichrome staining was used to construct anatomic "roadmaps" of the 18 AVN preparations. These were analysed using Matlab to study deformation. The tiny tissues of the AVN were dissected using laser-assisted microdissection

(LMD). Transitional tissue (TT), the inferior nodal extension including compact node- (INE), penetrating bundle (PB) and His bundle (HB), as well as atrial and right (RVS) and left (LVS) ventricular septal tissues were microdissected and mRNA extracted immediately.

mRNA quantity and integrity were assessed using the NanoDrop ND-1000 spectrophotometer and Agilent 2100 bioanalyzer. We obtained RNA quantity of ≥ 100 ng with an average 260/280 RNA absorbance ratio of 1.84 (RIN 2.4-8.8), and studied 37 ion channels and connexins with quantitative RT-PCR. Values are expressed as means $\pm$ SEM, compared by t-test unless otherwise stated.

Anatomically, HF hearts showed hypertrophy of the AVN at the PB and HB levels (combined HB+PB length 3.0 ± 0.186 mm (HF), 2.4 ± 0.231 mm (sham), $P=0.04$). The majority of transcripts were differentially expressed between working myocardium and the AVN conduction tissue ($\text{FDR}<0.05$). Steep ion channel gradients across the AVN itself (INE-PB-HB) were found for ANP, Kir3.1, HCN4, Kir2.1, Nav1.5 and TWIK-1 in both HF and sham. Kchip2 mRNA is significantly more abundant in RVS vs. LVS ($\text{FDR}<0.05$). In HF Cx43, HCN1 and Cav1.3 are down-regulated across all regions studied ($P<0.05$, 2-way ANOVA). Conversely, SUR2a, CLC-2, Nav β 1 and Nav1.1 are up-regulated ($P<0.05$, 2-way ANOVA). Down-regulation of Cx43, Cav1.3 and HCN1 may help explain the AVN dysfunction and slowing of conduction in HF. Up-regulation of ANP in response to mechanical stress is well characterised in HF and our study supports this. We hypothesise that up-regulation of SUR2a, CLC-2, Nav β 1 and Nav1.1 may act as a compensatory response to impaired AVN conduction.

Gervais R, Leclercq C, Shankar A, Jacobs S, Eiskjaer H, Johannessen A, Freemantle N, Cleland JG, Tavazzi L & Daubert C (2009). Surface electrocardiogram to predict outcome in candidates for cardiac resynchronization therapy: a sub-analysis of the CARE-HF trial. *Eur J Heart Fail* 11, 699-705.

National Institute for Health and Clinical Excellence (2010). Chronic Heart Failure: The Management of Adults with Chronic Heart Failure in Primary and Secondary Care (Partial Update). (CG 108). London: Royal College of Physicians.

Olshansky B, Day JD, Sullivan RM, Yong P, Galle E & Steinberg JS (2012). Does cardiac resynchronization therapy provide unrecognized benefit in patients with prolonged PR intervals? The impact of restoring atrioventricular synchrony: an analysis from the COMPANION Trial. *Heart Rhythm* 9, 34-9.

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PC37

Adaptation of the coronary microvascular system by a high-fat diet: cross-interaction study between diet and ageing

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Obesity is known to confer an increased risk for developing cardiovascular disease and is related to vasomotor dysfunction in the peripheral and cerebral circulations. However, the impact of obesity on the function of the coronary microvascular circulation is less known, especially regarding the overall vasodilator capacity of this system.

Our study aimed at evaluating the cardiac mechanical function and the coronary reactivity of Wistar rats after a short- (3 months) and a long-term (7 months) period of a high-fat (HF) or standard diet. The rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (30mg/kg) and their hearts were immediately perfused according to the Langendorff mode. After vasoconstriction with continuous infusion of a thromboxane A2 analogue (U46619, 30nM), the endothelial-dependent (EDD) and endothelial-independent (EID) dilations were determined in the beating heart by injections of increasing doses of acetylcholine (4, 10, 20, 40, 60, 80, 100 pmoles) and sodium nitroprusside (100, 200, 400, 600, 800, 1000 pmoles) respectively. The mitochondrial oxidative stress was then evaluated in the isolated cardiac mitochondria (rate of H₂O₂ production) and in the whole heart (aconitase-to-fumarase ratio).

In our study, the high-fat diet induced a reduction of the cardiac mechanical function which was not affected by ageing. The EDD was also increased by the diet at the early phase (+23.5%) and remained always higher compared to the control rats even though it was progressively decreased by ageing (-21.9% for the control and -19.2% for the HF rats). However, the EID was not affected neither by the diet neither by the ageing and it remained at the same levels for all the rat groups. These results indicate a functional adaptation of the coronary system induced by high-fat diet which is probably due to an enhanced function of their endothelial cells. The increased mitochondrial stress found either in the whole heart by a reduction of the aconitase-to-fumarase ratio (-41.4% for the control and -41.6% for the HF rats) either in the isolated mitochondria (+53% for the control and -41.6% for the HF rats when glutamate/malate/succinate were used as substrates) of rats following a long-term diet can only explain the progressive reduction of the EDD by ageing and not the diet effect. Thus, these data suggest the development of a compensatory adaptation of the coronary system shortly after the beginning of a high-fat diet. This phenomenon helps the heart to maintain the EDD and consequently an adequate tissue perfusion meeting the higher metabolic requirements of the organism. Finally, it seems that the process of ageing is more important than diet in affecting negatively the vascular function via the presence of oxidative stress.

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PC38

Sex difference in hypoxic tolerance of mouse sternohyoid muscle

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Research into sex differences in respiratory-related disorders such as chronic obstructive pulmonary disease and obstructive sleep apnoea syndrome suggest that differences at the level of the muscle are important (Skelly et al., 2010, Silverman et al., 2000). Hypoxia, a common feature of respiratory related diseases, has been observed to induce respiratory muscle dysfunction (Skelly et al., 2009) which is associated with deleterious clinical consequences such as dyspnoea, respiratory failure and premature death. We hypothesized that sex differences intrinsic to respiratory muscles will become apparent under hypoxic conditions.

Male and female C57Bl6/J mice were humanely killed and diaphragm and sternohyoid (a representative upper airway dilator) muscle strips were excised. Isometric and isotonic contractile properties of muscle strips were examined in physiological solution at 35°C in vitro. Muscle strips were incubated in tissue baths under control (95%O₂/5%CO₂) or hypoxic (95%N₂/5%CO₂) conditions. All data was normalised to optimal length, or cross-sectional area as appropriate and was analysed using two-way ANOVA.

Hypoxia alone, as expected, caused significant reductions in peak force, peak work and peak power in both muscles of both sexes. Sex alone, had no effect on peak work or peak power in either muscle group under control conditions. However female sternohyoid, but not diaphragm, peak force was significantly more tolerant under hypoxic conditions (Control v Hypoxia, mean ± SEM: 9.4±0.5N/cm² v 4.3±0.61N/cm² (male); 10.1±0.5N/cm² v 8.0±0.7 N/cm² (female); N= 6-7 per group). There was a significant sex-gas interaction (P=0.0143). There was a sex and a gas difference in diaphragm maximum shortening velocity (L₀/s=muscle lengths/second; Control v Hypoxia, mean ± SEM: 4.4±0.4L₀/s v 3.5±0.8L₀/s (male); 3.5±0.5L₀/s v 1.5±0.6L₀/s (female); N= 9,7,9,6 animals per group) but no interaction (P=0.3703). In contrast, neither sex nor gas had an effect on maximum shortening velocity in the sternohyoid (Control v Hypoxia, mean ± SEM: 5.5±0.8L₀/s v 7.5±1.3L₀/s; 5.4±0.5L₀/s v 5.1±0.8L₀/s; N= 6-7 animals per group) and there was no interaction (P=0.2172).

We conclude that there are sex differences in hypoxic tolerance of murine upper airway dilator muscle. These differences potentially contribute to development, progression and outcomes of several respiratory related diseases.

SILVERMAN, E. K., WEISS, S. T., DRAZEN, J. M., CHAPMAN, H. A., CAREY, V., CAMPBELL, E. J., DENISH, P., SILVERMAN, R. A., CELEDON, J. C., REILLY, J. J., GINNS, L. C. & SPEIZER, F. E. 2000. Gender-related differences in severe, early-onset chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, 162, 2152-8.

SKELLY, J. R., BRADFORD, A., JONES, J. F. X. & O'HALLORAN, K. D. 2009. Superoxide Scavengers Improve Rat Pharyngeal Dilator Muscle Performance. *American Journal of Respiratory Cell and Molecular Biology*, 42, 725-731.

SKELLY, J. R., BRADFORD, A. & O'HALLORAN, K. D. 2010. Intermittent hypoxia impairs pharyngeal dilator muscle function in male but not female rats. *Adv Exp Med Biol*, 669, 285-7.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC39

The effects of sprouty2 knockdown on vascular signalling from human bronchial epithelial cells

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Co-ordinated signalling between airway epithelium and surrounding mesenchyme tissue creates the conducting airway and vasculature of the fetal lung. The branching rate of both structures is precise and depends upon molecular "time keepers" such as Sprouty2 (Spry2) that determine the inter-branch length and branch positioning. However, the cues which link vascular growth to the airway branching process are not well understood. The primary inducer of airway tube elongation is fibroblast growth factor-10 (FGF-10) which induces growth of the airway epithelium by activating its receptor, FGFR2b. This

is antagonised by Spry2 which determines the duration of receptor signaling to the ERK1/2 and PI3-kinase pathways and, consequently, the length of each airway branch. Given its pivotal role in the airway branching process, we hypothesised that Spry2 would integrate FGF-10-stimulated airway outgrowth with the process of vascular signalling in airway epithelium. To test this, immortalised human bronchial epithelial cells (16HBE14o- or "HBE") were stably transformed with either non-genomic (control) shRNA or Spry2 shRNA. Cells were then exposed to a concentration range of FGF-10 (0.01–1 $\mu\text{g}\cdot\text{ml}^{-1}$) in serum-depleted medium at the PO₂ of the fetal lung (3% O₂) or at ambient PO₂ (21% O₂). Vascular signalling was measured using a luciferase reporter gene to report hypoxia inducible factor-1 α (HIF-1 α) activity and qPCR to report vascular endothelial growth factor-A (VEGF-A) mRNA abundance relative to 18S RNA. Spry2 mRNA abundance in Spry2-shRNA cells was <20% of that in control cells. This level of knockdown sustained HIF-1 α protein abundance irrespective of FGF-10 treatment and PO₂, but its effect on HIF-1 α -driven reporter gene activity was strongly oxygen dependent. Thus, at 3% O₂, FGF-10 evoked a concentration dependent decline in HIF-1 α activity in control cells whereas Spry2 knockdown abolished the FGF-10 effect and suppressed HIF-1 α activity by ~5-fold relative to control shRNA cells ($P < 0.05$; $n = 4$). At 21% O₂, however, HIF-1 α activity was significantly induced by Spry2 knockdown compared to the control cells and was associated with a corresponding increase in VEGF-A mRNA expression ($P < 0.05$; $n = 6$). We conclude that Spry2 participates in the regulation of vascular signalling from airway epithelial cells and that its major effect is to repress HIF-1 α mediated gene expression at elevated PO₂.

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PC40

Sprouty2 regulates vascular signalling in the developing lung through a direct interaction with regulatory sequences in the VEGF-A promoter

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Airway branching and vascular growth in the developing lung are co-ordinated processes which involve cross-talk between the primary inducer of airway tube elongation, fibroblast growth factor 10 (FGF-10), and the pro-vasculogenic transcription factor, hypoxia inducible factor-1 α (HIF-1 α) (Scott *et al.*, 2010). The FGF receptor antagonist, Sprouty2 (Spry2), influences the duration of this signalling activity and so governs the periodicity of airway and vascular branching along the respiratory tree. However, the mechanism through which Spry2 co-ordinates these events is unknown. Spry2 occurs in the nuclei of both human bronchial epithelial cells (HBE) and fetal distal lung epithelial cells (FDLE), which suggest that it could influence vascular signalling at an epigenetic level. To test this, we hypothesised that Spry2 suppresses transcriptional activity of HIF-1 α by direct interaction with the vascular endothelial growth factor A (VEGFA) promoter and so governs the vascular development of the fetal lung. Primary cultures of rat FDLE were isolated from gestation day 19 (E19) fetal Sprague Dawley rats and used to investigate the influence of FGF-10 on

Spry2 nuclear function and interaction with the VEGF-A promoter. Immortalised HBE (16HBE14o-) were used for Spry2 overexpression studies. All experiments were conducted in serum-depleted medium at the PO₂ of the fetal lung (3% O₂). FGF-10 induced a concentration dependent cleavage of nuclear Spry2 which corresponded with an increase in Histone-3 (H3) phosphorylation (pS10) and acetylation (acK14), decreased histone deacetyltransferase 1-3 (HDAC1, HDAC2 and HDAC3) abundance and increased VEGFA mRNA and protein expression. Chromatin immunoprecipitation (ChIP) assays revealed a dominant interaction between Spry2 and a GC rich region of the VEGF-A promoter spanning -124 to -403bp downstream from the HIF response element (HRE). This was associated with enhanced CBP/p300 histone acetyltransferase binding to this same region. Bioinformatic analysis identified a putative Zn²⁺ finger region in the Spry2 cysteine rich domain; therefore, we tested the effect of mutations to this region upon Spry2-DNA interaction and associated H3 modifications. When overexpressed in HBE, wild-type Spry2 bound strongly to regions spanning the HRE and GC-rich domains of the VEGF-A promoter and this was abolished by C218A or C221A mutation, which also raised H3 pS10, acK14 modification. We conclude that Spry2 directly participates in the epigenetic regulation of VEGFA gene expression. Given its established role as a regulator of airway branching periodicity, we propose that Spry2 links this process to vascular gene expression and so accounts for the tightly co-ordinated development of both structures in the respiratory tree.

Scott CL, Walker DJ, Cwiklinski E, Tait C, Tee AR, Land SC (2010). Control of HIF-1 α and vascular signaling in fetal lung involves cross talk between mTORC1 and the FGF-10/FGFR2b/Spry2 airway branching periodicity clock. *Am J Physiol Lung Cell Mol Physiol.* **299**(4):L455-71.

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PC41

Relationship between Bradykinin-induced relaxation in human bronchi and endogenous epoxy-eicosanoid synthesis

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Asthma is an inflammatory disease characterised by bronchoconstriction and increased mucus secretion which may be modulated by endogenous lipids (Prostaglandins, Leukotrienes and Eicosanoids). Epoxy-eicosanoids (EETs) have been shown to play key roles in inflammation resolution and broncho-relaxation. EETs are produced in epithelial cells by CYP 450 epoxygenase and are metabolized by soluble Epoxide Hydrolase (sEH) in non-active dihydroxyeicosatrienoic acid (DHET). While the mode of action and physiological involvements of EETs are well established (Morin *et al.*, 2007, 2008, 2010 AJRCMB), it is still unknown what triggers their endogenous production. The relaxing effect of Bradykinin is already known to be related to endogenous production of epithelial derived hyperpolarising factors (EpDHF), nitric oxide (NO) and Prostacyclin (Pgl₂). Because of their effects on membrane potential, EETs have been reported to be a candidate for EpDHF. Working hypothesis: There is a relationship between Bradykinin stimulation

and endogenous EET production in human bronchi. Specific objectives: 1) To quantify relaxing effects of exogenous Bradykinin and 14,15-EET on human bronchi. 2) To assess the effects of various enzymatic inhibitors on the relaxing effects of Bradykinin and 14,15-EET on human bronchi. 3) To evaluate the hyperpolarising effects of these compounds on airway smooth muscle cells (ASMC) in presence (or absence) of specific CYP 450 epoxygenase inhibitors. Methods and Results: Isometric tension measurements were performed on distal human bronchi recovered from pulmonary resections, in an isolated organ bath system. All procedures were approved by the Ethics Committee of our institution (protocol Nb: 05-088-S1-R2). 1 μ M of Bradykinin or 1 μ M of 14,15-EET induced 50% relaxation (n=15) of tension induced by 30 nM of U-46619 (a Thromboxane A₂ analogue). These relaxing effects of Bradykinin were reduced by 50% upon addition of 10 nM Iberitoxin (a BKCa channel blocker, n=6), by 40% following addition of 10 μ M 14,15-EEZE (an EET antagonist, n=9) and by 35% with 3 μ M MS-PPOH (an epoxygenase inhibitor, n=15). Hence, Bradykinin and EET display net hyperpolarising effects on ASMC, which are related to the activation of BKCa channels and yield relaxation. Moreover, preliminary results indicate that 3 μ M MS-PPOH reduces the hyperpolarising effects of Bradykinin (n=2) on ASMC by 50%, as assessed by microelectrode measurements. All results were subjected to a Wilcoxon Signed Rank test and were significant (p<0.05). Together, our data support the working hypothesis and suggest a relationship between Bradykinin and endogenous EET production. Because of their potent anti-inflammatory and relaxing properties, epoxy-eicosanoids signalling is a promising target in asthma and COPD.

Morin C, Sirois M, Echave V, Gomes MM, Rousseau E. Epoxyeicosatrienoic

acid relaxing effects involve Ca²⁺-activated K channel activation and CPI-17 dephosphorylation in human bronchi. *Am J Respir Cell Mol Biol* 36: 633–641, 2007.

Morin C, Sirois M, Echave V, Gomes MM, Rousseau E. EET displays anti-inflammatory effects in TNF- α stimulated human bronchi: putative role of CPI-17. *Am J Respir Cell Mol Biol* 38: 192–201, 2008.

Morin C, Sirois M, Echave V, Albadine R, Rousseau E. 17,18-EpETE Targets PPAR γ and p38MAPK to mediate its anti-inflammatory effect in lung: role of sEH. *Am J Respir Cell Mol Biol* 43: 564–575, 2010. Farid Yannick Senouvo,1 Yacine Tabet,1 Caroline Morin,1 Roula Albadine,2 Chantal Sirois,3

and Eric Rousseau.1 Improved bioavailability of epoxyeicosatrienoic acids reduces TP-receptor agonist-induced tension in human bronchi. *Am J Physiol Lung Cell Mol Physiol* 301: L675–L682, 2011.

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PC42

Forced swim stress is as an effective physical stress in reinstating morphine-induced place preference in rats

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Several lines of evidence have shown that stress has key role in different aspect of physiological process. Relapse to drug-seeking in abstinent morphine addicts and reinstatement in experimental animals are observed when exposed to drug-associated stimuli or cues, the drug itself, and stressful events. However, the effect of forced swim stress (FSS) as a physical stress on the reinstatement of opiate-seeking after extinction has not been fully studied. Therefore, we tried to investigate the effects of FFS in induction of morphine conditioned place preference (CPP) reinstatement. In this present study, the CPP paradigm was done and animal displacement, conditioning score and locomotor activity were recorded by Ethovision software. Forced swim stress was induced by putting the animal in a cylinder filled with water (27 °C) for 6 minutes. After 10 minutes, morphine (5mg/kg) was administered subcutaneously and the animal was inserted in to its assigned compartment in CPP box for 30 minutes. All animals underwent extinction sessions until the CPP was extinguished; rats were confined to the previous morphine- or saline-paired compartment for 30 min a day for 8 days. The next day following the last extinction session, morphine CPP reinstatement was induced by FSS as stress-induced reinstatement. In FSS protocol, each animal was put in a cylinder filled with water (23–27 °C) and was forced to swim for 6 min. The results showed that FSS could induce reinstatement of morphine CPP. It is supposed that there is a relationship between this model of physical stress and the level of glucocorticoids during forced swim stress protocol in the rats.

HUMS

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PC43

A general mathematical model of transduction events in mechano-sensory stretch receptors

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Crayfish (*Astacus astacus*) muscle stretch receptors show strong homology to mammalian muscle spindles and bipolar neurons in *D. melanogaster*. All are typical, non-ciliated, stretch-sensitive, afferent neurons. Such receptors are observed in many species and perform an important sensory role. However, they are poorly characterised. A previous study reported

a bio-mechanical and behavioural model of *A. astacus* stretch receptors, which used the principles of elasticity and tension in a spring to describe the adaptation of a mechano-sensory ending. This model described the changing mechano-sensory currents in the receptor when subjected to a stretch protocol. This model was re-implemented and extended. Notably, additional descriptions of voltage-gated channels, that are suggested to contribute to stretch receptor mechano-transduction, were introduced.

Our model now presents a more complete picture of the initiation of the mechano-receptor potential in response to a stretching stimulus. It describes the initial, stretch-mediated depolarisation; subsequent, voltage-activated channel activity and the transition from dynamic to static stretching. The inclusion of voltage-dependent sodium and potassium currents in addition to the initial mechano-sensitive sodium current allowed the model to account for most of the initial stretch response of the receptor. A further stretch-activated potassium channel is also suggested to be present. This preliminary model has potential for extension to describe fully the behaviour of non-ciliated mechano-sensors across species and predict the molecular mediators of mechano-transduction.

Swerup C & Rydqvist B. (1996). *J. Neurophysiol.* 76, 2211–20.

Platkiewicz J & Brette R. (2010). *PLoS Comput. Biol.* 6, e1000850.

Angelino E & Brenner MP. (2007). *PLoS. Comput. Biol.* 3, 1751–60.

Hunt CC, Wilkinson RS & Fukami Y. (1978). *J. Gen. Physiol.* 71, 683–98.

Ottoson D & Swerup C. (1985). *Brain Res.* 336, 1–8.

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PC44

Ca²⁺ influx evoked by high-frequency sustained firing in rat retinal ganglion cells is modulated by Kv3 conductance

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Expression of non-inactivating Kv3.1/Kv3.2 potassium channels is necessary for the fast-spiking phenotype of many types of central neurons including retinal ganglion cells RGCs (1;2). Another reported function of Kv3 channels is to regulate neurotransmitter release from presynaptic terminals (3). In the present study we investigated how Kv3 channel block by low TEA concentrations alters firing properties and Ca²⁺ influx in the rat RGCs somata. Experiments were carried out on the whole-mount retinal preparations from killed (by decapitation after CO₂-anesthesia) Wistar rats (4-6 weeks old) using simultaneous whole cell patch clamp techniques and intracellular Ca²⁺ measurements. All of the tested cells demonstrated high-frequency sustained firing behaviour at a mean frequency of 60±13Hz (n=19). Mean action potential (AP) parameters were: half-width 1.59±0.12ms; peak amplitude 87±1mV and after-hyperpolarization amplitude -17±1mV (n=19). Bath applications of TEA (250µM, 500µM and 1mM) led to significant

(P<0.05, ANOVA) changes in firing properties: firing frequency was decreased by 42%, 53% and 54%; mean half-width increased by 41%, 74% and 86%; mean afterhyperpolarization amplitude decreased by 41%, 59% and 68%, respectively (n=6). Either bath application of the Kv1 channel blocker α -dendrotoxin (100nM, n=5) or Cd²⁺ (200µM, n=4), which blocks Ca²⁺ entry and consequent activation of K_{Ca} channels, had no significant effect on firing properties. This indicates that the observed effects of TEA are not produced by Kv1 or K_{Ca} channels blocking. We observed a linearly-increasing Ca²⁺ signal during depolarization-evoked AP firing. After rapid termination of firing the intracellular Ca²⁺ concentration declined exponentially with a time constant of 5.5±0.5s (n=7). The amplitude of the Ca²⁺ signals depended quite linearly on number of APs with a mean slope of 7±1nM per one AP (n=7) and zero intercept. Since in adult mammalian RGCs the Ca²⁺ binding ratio is not too high (4), the short AP duration should be an important factor in limiting the intracellular Ca²⁺ rise during fast firing under natural conditions. Indeed, AP widening by TEA led to an increase in the Ca²⁺-transient amplitude at a given firing frequency. Bath application of TEA in a dose-dependent manner affected the slope of the amplitude vs AP number plots: 250µM of TEA increased the mean slope value to 11±1nM/AP, 500µM to 14±2nM/AP and 1mM to 16±3nM/AP (n=7). These were all significantly (P<0.05) different from controls and from each other except between 500µM and 1mM. This is consistent with the pharmacological properties of Kv3.1/Kv3.2 channels: the TEA IC₅₀ is in the range 150-300µM with almost complete block at 1mM (2). Thus, Kv3.1/Kv3.2 channels underly the fast firing of the rat RGCs, and restrict Ca²⁺ influx, thus protecting the cells from its cytotoxic action (5).

Henne J, Jeserich G., *J Neurosci Res* 2004 Jan 1;75(1):44-54.

Rudy B, Chow A, Lau D, Amarillo Y, Ozaita A, Saganich M, et al., *Ann N Y Acad Sci* 1999 Apr 30;868:304-43.

Goldberg EM, Watanabe S, Chang SY, Joho RH, Huang ZJ, Leonard CS, et al., *J Neurosci* 2005 May 25;25(21):5230-5.

Mann M, Haq W, Zabel T, Guenther E, Zrenner E, Ladewig T., *Eur J Neurosci* 2005 Dec;22(11):2735-43.

Szydlowska K, Tymianski M., *Cell Calcium* 2010 Feb;47(2):122-9.

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PC45

The AMP-Activated Protein Kinase (AMPK)-Uncoupling Protein 2 (UCP2) pathways controls glucose sensing and whole body glucose counterregulation in mouse

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Certain brain regions contain specialised glucose sensing (GS) neurons that detect falling blood glucose concentrations. Recent studies suggest that there are parallels between these GS neurons and pancreatic β -cells, in that glucokinase, the ATP-sensitive K⁺ channel and AMPK are all thought to play an integral part (1). AMPK is a serine/threonine kinase composed of

α , β and γ subunits, of which there are multiple isoforms (2 α , 2 β and 3 γ -subunits), and has been described as an intracellular “fuel gauge”, activated by either an increase in AMP, or a decrease in the ATP:AMP ratio. Several groups have suggested that AMPK controls GS at the cellular and whole body level (2, 3), yet little is known about the downstream mechanisms of AMPK in these neurons. We have been studying hypothalamic GS using the gonadotropin-releasing hormone (GnRH)-secreting GT1-7 cell line allowing detailed examination of the molecular mechanisms of GS and the role of AMPK.

We have assessed GT1-7 cell GS by electrophysiological recording using the perforated patch configuration and gene/protein expression using qRT-PCR and Western blotting, respectively. Kinase activity was measured by radiolabelled activity assay. Furthermore, we have assessed whole body glucose counterregulation in mice using single-catheter hyperinsulinaemic-hypoglycaemic clamp study.

To investigate the role of AMPK in GT1-7 cell GS, we generated stable cell lines with knockdown of α 2AMPK using lentiviral delivery of short hairpin RNA (shRNA) or a control non-targeting shRNA. Suppression of α 2AMPK expression led to decreased AMPK activation during low glucose treatment of infected GT1-7 cells (0.5 mM; n=6). Furthermore, GS, as measured by changes in electrical activity, was significantly blunted and shifted toward a lower glucose concentration, correlating with previous studies of GS neurons in ex vivo slices. We also observed a significant reduction in UCP2 mRNA expression in α 2AMPK shRNA-treated cells (n=3), indicating that UCP2 may be involved in GS. To investigate this, we utilised genipin, a pharmacological inhibitor of UCP2 on wildtype GT1-7 cells. Genipin reversed the effects of low glucose on electrical activity (n=5), indicating that UCP2 activity may be required for GS. To determine whether UCP2 activity is required for whole body GS, whole body UCP2 knockout mice underwent hyperinsulinaemic-hypoglycaemic clamp study. UCP2 $-/-$ mice required significantly greater exogenous glucose during the clamp study, indicating a poor response to hypoglycaemia. This was mediated by blunting of the glucagon and adrenaline response to hypoglycaemia (n=8). Together, these data indicate that the AMPK-UCP2 pathway may be important for GS and hypoglycaemia counterregulation in diabetes.

1. Beall C, Ashford ML, and McCrimmon RJ. The physiology and pathophysiology of the neural control of the counterregulatory response. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 302: R215-R223, 2012.

2. Beall C, Piipari K, Al-Qassab H, Smith MA, Parker N, Carling D, Viollet B, Withers DJ, and Ashford MLJ. Loss of AMP-activated protein kinase α 2 subunit in mouse beta-cells impairs glucose-stimulated insulin secretion and inhibits their sensitivity to hypoglycaemia. *Biochemical Journal* 429: 323-333, 2010.

3. Claret M, Smith MA, Batterham RL, Selman C, Choudhury AI, Fryer LG, Clements M, Al-Qassab H, Heffron H, Xu AW, Speakman JR, Barsh GS, Viollet B, Vaulont S, Ashford ML, Carling D, and Withers DJ. AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. *J Clin Invest* 117: 2325-2336, 2007.

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PC46

Pharmacological profile of non-canonical mGluR regulating mechanosensory nerve terminal firing

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The system involved in mechanotransduction by proprioceptive sensory organs, such as muscle spindles, is poorly understood. We recently reported that stretch releases glutamate from synaptic-like vesicles within spindle terminals, and activates a non-canonical mGluR (Bewick et al, 2005). To further investigate the receptor's pharmacology, ligands selective for classical mGluRs were screened for their ability to alter stretch-evoked spindle firing. In addition, due to kainate's potency and its ease of chemical modification, novel kainate-derived compounds were tested to find more potent analogues suitable for “click chemistry” (Kolb et al, 2001).

4th lumbrical nerve-muscle preparations were excised from humanely killed (Schedule 1, ASPA, 1986) adult male Sprague-Dawley rats (305-488 g). Spindle discharges were recorded en passant from the muscle nerve during 1 mm stretch-and-hold cycles. The stretch-evoked firing in compounds applied for 60 min was compared to that in saline alone, with differences in mean firing frequencies (impulses/sec) evaluated by ANOVA with Bonferroni post test (significance threshold $P = 0.05$). Glutamate (1 mM) increased afferent firing by $52.3 \pm 13.0\%$ (mean $\pm$ S.E.M; n = 10; $P < 0.01$). PCCG-13 (10 μ M), a selective PLD-coupled mGluR antagonist, decreased firing by $34.6 \pm 4.4\%$ (n = 6; $P < 0.01$). These confirmed previous results and served as a comparison with new compounds. Quisqualate and kainate were more potent agonists, with 10 μ M increasing firing by $56.3 \pm 11.9\%$ (n = 7; $P < 0.001$) and $29.4 \pm 7.2\%$ (n = 7; $P < 0.05$) respectively.

Novel kainate analogues ZCZ49 and ZCZ50 produced no significant change in afferent firing. However, ZCZ90 was more potent than kainate, increasing firing by $34.3 \pm 6.5\%$ (n = 8; $P < 0.001$) at 1 μ M and by $42.0 \pm 7.1\%$ (n = 8; $P < 0.001$) at 10 μ M.

Many compounds with activities at cloned mGluRs had no significant effect on afferent firing (all $P > 0.05$ at 10 μ M); ibotenate (n = 5), DCG-IV (n = 6), ACPD (n = 8), L-AP4 (n = 8) and, importantly, 100 μ M LY341495 (n = 13), a concentration that blocks all cloned mGluRs (Kingston et al, 1998). Furthermore, while 200 μ M S-DHPG (n = 5) had no effect on firing, 1 μ M R-DHPG decreased firing by $20.9 \pm 3.7\%$ (n = 7; $P < 0.05$).

These data are further evidence mechanosensory terminals contain an atypical mGluR, with a ligand-activity profile distinct from cloned mGluRs but potentially antagonised by PCCG-13, the specific antagonist of PLD-coupled mGluR (Albani-Torregrossa et al, 1999). Most interestingly, quisqualate is the most potent agonist, LY341495 (antagonist of all cloned mGluRs) has no effect and R-DHPG (c.f. the group I agonist, S-DHPG) is an antagonist. ZCZ90, our novel kainate analogue, is amenable to the addition of fluorescent and crosslinker tags for use in receptor visualisation and “pull down assays” in future studies.

Bewick GS et al. (2005). *J Physiol* 562(2), 381-394

Kolb HC et al. (2001) *Angew Chem Int Edit* 40(11), 2004-2021

Kingston AE et al. (1998) *Neuropharmacology* 37(1), 1-12

Albani-Torregrossa S et al. (1999) *Mol Pharmacol* 55(4), 699-707

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PC47

Low open probability receptor activation reveals a mechanism for the partial agonist action of sarcosine at rat GluN1/GluN2B NMDA receptors

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N-methyl-D-aspartate receptors (NMDAR) are part of the ionotropic glutamate receptor family and are important in several physiological and pathophysiological processes in the mammalian central nervous system. The binding of both glutamate and the co-agonist glycine in order to activate NMDAR opening is a unique property of this receptor type. Consequently, various endogenous ligands at the NMDAR glycine-binding site such as D-serine have been studied extensively and are thought play important roles in synaptic function (Paoletti & Neyton, 2007). Moreover, the clinical use of NMDAR-glycine site agonists or glycine uptake transporter antagonists has been explored as these actions may be expected to enhance excitatory neurotransmission mediated by NMDARs in the central nervous system and could alleviate the hypofunction of NMDARs implicated in schizophrenia (Lane et al. 2005). One such agonist that is undergoing clinical trials is sarcosine (N-methylglycine) an endogenous amino acid in the brain that takes part in the metabolic pathways of glycine biosynthesis and metabolism. Sarcosine is a substrate inhibitor of the Type I glycine transporter (GlyT1) but also acts as an NMDAR co-agonist (Zhang et al. 2009). The mechanism of NMDAR activation by sarcosine remains to be elucidated and the purpose of this study was to improve our understanding of NMDAR activation by sarcosine. Macroscopic and single channel NMDAR currents were recorded from *Xenopus laevis* oocytes expressing recombinant GluN1 and GluN2B subunits. Dose-response curves from oocytes injected with recombinant NMDARs in the presence of saturating concentrations of glutamate demonstrated that sarcosine (sarcosine EC_{50} = $28.1 \pm 2 \mu\text{M}$, $n=8$) was approximately 100-fold less potent than glycine (glycine EC_{50} = $0.2 \pm 0.01 \mu\text{M}$, $n=5$). Furthermore, the maximal response exhibited by sarcosine at $300 \mu\text{M}$ was approximately 20 % lower than the maximal response evoked by saturating ($50 \mu\text{M}$) concentrations of glycine. These data confirm that sarcosine is acting as a partial agonist (relative to glycine) at GluN1/GluN2B NMDARs. Single channel recordings show that sarcosine did not significantly change the amplitude or duration of individual channel openings relative to glycine and that the partial agonism exhibited by sarcosine results from a combination of reduced channel open probability during the receptor activation and shortened duration of receptor activation. Lane HY et al. (2005). *Arch Gen Psych* **62**, 1196-1204.

Paoletti P & Neyton J (2007). *Curr Opin Neurobiol* **7**, 39-47.

Zhang HX et al. (2009). *J Physiol* **587**, 3207-3220.

This work was funded by grants from the Royal Society and the Royal Holloway Research Strategy Fund.

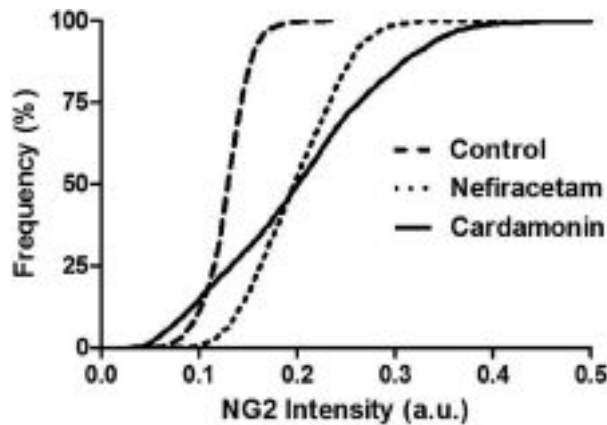
PC48

Exploring the mechanism of action of a novel remyelinating therapeutic

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Enhancing the rate of myelination and subsequent myelin repair could potentially arrest and reverse the disease progression of demyelinating disorders. To date, no such therapeutics with pro-remyelinating properties have been identified. We investigated the pro-myelinating properties of nefiracetam, and whether it exerts these effects by modulating Wnt signalling, which is known to play a role in oligodendrocyte development (Feigenson et al, 2009). To investigate this, myelin formation, as assessed by immunofluorescent staining for MBP, was examined in organotypic hippocampal cultures from Wistar rats. When nefiracetam ($1 \mu\text{M}$) was applied to the medium from the first day of culture (1DIV), the density of MBP staining was increased at 6DIV (209.2 ± 47.7 , $n=18$, $p<0.05$, ANOVA) relative to untreated control (100 ± 14 , $n=18$). Treatment with $10 \mu\text{M}$ cardamonin, an inhibitor of β -catenin signalling did not cause a change in myelin density (109.2 ± 30.3 , $n=18$, $p>0.05$). However, both nefiracetam and cardamonin increased immunofluorescence of NG2, an oligodendrocyte precursor marker ($p<0.001$, Kolmogorov-Smirnov test, $2825 \leq n \leq 3622$, fig.1). This suggests that, while modifying Wnt signalling may impact on the development of precursor cells, this does not correspond to an increase in mature myelinating cells. Therefore, the pro-myelinating effects of nefiracetam must involve some additional mechanism. We next investigated the effect of nefiracetam on spontaneous activity, which also modulates myelination (Demerens et al, 1996). Cultures were exposed to $250 \mu\text{g/ml}$ lyssolecithin (LPC) for 18 hours at 13DIV. After LPC exposure, cultures recovered in LPC-free medium $\pm 1 \mu\text{M}$ nefiracetam. After 24 hours, cultures were loaded with Fluo4-AM, and the number of cells displaying spontaneous calcium activity measured. Cultures not exposed to LPC displayed low levels of activity (1.7% of 5174 cells), which was unchanged by nefiracetam treatment (1.7% of 4819 cells, $p=0.93$, X2 test). LPC exposure caused an increase in activity (4.7% of 5608 cells, $p<0.001$), which was enhanced by nefiracetam treatment (12.9% of 4506 cells, $p<0.001$). When MBP density was measured in these cultures, LPC caused decreased MBP density (49.9 ± 5.3 , $n=27$, $P<0.05$, ANOVA) compared to control (100 ± 11 , $n=27$), but nefiracetam exposure post LPC caused a return to control levels (80 ± 10.7 , $n=24$, $p>0.05$), suggesting that the increase in post LPC activity may be an adaptive response related to myelin repair, which is enhanced by nefiracetam. Together, these data suggest a complex mechanism of action for nefiracetam with respect to its action on myelin.



Feigenson K, Reid M, See J, Crenshaw E.B, Grinspan J.B. 2009. Wnt signalling is sufficient to perturb oligodendrocyte maturation. *Molecular and Cellular Neuroscience*. 42:255-265.

Demerens, C, Stankoff B, Anglade P, Allinquant B, Couraud F, Zalc B, Lubetzki C. 1996. Induction of myelination in the central nervous system by electrical activity. *Proceedings of the National Academy of Sciences (USA)*. 93:9887-9892

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PC49

The glycogen contribution to lactate release from adult mouse optic nerve is dependent upon tissue energy demand

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The presence of glycogen in the central nervous has been known for many years, but assigning a physiological role has been difficult. Recent experiments in adult rodent optic nerve have shown that glycogen supports axonal conduction during periods of decreased glucose availability and increased energy demand under normoglycaemic conditions (Brown et al., 2003). It is accepted that glycogen is metabolized to lactate in astrocytes, and released into the extracellular space, for uptake by axons. Although lactate has been measured in the hundreds of micromolar range in the brain extracellular space (Hu & Wilson, 1997), the dynamics of lactate release and the relative contribution of glycolysis and glycogenolysis are unknown. In this study we used lactate biosensors placed against the optic nerve to measure real-time lactate levels in response to a variety of metabolic interventions.

We used enzyme-based sensors to measure lactate release from adult mouse optic nerve, while simultaneously recording the stimulus evoked compound action potential (CAP), to correlate axon conduction with lactate concentration ([lactate]_o). Recordings were carried out in vitro in a superfusion chamber at 37°C. The basal [lactate]_o recorded from nerves perfused with 10 mM glucose aCSF was $410.2 \pm 28.3 \mu\text{M}$ (n = 18), implying continuous release of lactate under resting conditions. The [lactate]_o did not change when glucose was increased above 5 mM, but decreased with [glucose] of 2, 1 and 0.5 mM. Glucose withdrawal led to a rapid fall in [lactate]_o but a delayed fall in the CAP (Brown et al., 2003), confirming glucose as the source of lactate. Both the [lactate]_o and CAP

were rapidly abolished in the presence of the glycolytic inhibitor iodoacetate, implying an absolute requirement for glycolytic metabolism of glucose for both axon conduction and lactate production. In the presence of the glycogen phosphorylase inhibitor DAB [lactate]_o decreased by $22.2 \pm 1.6 \%$ (n = 3) indicating glycogen metabolism contributes to the lactate signal. Imposing a 10 kHz stimulus on the nerve resulted in a $14.7 \pm 2.3 \%$ increase in [lactate]_o with subsequent DAB introduction resulting in a fall of $33.9 \pm 2.0 \%$ (n = 3) in [lactate]_o, implying that increased tissue energy demand activates glycogenolysis, such that the contribution of glycogen-derived lactate to the [lactate]_o increases relative to tissue energy demand.

These data indicate that mouse optic nerve continually releases lactate into the extracellular space. The source of lactate is via both metabolism of glucose and glycogen, but the contribution of glycogen-derived lactate increases in line with tissue energy demand.

Brown AM, Baltan Tekkök S & Ransom BR. (2003). Glycogen regulation and functional role in mouse white matter. *Journal of Physiology* 549.2, 501-512.

Hu Y & Wilson GS. (1997). A temporary local energy pool coupled to neuronal activity: fluctuations of extracellular lactate levels in rat brain monitored with rapid-response enzyme-based sensor. *Journal of Neurochemistry* 69, 1484-1490.

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PC50

A confocal study of the coexistence of neuronal nitric oxide synthase and tyrosine hydroxylase in the rat nigrostriatal pathway

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Nitric oxide is highly mobile and unconstrained by cell membranes. As such, it can therefore act across a long distance as a volume transmitter. Spillover of nitric oxide between neurons might have a major impact on central nervous system diseases and particularly on neurodegeneration. In this sense, there is abundant evidence indicating that the communication between nitrergic and dopaminergic systems plays an essential role in the control of motor function. Dopaminergic boutons represent nearly 10% of all striatal synapses (Arbuthnott and Wickens 2007). The release of a single vesicle of dopamine could modulate the excitability of tens to thousands of synapses within distances a few micrometers of a release site, in both substantia nigra compacta and striatum (Cragg et al., 2001). It is predicted that the physiological volume of influence of a single source of nitric oxide that emits for 1 to 10 seconds has a diameter of about 200 μm, corresponding to a volume of brain enclosing 2 million synapses (Wood and Garthwaite 1994). However, there is sparse information available for either the coexistence or overlap between nitric oxide and dopamine in the nigrostriatal region, which in turn may be a mechanism of functional significance. The dual localization of immunoreactivity for nitric oxide synthase and tyro-

sine hydroxylase, enzymes responsible for the synthesis of nitric oxide and dopamine, respectively, in the neurons of the nigrostriatal pathway in the rat brain was examined by means of a double-immunohistochemical method and confocal laser scanning microscopy. The study was performed on six adult Wistar rats. After perfusion and fixation, the brains were cut, immuno-stained for tyrosine hydroxylase and nitric oxide synthase. We investigated neuronal populations in the frontal cortex, striatum (ventral and dorsal), globus pallidus, subthalamic nuclei, substantia nigra and in the pedunculopontine nuclei. High-resolution confocal laser scanning microscopy revealed nitric oxide synthase immunoreactive fiber buttons in submicrometer proximity to both the axon/dendrite and soma of tyrosine hydroxylase immunoreactive neurons and fibers of each studied region. Among them, a considerable number showed close proximity of both neurotransmitters. Only a small portion of the widely co-distributed cells was double labeled with both antibodies. Pharmacological manipulation of nitric oxide signaling affected the response of experimental Parkinsonism (Gomes et al., 2008) and levodopa-induced dyskinesia (Padovan-Neto et al., 2009). 1). Based (i) on the localization of tyrosine hydroxylase-nitric oxide synthase-positive processes/neuronal body and vice versa, and (ii) the similar physiological responses effected in pharmacological studies of either transmitter system in the nigrostriatal organization, we have identified anatomical/physiological sites where the two neurotransmitter types interact with each other. Arbutnott GW, Wickens J. 2007. Space, time and dopamine. Trends Neurosci. 30:62–69

Cragg, S.J., Nicholson, C., Kume-Kick, J., Tao, L., Rice, M.E., 2001. Dopamine-mediated volume transmission in midbrain is regulated by distinct extracellular geometry and uptake J. Neurophysiol 85, 1761–1771

Gomes, MZ, Raizman-Vozari, R Del-Bel, EA (2008) A nitric oxide synthase inhibitor decreases 6-hydroxydopamine effects on tyrosine hydroxylase and neuronal nitric oxide synthase in the rat nigrostriatal pathway. Brain Research. 01:12 – 24.

Padovan-Neto, F.E., Echeverry, M.B., Tumas, V., Del-Bel, E.A. (2009) Nitric oxide synthase inhibition attenuates L-DOPA-induced dyskinesias in a rodent model of Parkinsons disease. Neuroscience 159(3):927–35.

Wood J Garthwaite J (1994) Models of the diffusional spread of nitric oxide: implications for neural nitric oxide signalling and its pharmacological properties. Neuropharmacology.33(11):1235–44

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PC51

Model of organophosphorus toxicity in mice

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Organophosphorus (OP) pesticide toxicity is a global health problem. However, the underlying mechanism is still unclear. OP pesticides are inhibitors of cholinesterases. Hypothesized mechanisms for respiratory failure include overstimulation of nicotinic acetylcholine receptors (nAChRs) and down regula-

tion of post synaptic nAChRs, (De Bleeker, 1995, Senanayake and Karalliedde, 1987). We are addressing these hypotheses by studying OP toxicity in vivo and in vitro. Mice anesthetized with isoflurane and medical oxygen (0.4L/min) were treated with either saline placebo or the commercial OP pesticide dimethoate EC40 (emulsifiable concentrate; 0.12mg–0.25mg/kg IP) and mechanically ventilated throughout the experiment. Noradrenaline (4mg%–20mg%) was used to maintain the mean blood pressure above 75mmHg. Mechanical muscle twitch strength was measured in response to indirect stimulation via the tibial nerve (train-of-four stimuli). Animals were maintained for 4–5hrs and showed a decline in neuromuscular function that was consistent with failure of neuromuscular transmission. To test the direct effect of OP compound in NMJ transmission, we made intracellular electrophysiological recordings of resting membrane potential, spontaneous miniature endplate potentials (MEPPs) and evoked endplate potentials (EPPs) in isolated sciatic nerve/flexor digitorum brevis muscles using different treatments in vitro. Experiments were conducted in Hepes buffered mammalian physiological saline (MPS) and action potentials were abolished with μ -conotoxin (2 μ M). We compared the effects of toxic minipig plasma (extracted in-house from minipigs treated with (orally) either dimethoate EC40, or non-formulated dimethoate active ingredient (AI) alone) with neostigmine as positive control.

Toxic plasma abolished synaptic transmission within 30–60 minutes of bath application with little change in the time course of MEPPs or EPPs. By contrast, an increased half decay time was detected in neostigmine and dimethoate AI treated preparations, in both EPPs (Hepes MPS, 3.44 $\pm$ 0.60sec; control plasma, 3.24 $\pm$ 0.49sec; EC plasma, 4.10 $\pm$ 2.38sec; AI plasma, 6.91 $\pm$ 2.04sec; neostigmine, 7.74 $\pm$ 2.16sec; $p < 0.001$, ANOVA, $n = 20$ –30 fibres in each of 2–4 mice) and MEPPs (Hepes MPS, 2.56 $\pm$ 0.76sec; EC plasma, 2.81 $\pm$ 0.67sec; AI plasma, 4.55 $\pm$ 0.90sec; neostigmine, 3.66 $\pm$ 0.81sec; $p < 0.001$ ANOVA). However, there was no significant change in half-decay time in dimethoate EC plasma compared to control, indicating involvement of complex pathway in transmission failure. Furthermore, dimethoate EC plasma produced increased spontaneous activity compared to other treatments (MEPP frequency: Hepes MPS, 0.86 $\pm$ 0.45sec⁻¹; EC plasma, 3.04 $\pm$ 0.91sec⁻¹; AI plasma, 0.63 $\pm$ 0.27sec⁻¹; neostigmine, 0.66 $\pm$ 0.44sec⁻¹; $p < 0.001$ one way ANOVA). The data indicate that neuromuscular transmission failure with commercially available OP pesticides cannot be explained solely by inhibition of acetylcholinesterase.

De Bleeker JL (1995) The intermediate syndrome in organophosphate poisoning: an overview of experimental and clinical observations. J Toxicol Clin Toxicol 33:683–686

Senanayake N, Karalliedde L (1987) Neurotoxic effects of organophosphorus insecticides. An intermediate syndrome. N Engl J Med 316:761–763

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PC52

Short-term synaptic plasticity in the sensory thalamus controlled by sleep state-related neuromodulators

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The molecular and cellular processes controlling the rapid change among different sleep-wakefulness stages in mammals are not fully clear. Although the sleep-relevant neuromodulators have been shown to alter membrane properties and thus firing patterns of thalamic neurons, their modulatory effect on thalamic synaptic transmission is less noted. At the retinogeniculate synapse that shows characteristic short-term synaptic depression (paired-pulse ratio of 0.33 ± 0.08 , $n=15$), we investigated the modulatory effect of combined neuromodulators mimicking different sleep-wakefulness states. The whole brain from the C57/Bl6 mouse (p21-30) was rapidly removed under 1-3% isoflurane inhalation anesthesia, and brain slices containing the retinogeniculate synapse were freshly prepared. We examined the effect of serotonin plus acetylcholine to mimic the awake state (in which the concentration of adenosine should be very low) and the effect of adenosine plus acetylcholine to mimic the paradoxical (rapid eye movement, or REM) sleep state (in which monoamine level is decreased to almost zero). We found that bath application of both 10 nM 5-carboxytryptamine (5-CT, the 5-HT₁ receptor agonist) and 3 μ M (+)-anabasine (ABS, the nicotinic receptor agonist) or of both 10 μ M N6-cyclopentyladenosine (CPA, a potent and selective adenosine A₁ receptor agonist) and 3 μ M ABS results in similar-size postsynaptic AMPA receptor currents in response to paired-pulse stimulation (separated by 50 ms) of the optic tract. The paired-pulse ratio is 1.09 ± 0.11 ($n=5$) and 0.88 ± 0.14 ($n=6$) for the former and the latter combinations, respectively. Moreover, paired stimulation separated by ISIs of a wide range (20-2000 ms) always evokes second EPSC amplitude similar to the first EPSC in the presence of the foregoing combinations of neuromodulators. We further examined the EPSCs in response to a 10 Hz- or 50 Hz-train of 30-stimuli. In control, the postsynaptic currents are more reliably elicited by the earlier than by the later, and by 10 Hz- than 50 Hz-train of, stimuli. This is consistent with an actual "low-pass" transmission through a synapse with short-term depression. In contrast, the elicited synaptic currents all become relatively similar in amplitude in response to either 10 Hz or 50 Hz-train of stimuli and to either earlier or later stimuli in a train in the presence of both nicotinic and adenosine receptor agonists. Thus, short-term plasticity at the retinogeniculate synapse could be essentially abolished over a wide frequency range of presynaptic activity in both awake and paradoxical sleep states. These properties are consistent with a more faithful relay of sensory information through the thalamus during wakefulness and REM sleep, and provide a partial explanation for why we need both serotonin and acetylcholine during wakefulness.

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PC53

The acetylcholine receptor current of neonatal mouse inner hair cellsM.G. Evans¹ and H.J. Kennedy²¹ISTM, Keele University, Keele, UK and ²Physiology and Pharmacology, University of Bristol, Bristol, UK

Outer hair cells (OHCs) function as electromotile amplifiers of basilar membrane motion, thereby increasing the mechanical sensory input to the inner hair cells (IHCs) that function as the primary auditory receptors in the cochlea. This action of OHCs is voltage-dependent, and is thought to be under efferent control via the olivocochlear pathway that in mammals targets the OHCs. The efferents exert inhibitory synaptic control via an acetylcholine receptor (AChR) in the OHCs that functions as a calcium influx pathway, leading to activation of calcium-activated potassium channels which increases cell conductance thereby reducing both the receptor potential and electromotility. During cochlear development in mice, which continues during the first two weeks after birth, the efferents transiently innervate the IHCs before reaching the OHCs. This might serve to modulate the firing properties of immature IHCs which are thought to help strengthen the formation of mature auditory neuronal connections. In this study we have investigated responses to ACh in mouse IHCs at 6-10 days after birth, when efferent-IHC synapses are present. The apical turn of the cochlea was isolated and the basal surfaces of the IHCs exposed by mechanically stripping away the outer rim of the organ of Corti, following removal of the tectorial membrane. Whole-cell voltage-clamp recordings were made in artificial perilymph and ACh (0.1 mM) was applied from a puffer pipette positioned nearby. The current-voltage (I-V) relation for the ACh-sensitive current showed inward and outward rectification with a reversal potential at 0 mV (cesium-based internal filling solution with 10 mM BAPTA). The current activated over about 0.5s. The current was cationic since in low (30 mM) external sodium (replaced by n-methyl-glucamine) the reversal potential was -19 mV and currents were much smaller, particularly at negative voltages. Preliminary data is suggestive of a block of the AChR by external divalent cations since the largest responses were observed in solutions lacking calcium or magnesium. The I-V data was fitted with a single energy barrier model, which assumes a single blocking site within the channel pore, usually assumed to be occupied by divalent cations. The fit indicated that the blocking site was 0.3-0.4 of the way through the channel from the outside. In comparison with data from adult outer hair cells the main difference is that AChRs in IHCs produce larger and more slowly activating cationic currents, although it is unclear whether this reflects differences in the respective AChRs. If so, this might indicate that the hair cell AChR itself undergoes maturation so that it is appropriately matched to modulate the function of IHCs and OHCs during development and in the mature cochlea respectively.

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PC54

Acute metabotropic glutamate receptor-dependent synaptic plasticity in a mouse model of fragile-X syndrome

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Fragile X syndrome (FXS) is the most common genetically inherited form of mental retardation affecting approximately 1 in 3-4000 boys in the UK. FXS results from the silencing of the fragile X mental retardation 1 gene (FMR1) and loss of the protein it encodes (fragile X mental retardation protein, FMRP). FMRP is believed to play an important role in the development and regulation of excitatory synaptic transmission (Pfeiffer & Huber, 2009) and in particular, loss of FMRP has been reported to impair synaptic plasticity (Huber et al. 2002) and basal synaptic transmission in the hippocampus of *Fmr1*-null mutant mice.

At Schaffer collateral/commissural inputs to hippocampal CA1 pyramidal neurones, we have examined metabotropic glutamate receptor (mGluR)-induced long-term depression (LTD) using the group 1 mGluR agonist (S)-3,5-dihydroxyphenylglycine (DHPG; 30 or 100 μ M).

In vitro, extracellular field recordings from hippocampal slices revealed that mGluR-LTD is enhanced in male *Fmr1*^{-/-} mice relative to wild type (WT) littermates (30 μ M 80.5 $\pm$ 9 %, *Fmr1*^{-/-}, n = 7 versus 99.1 $\pm$ 9 %, WT, n = 9; 100 μ M 63 $\pm$ 4 %, *Fmr1*^{-/-}, n = 19 versus 77 $\pm$ 5 %, WT, n = 17). In *Fmr1*^{-/-} mice this form of synaptic plasticity was independent of new protein synthesis since in the presence of anisomycin (20 μ M) LTD was still induced and maintained at similar levels (65 $\pm$ 7 %, n = 9) as were observed in recordings in the absence of anisomycin. In slices from WT mice anisomycin prevented the maintenance of LTD (91 $\pm$ 5 %, n = 8).

In addition, we examined mGluR-induced alterations in intrinsic membrane properties – a recently characterized form of plasticity that has not previously been examined in *Fmr1*^{-/-} mice (Brager & Johnston, 2007). No detectable impairments were evident among our data, which measured the relative change in input resistance, resting potential, sag ratio and rheobase before and after DHPG application (WT, n = 19; *Fmr1*^{-/-}, n = 17). These findings suggest that disruptions in mGluR-LTD in *Fmr1*^{-/-} mice are due to changes at the synapse rather than alterations in the intrinsic properties of CA1 pyramidal neurones. Currently, we are investigating mGluR-dependent signaling cascades that may be implicated in synaptic plasticity deficits observed in our mouse model of FXS.

Pfeiffer BE & Huber KM. (2009) *Neuroscientist* **15**, 549-567.

Huber KM et al. (2002). *Proc Natl Acad Sci USA* **99**, 7746-7750.

Brager DH & Johnston D. (2007). *J Neurosci* **27**, 13926-13937.

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PC55

GPCR constitute the local Ca²⁺ signaling which modulate the activation of Ca²⁺ activated Cl⁻ channel

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Proteins of Tmem16 family are the candidate subunits for Ca²⁺-activated Cl⁻ channels (CaCC). We show that CaCC in nociceptors is activated by the release of Ca²⁺ from the IP3-sensitive intracellular stores in response to bradykinin(BK) or proteases (through PAR-2 receptors). Interestingly, while in the majority of rat small DRG neurons CaCC was induced by Ca²⁺ release from the stores, only in 12/58 neurons CaCC was activated by the Ca²⁺ influx through the voltage-gated Ca²⁺ channels. Chelating intracellular Ca²⁺ with the slow Ca²⁺ buffer EGTA did not affect CaCC activation by PAR2 while BAPTA abolished such activation suggesting a close proximity of the Ca²⁺ release sites and CaCC. Acute treatment of rat DRG neurons with lipid raft disrupting agent methyl-beta-cyclodextrin partially restored coupling of CaCC to VGCC but disrupted coupling with the intracellular stores. Thus, after the methyl-beta-cyclodextrin treatment activation of VGCC by voltage pulse CaCC currents in majority of neurons while BK and PAR2-AP failed to induce measurable CaCC in the majority of tested neurons. Membrane fractionation demonstrated that in DRG TMEM16A can be co-purified with lipid raft marker caveolin.

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Role of the neuronal glutamate transporter, EAAT4, in cerebellar Purkinje neuron intrinsic activityE.M. Perkins¹, T. Van Slageren¹, Y.L. Clarkson¹, K. Tanaka² and M. Jackson¹

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Cerebellar Purkinje neurons express the glutamate transporter, EAAT4, which is critical for the removal of glutamate from excitatory synapses, thus maintaining normal neurotransmission by preventing synapse spill-over and glutamate mediated excitotoxicity. In addition, EAAT4 has a prominent glutamate-gated chloride conductance of unknown physiological relevance. Purkinje neurons fire action potentials (AP) tonically in the absence of synaptic activity, but this firing rate is modulated by excitatory and inhibitory synaptic inputs to produce the functional output. The influence of EAAT4 on intrinsic excitability of Purkinje neurons is unknown. Here we investigate the effects of EAAT4 reduction and absence on spontaneous firing activity of current-clamped Purkinje neurons using acute cerebellar slices from EAAT4^{-/-} and EAAT4^{+/-} mice (Huang et al, 2004).

Mean spontaneous Purkinje neuron firing rates in EAAT4^{-/-} and EAAT4^{+/-} mice appeared higher than wildtype (WT) controls although this effect was not significant (WT, 34.6 $\pm$ 1.7 Hz, n = 5; EAAT4^{+/-}, 56.8 $\pm$ 9.5 Hz, n = 7; EAAT4^{-/-}, 54.2 $\pm$ 10.1 Hz, n = 9, data are mean $\pm$ S.E.M). However, firing rates observed in EAAT4^{-/-} and EAAT4^{+/-} mice were variable, with some cells firing at similar levels to WT, whereas some showed much

higher rates. Furthermore, a subset of Purkinje cells from both EAAT4^{-/-} and EAAT4^{+/-} mice displayed bursting patterns of spontaneous activity rather than the regular tonic firing always observed in WT mice (bursting observed in 0/5 WT, 4/9 EAAT4^{+/-} and 5/15 EAAT4^{-/-} cells). This variation in firing rate and bursting activity patterns could be related to the non-uniform expression of EAAT4 in the cerebellum, with higher and lower expression occurring in a banding pattern (Dehnes et al, 1998). In cells showing a bursting pattern of AP activity, blockade of voltage-gated sodium channels by tetrodotoxin (TTX) revealed a TTX-insensitive 'spike' not observed in WT cells, indicating it is not mediated by sodium currents but likely to be due to dendritic calcium channel activity.

The data presented suggest wild type levels of EAAT4 are required for normal Purkinje cell intrinsic excitability. However, the ionic mechanisms underlying the bursting pattern as well as verification of a correlation between EAAT4 expression levels and spontaneous firing rate require further investigation. Dehnes Y., Chaudhry F.A., Ullensvang K., Lehre K.P., Storm-Mathisen J. and Danbolt N.C. (1998) The Glutamate Transporter EAAT4 in Rat Cerebellar Purkinje Cells: A Glutamate-Gated Chloride Channel Concentrated near the Synapse in Parts of the Dendritic Membrane Facing Astroglia. *The Journal of Neuroscience*, 18(10): 3606-3619.

Huang Y.H., Dykes-Hoberg M., Tanaka K., Rothstein J.D. and Bergles D.E. (2004) Climbing Fiber Activation of EAAT4 Transporters and Kainate Receptors in Cerebellar Purkinje Cells. *The Journal of Neuroscience*, 24(1):103-111.

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PC57

Control of hippocampal theta oscillations by rhythmic hyperpolarization

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Network oscillatory activity reflects the dynamic interactions of ensemble neurons in information coding. Theta oscillations are a dominant oscillatory pattern of local field potentials (LFPs) in rodent hippocampus during voluntary behaviours as well as rapid-eye-movement (REM) sleep. Diverse hippocampal interneurons fire phase-locked to theta oscillations with distinct temporal patterns, and have been considered playing important role in shaping theta oscillations. However, their specific contribution to theta generation has yet to be determined. We here recorded interneurons in the hippocampal CA1 area of freely behaving mice, and identified a unique group of theta-locked interneurons. These cells fired at extremely high firing rates (88.05 ± 4.45 Hz) and in reliable theta-rhythmic-bursts with 5-15 spikes per theta cycle during exploration. Granger causality is a statistical concept of causality based on prediction (Geweke, 1982; Geweke, 1984; Granger, 1969; Brovelli et al., 2004), and has been applied to evaluate the causal influence among simultaneous neural signals. We therefore employed the Granger causality method to identify directionality of neuronal interactions between spike activity of these theta-locked interneurons and simultaneous LFPs. Our results showed that theta rhythmic spiking activity of these cells always preceded the theta rhythm of LFPs recorded from the same electrodes. Evaluation of Granger causality relationship between spike and LFP showed over-threshold (per-

mutation test $P < 0.001$) peaks within the theta band, and the peak in the spike-to-LFP direction (peak = 0.90) was much higher than that in the reverse direction. The average values from the five cells confirmed the asymmetric directional influence (Spike-to-LFP: 0.94 ± 0.08 v.s. LFP-to-Spike: 0.19 ± 0.04 , $n=5$, $P < 0.001$, paired t test). Furthermore, higher Granger causality values in the Spike-to-LFP direction in the theta band were also found in the interactions between spike activity and the concurrent LFPs recorded from all of the ipsilateral CA1. These results suggested that these theta-locked cells could generate a strong Granger causal influence on theta oscillations, and thereby could be involved directly in theta generation. And those cells remained significant Granger causal influences on LFP residue theta during slow wave sleep when theta oscillations became not dominant. On the contrary, the other theta-locked interneurons were mainly Granger-causally influenced by, rather than imposing the influence on, LFP theta. Computational simulations demonstrated that the firing of theta-generating cells could uniquely evoke intracellular hyperpolarized theta oscillations of the somatic membrane potential in a model CA1 pyramidal cell, which were highly correlated with recorded LFP theta. The results suggest that this type of theta-locked interneurons could serve somatically as theta-generating cells in shaping hippocampal theta oscillations.

Buzsaki G (2002). *Neuron* 33(3), 325-340.

Klausberger T et al. (2003). *Nature* 421(6925), 844-848.

Czurko A et al. (2011). *J Neurosci* 31(8), 2938-2947.

Ylinen A et al. (1995). *Hippocampus* 5(1), 78-90.

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PC58

Dopamine-induced regulation and de-regulation of catabolism of cyclic ADP-ribose, an intrinsic mTOR signal inhibitor, during development in the rodent striatum

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Abnormal activity of the DA system has been implicated in neurological and psychiatric disorders, such as Parkinson's disease, schizophrenia, bipolar disorder and attention deficit hyperactivity disorder. Thus, the study of DA receptor-mediated intracellular signal transduction has been a primary approach toward understanding the physiological functions as well as pathophysiological aspects of DA-related cellular responses. D1- and D2-class DA receptor subtypes positively and negatively regulate adenylyl cyclase, respectively, and D2 receptors are coupled to phospholipase C. Although the contribution of D1 receptors is unclear, stimulation of D2 receptors can increase intracellular Ca²⁺ concentrations by mobilization of Ca²⁺ from inositol-1,4,5-trisphosphate-sensitive stores, followed by activation of ryanodine-sensitive Ca²⁺ pools. Increased intracellular free calcium concentration ([Ca²⁺]_i) seem to be mediated by interaction of DA receptors with DA receptor interacting proteins, such as neuronal calcium sensor-1 or calycon. Ca²⁺-dependent cellular responses are also mediated by calcineurin to dephosphorylate DPADPP.

Therefore, it is of interest to determine any crosstalk between Ca^{2+} and other second messengers, such as cyclic ADP-ribose (cADPR), in the downstream signalling of DA receptors.

The mTOR pathway has emerged as a regulator of neuroplasticity in the central nervous system. It has been shown that administration of L-DOPA in a mouse model of Parkinsonism leads to dopamine D1 receptor-mediated activation of the mTOR complex 1 (mTORC1), which has been implicated in several forms of synaptic plasticity. The L-DOPA-mediated activation of mTORC1 persisted in mice that developed dyskinesia. Moreover, the mTORC1 inhibitor rapamycin prevented the development of dyskinesia without affecting the therapeutic efficacy of L-DOPA. Thus, the mTORC1 signalling cascade represents a promising target for the design of anti-parkinsonian therapies. Here, we addressed whether cADPR plays a role as a second messenger and modulator of the mTOR pathway downstream of DA receptors through FKBP12.6 or FKBP12 in the rodent striatum. For this purpose, ADP-ribosyl cyclase activity was measured in the crude membrane fractions of rats and mice in the presence or absence of DA, and phosphorylation of S6K in whole homogenates of the isolated mouse striatum after incubation with or without cADPR was measured. mTOR1 activates the S6 K, which is responsible for phosphorylation of the ribosomal protein S6, a component of the 40S ribosomal subunit. cADPR significantly decreased in S6K phosphorylation, which was reversed by the prior administration of 8-bromo-cADPR, a cADPR antagonist. These results suggest that the abundant cADPR seems to be an intrinsic regulator of the mTOR signal in the adult striatum.

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PC59

Localised calcium signalling at the inner hair cell ribbon synapse of the adult mouse

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The inner hair cell (IHC) is the primary sensory cell of the mammalian cochlea, each cell forming multiple synaptic contacts with the auditory nerve. Their ribbon synapses are characterised by dense bodies surrounded by numerous vesicles and capable of multivesicular release precisely linked to depolarization induced by the sound stimulus (Goutman & Glowatzki, 2007). The mechanism for the control of release is incompletely understood. To this end we have been using multiphoton imaging to identify the calcium entry sites near the ribbon when the cell is depolarized under whole cell voltage clamp. Cochlear IHCs were imaged and recorded using an in situ preparation of the CaJ mouse inner ear allowing adult tissue to be studied whilst preserving cochlear geometry. Animals aged P28 and above were used. The temporal bone was removed after killing the animals by cervical dislocation and stuck to the base of a chamber containing artificial perilymph (in mM, NaCl 135, KCl 5.4, MgCl_2 1, CaCl_2 1.3, Na_2HPO_4 0.7, hepes 10, to pH 7.3). Access to the cells was possible through a small opening in the apical turn which exposed the 5-15 kHz region of the cochlear spiral. The ribbon constituent peptide CtBP2 was subsequently identified by immunohistochemistry. In this region we found 6-20 ribbons per IHC (mean 12.2 ± 2.9 S.D., $n = 83$ IHCs) using reconstructed z-stacks to identify the ribbon distribution.

IHCs were recorded with pipettes containing K^+ channel blockers to reduce the large outward currents. Pipettes contained (in mM): CsMeSO₃ 130, TEACl 13, EGTA 1, Mg_2ATP 3, hepes 5, to pH 7.3). OGB-5N (200 μM), a low affinity fluorescent indicator, was included in order to measure intracellular calcium. Inward calcium currents could be recorded on depolarization from -60 to 0 mV. In many cases, simultaneous imaging of the basal pole of the IHC with the multiphoton upright microscope, exciting the dye at 935 nm, revealed large increases in the baseline fluorescence. The largest increases occurred at highly localised regions ('hotspots'). Variability in the size of individual responses was partly ascribed to different amplitude calcium signals arising from presumed ribbon sites at opposite sides of the IHC (Culley & Ashmore, 2010). The voltage dependence of the peak calcium signal at different hotspots was indistinguishable, suggesting the underlying calcium channel type to be identical. Calcium signals also increased with the duration of depolarization and spreading along the IHC axis by about 3 μm for prolonged depolarizations (500 ms). In some cells, a 10ms depolarization generated a calcium signal with $\Delta F/F = 0.27$, rising to 3.2 for 200 ms steps. The results are compatible with a calcium entry highly localised at the ribbon synapse, and strongly buffered around the site of entry into the IHC.

Goutman JD & Glowatzki E. (2007) *Proc Natl Acad Sci USA*. 104:16341-6.

Culley S & Ashmore JF (2010) *Proc Physiol Soc* 19 PC37

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PC60

Expression profile of large conductance calcium-voltage operated potassium channels in an animal obesity model

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Large conductance calcium-voltage operated potassium (BK) channels are a diverse ion channel family that features in many cellular processes, mainly serving as regulators of Ca^{++} entry mediated processes such as neurotransmission and exocytosis. The pore forming α -subunit is coded for by the single gene KCNMA1 which undergoes extensive alternative splicing, imparting differential properties to the overall channel kinetics. One such splice variant, STREX greatly enhances channel Ca^{++} sensitivity thus increasing channel excitability and promoting cell excitability. Aberrant changes in spinal cord BK channel levels are now thought to contribute to development of pathological pain. In obesity, nociceptive responses and inflammatory pain are potentiated but the underlying mechanism is not known. It is hypothesized that alterations in the relative expression of STREX in spinal cord may contribute to changes in nociceptive processing with obesity. The aim of this study was to investigate differential expression of BK and STREX channels in spinal cord and white adipose tissue (WAT) in the Zucker rat model of genetic obesity.

WAT and spinal cord tissues from adult male and female lean ($n = 10$ and 9, respectively) and obese ($n = 10$ and 9, respectively) Zucker rats were collected after schedule 1 killing. Total RNA was extracted, reverse transcribed and using real-time PCR the relative expressions of BK, STREX mRNA were quantified. For relative quantitative analysis of target gene mRNA

the comparative Ct method was utilized which normalizes the number of copies of the target gene to the reference gene cyclophilin. Data was analyzed using either a student's t-test or ANOVA.

Both BK and STREX mRNA were found to be constitutively expressed in WAT and spinal cord. BK and STREX mRNA was more highly expressed in spinal cord tissue compared to WAT (2 fold higher; $p < 0.05$). Levels of expression of BK and STREX mRNA were unchanged in obese rat spinal cord, and there was no difference between sexes. In WAT, BK and STREX mRNA expression were similar in obese and lean rats but were significantly down-regulated in both lean and obese females compared to lean and obese males (4.5 fold lower; $p < 0.05$). The current study failed to detect any alteration in BK or STREX mRNA levels in spinal cord from obese rats, suggesting that another mechanism is responsible for plasticity-related changes in spinal nociceptive processing. This is the first study to detect BK and STREX mRNA in WAT. Their functional role in this tissue is not known, but could influence the excitability of adipocytes to affect secretion rates of inflammatory adipokines. The finding that BK channel genes are suppressed in female white adipose tissues, suggests that these channels are under hormonal regulation and could underlie a sex specific mechanism of regulating adipokine release.

Chen, L., L. Tian, et al. (2005). *J Biol Chem* 280(39): 33599-33609.

Chen, S. R., Y. Q. Cai, et al. (2009). *J Neurochem* 110(1): 352-362.

Cui, J., H. Yang, et al. (2009). *Cell Mol Life Sci* 66(5): 852-875.

Janke, E. A., Collins, A., & Kozak, A. T. (2007). *J Rehabil Res Dev*, 44(2), 245-262.

Ocana, M., C. M. Cendan, et al. (2004). *Eur J Pharmacol* 500(1-3): 203-219.

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PC61

A proteomic investigation of postmortem prefrontal cortex of human alcoholics reveals insights into structural and behavioural brain changes

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Chronic excessive alcohol consumption evokes progressive and cumulative damage to tissues and organs. Global and regional brain alcoholic-induced pathology may impact upon general motor-neuron function, and also influence cognitive behaviour. In order to investigate a molecular basis for loss or altered function associated with a brain region involved in cognitive and social behaviour, we examined the postmortem prefrontal cortex (Brodmann's area 9) from 10 human alcoholics and 10 age-, gender- and postmortem delay- matched control subjects.

H & E staining and light microscopy of this prefrontal cortex region revealed a disorder of cellular alignment in the alcoholics. Brain tissue homogenisation and then one dimensional polyacrylamide gel electrophoresis proteomics of cytosolic proteins identified dramatic changes in protein profiles between these two groups. We identified several of the major protein changes, and these were validated and quantitated by Western blotting. This proteomic approach was extended to

an evaluation of membrane proteins which also revealed protein profile changes, but moreover, distinct post-translational modifications seen with control subjects that were absent from alcoholic tissues.

In summary, this proteomic approach suggests that cumulative alcohol abuse results in dramatic structural changes of brain protein profiles, and alterations of novel post-translational modifications; that collectively contribute to alcohol-induced anatomical and presumed behavioural abnormalities.

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PC62

Co-existence of theta-nested gamma oscillations and grid firing fields in an attractor network model

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Grid cells in the medial entorhinal cortex (MEC) encode location through firing fields that form grid-like maps of the environment. At the same time network activity in the MEC is dominated by oscillations in the theta (4-12 Hz) and gamma (30-100 Hz) bands. The relationship between oscillatory activity and grid firing is not known. Our recent experimental data establishes that feedback inhibition between excitatory stellate cells and inhibitory fast spiking interneurons is the dominant form of synaptic connectivity within layer II of the MEC. To determine if this synaptic architecture is sufficient to explain network oscillations or grid firing fields, we constructed a network of model stellate cells and interneurons. The model contains 4096 excitatory stellate cells and 1024 inhibitory interneurons. In this model, stellate cells connect exclusively to interneurons, while interneurons contact only stellate cells. We show that external excitatory conductances can drive the network into an attractor state. Feedback inhibition onto the model stellate cells has a synchronising effect. The activity of both populations discharged in the gamma frequency range (30 - 100 Hz), and when coupled with a theta modulated external drive (8 Hz), we observed the synchronisation during the trough of the theta signal only. When the borders of the network are connected with a twisted torus topology and velocity modulated inputs are applied to the circuit, excitatory neurons in the circuit generate grid-like firing fields. Due to only a partial stability of the attractor state the grid fields appeared noisy. These results have implications for mechanisms of both formation of grid-like receptive fields and also population coding and information transmission between brain areas.

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PC63

Commissural control of frequency selectivity in the inferior colliculi

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The commissure of the inferior colliculi (CoIC) reciprocally innervates the inferior colliculi (ICs), the principal midbrain auditory nuclei. The CoIC interconnects the ordered representation of sound frequency in the ICs, but its function is not understood. We investigated the role of these connections in the processing of sound by reversibly deactivating spiking activity in one IC using a cryoloop while recording sound driven responses in the other. Experiments were performed in guinea pig anaesthetised with urethane (1.0 g/kg, 20% solution, i.p.) and Hypnorm (1 mg/kg i.m.). Thermocouple measurements (Hyp-0, Omega) determined that cooling was restricted to the directly cooled IC. Single and multi-unit recordings revealed a gradient of deactivation through the cooled IC with deactivation maximal dorsally, adjacent to the cryoloop, while more ventral areas were less affected by cooling. A linear regression fitted the maximal change in firing rate as a function of characteristic frequency ($r^2 = 0.82$, $P < 0.001$). Averaged auditory evoked potentials to clicks showed that the amplitude of the afferent volley to the IC was unchanged by cooling (Wilcoxon matched-pairs test; $P = 0.0825$) suggesting that lower auditory nuclei were unaffected. This was corroborated by thermocouple measurements in the cochlear nucleus ipsilateral to cooling. Together these observations suggest that the effects of cooling on the contralateral IC are mediated via the CoIC and not by descending connections from the IC. The frequency response areas (FRAs) of most single units (57/94) in the IC contralateral to cooling were modulated ($> 20\%$ area change) by deactivation. A one sample Wilcoxon signed rank test showed that cooling produced a significant reduction in the area of FRAs ($P = 0.0017$). Cooling changed the areas of 39/72 V-shaped FRAs and across the population there was a significant reduction in area (Wilcoxon matched-pairs; $P = 0.036$). The change in area of non-V-shaped FRAs did not reach significance for the population (Wilcoxon matched-pairs; $P = 0.08$) despite a change in 18/23 FRAs. Of the 18 FRAs that changed with cooling, 8 increased and 10 decreased in area. When the direction of change in the FRA area was ignored, there was a significant difference between the change in V-shaped and non-V-shaped FRAs (Mann Whitney test; $P = 0.0052$), with non-V-shaped FRAs showing a bigger area change with cooling. These results show that cryoloop cooling offers a focal, reversible deactivation technique, suitable for IC deactivation in guinea pig. Recordings contralateral to the deactivated IC indicate that projections via the CoIC can modulate the encoding of spectral cues in IC neurons. Differential changes in V-shaped and non-V-shaped units suggest that the CoIC selectively modulates frequency receptive fields, possibly sub serving intercollicular communication between cells possessing similar properties.

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Dynamins and myosin-II may regulate the switching between the distinct modes of exocytosis in control and diabetic nerve terminals in a Ca^{2+} dependent manner

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There is much debate as to whether SV fusion in nerve terminals can occur via a "kiss-and-run" (KR) mode of exocytosis and this has been studied, herein, using cerebrocortical synaptosomes from adult male Wistar rats. The synaptosomes were stimulated with 30 mM K^+ (HK) and 5 mM $[\text{Ca}^{2+}]_o$ allowing them to be maximally loaded with FM2-10 styryl dye and were then studied for exocytosis of the dye. Maximal FM2-10 dye release occurs only when SVs fuse via Full Fusion (FF) whereas glutamate (Glu; the major neurotransmitter present) can be fully released by both, KR and FF. In this study, various secretagogues [HK, 1 mM 4-aminopyridine (4AP) and 5 μM ionomycin (ION)] in the presence of 5 mM $[\text{Ca}^{2+}]_o$ were used to stimulate a single round of SV exocytosis from both the readily releasable pool (RRP) and reserve/recycling pool (RP) of SVs, and FM2-10 dye and Glu release measured. We found that inhibition of dynamins by dynasore (160 μM) causes more dye to be released with 4AP ($p < 0.05$) and ION ($p < 0.05$) stimulation but there was no extra dye release when HK was employed. On the other hand, inhibition of myosin-II by blebbistatin (50 μM) increased the dye release induced by HK stimulation ($p < 0.05$) but had no effect on 4AP or ION evoked release. Neither dynasore nor blebbistatin had an effect on Glu release evoked by any of the stimuli. This indicates that dynamins or myosin-II can be responsible for the closure of the SV pore during KR depending upon the level of $[\text{Ca}^{2+}]_i$ that the exocytosing vesicles are exposed to. The results suggest that dynamins are functional at active zones only at lower $[\text{Ca}^{2+}]_i$ that can be produced by 4AP or ION stimulation whilst myosin-II is only activated at higher $[\text{Ca}^{2+}]_i$ at the active zone that can be produced by HK stimulation. In order to investigate further such Ca^{2+} dependencies, synaptosomes prepared from streptozotocin-treated rats – a model of type 1 diabetes – were used as these exhibited both a larger amount of KR release (due to some RP undergoing KR fusion) and a larger change in $[\text{Ca}^{2+}]_i$ upon stimulation. Interestingly, it was found that myosin-II was responsible for the KR during 4AP stimulation (due presumably to higher $[\text{Ca}^{2+}]_i$ being achieved at the active zones). In such diabetic rats, myosin-II were responsible for the KR of the RRP during HK stimulation but dynamins were responsible for the regulation of the mode of exocytosis of the RP as such vesicles never get exposed to the high $[\text{Ca}^{2+}]_i$ required for the activation of myosin-II. This idea of Ca^{2+} dependent regulation of the two proteins is being further investigated by regulating the $[\text{Ca}^{2+}]_i$ by other means. Furthermore, The specific role of dynamin I or dynamin II in the closure of the fusion pore is currently being studied.

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TGF- β 2 acutely reverses some of the deficits in neuromuscular transmission in a mouse model of motor neurone diseaseJ. Reid¹, G.B. Miles² and G.S. Bewick¹¹*School of Medical Sciences, University of Aberdeen, Aberdeen, UK* and ²*School of Biology, University of St Andrews, St Andrews, Scotland, UK*

The cytokine Transforming Growth Factor- β 2 (TGF- β 2) is highly expressed in the nervous system of healthy adult mammals. The source of TGF- β 2 in the CNS is unknown but it enhances postsynaptic currents when acutely applied to hippocampal synapses in culture (Fukushima *et al.*, 2007). At mammalian neuromuscular junctions (NMJs), TGF- β 2 is highly localised to the endplate region, while the overlying motor terminal expresses TGF- β 2 receptors. We recently showed TGF- β 2 enhances neuromuscular transmission by increased loading of neurotransmitter into synaptic vesicles (increasing quantal size; Fong *et al.*, 2010). TGF- β 2 also increases evoked postsynaptic potentials, but reduces the number of vesicles released per action potential (quantum content) through an atropine-sensitive pathway. Thus, TGF- β 2 increases both efficacy (bigger potentials) and efficiency (fewer vesicles required). Here we ask if this is how TGF- β 2 injections reduce muscle weakness and fatigue in a mouse model of motor neurone disease expressing a high copy number of the human G93A-SOD1 gene (Day *et al.*, 2005).

hG93A-SOD1 mice (either sex) at 4, 8 and 12 wks were humanely killed by cervical dislocation (Schedule 1, ASPA 1986). Spontaneous (miniature end-plate potentials, mEPPs) and nerve-stimulation evoked ACh release (EPPs) was recorded from NMJs in hemidiaphragm/phrenic nerve preparations (4-8 per condition). Muscle contraction was blocked by μ -conotoxin GIIIB. At 4 and 8 wks, mEPP amplitudes in hG93A-SOD1 mice were significantly bigger ($p < 0.05$) than in age-matched background strain (C57/BL6J) control mice. However, by 12 wks mEPPs were significantly smaller (C57 - 1.30 ± 0.05 mV, $n = 47$ vs SOD1 - 1.13 ± 0.06 mV, $n = 78$; $p < 0.05$). At 4 and 8 wks EPP amplitudes were not significantly different from controls but also became significantly smaller at 12 wks (reduced from 38.1 ± 3.5 mV, $n = 47$ to 31.4 ± 1.1 mV, $n = 78$; $p < 0.001$). TGF- β 2 (1 ng/ml, 1 hr) reversed the deficit in mEPP amplitude (to 1.21 ± 0.05 mV, $n = 88$; $p = 0.23$) but did not affect EPP amplitude ($p = 0.7$). The decrease in potential amplitudes at 12 wks correlates with the onset of MND symptoms (weakness, tremor, and disrupted hindlimb splay reflex) in the hG93A-SOD1 mice, and disruption of NMJ ACh receptor distribution (extrajunctional clusters, reduced labelling density), assessed with fluorescent α -bungarotoxin. These data show hG93A-SOD1 mice have significantly smaller mEPP and EPP amplitudes compared to age-matched C57/BL6J mouse NMJs at 12 wks. TGF- β 2 (1 ng/ml, 1 hr) application reversed the reduced mEPP amplitude but not that in EPP amplitude. Further experiments will examine when enhanced mEPP amplitudes first appear, and if TGF- β 2 helps both deficits at later time-points and EPP amplitude rundown during stimulus trains (see Tam *et al.* this meeting).

Day WA *et al.* (2005). *Neurobiol Dis* **19**, 323-330.Fong SW *et al.* (2010). *Proc Natl Acad Sci* **107**, 13515-13519.Fukushima T *et al.* (2007). *Hippocampus* **17**, 5-9.

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PC66

Electrical stimulation applied to the amygdala facilitates secretion of endogenous opioids in the periaqueductal grayT. Nakamura¹, M. Tomida¹, H. Ando¹, N. Asanuma¹, T. Takamata² and E. Kondo³¹*Department of Oral Physiology, Matsumoto Dental University, Shiojiri-shi, Japan*, ²*Department of Oral and Maxillofacial Biology, Graduate School of Oral Medicine, Matsumoto Dental University, Shiojiri-shi, Japan* and ³*Department of Oral Anatomy, Matsumoto Dental University, Shiojiri-shi, Japan*

Although many studies have demonstrated that amygdala is involved in the antinociception, the mechanism has not yet been clarified. Previous studies using rats demonstrated that brief electrical stimulation to the amygdala depressed neural discharge in the cingulate areas while noxious stimulation was being applied to peripheral tissues. We investigated the distribution and the quantitative changes of endogenous opioids secreted in the periaqueductal gray (PAG) when electrical stimulation was applied to the amygdala. Male wistar rats (250-320g) were anesthetised with pentobarbital sodium (55-95 mg/kg, i.p) and placed in a stereotaxic frame. Microelectrode was inserted into the central nucleus of the right amygdala and electrical stimulation (2 μ A, 100 Hz, 15 seconds) was applied ($n = 5$). After 60 minutes, the rats were perfused with normal saline followed by 4% paraformaldehyde. The brain was removed, and cut into 20 μ m-thick transverse sections using cryostat. Untreated rats were used as controls ($n = 5$). Some sections were stained with hematoxylin-eosin to confirm the stimulated area. The distribution and the quantitative changes of endogenous opioids (β -endorphin, enkephalin, dynorphin) secreted in the PAG were investigated immunohistologically. Immunopositive amount in a square of 100 μ m was determined by using appropriate software (Adobe Photoshop CS3 Extended, Adobe Systems Incorporated, USA). Statistical difference between stimulated and control rats was ascertained by Mann-Whitney U test. Conspicuous amount of β -endorphin, enkephalin and dynorphin was observed in PAG. Dynorphin was significantly increased in the entire area of the PAG in the stimulated rats (stimulated rats, immunopositive area of $140.63 \pm 112.04 \mu\text{m}^2$ (mean $\pm$ S.D.) out of the 100 μ m square, and controls, $72.45 \pm 76.53 \mu\text{m}^2$, $p < 0.05$). Furthermore, dynorphin was significantly increased both in ipsilateral and in contralateral side in the lateral PAG when electrical stimulation was applied only to the right amygdala (ipsilateral side : stimulated rats, $143.66 \pm 107.22 \mu\text{m}^2$, and controls, $56.90 \pm 32.28 \mu\text{m}^2$, $p < 0.005$; contralateral side : stimulated rats, $152.53 \pm 84.86 \mu\text{m}^2$, and controls, $66.70 \pm 44.52 \mu\text{m}^2$, $p < 0.005$). Enkephalin in the stimulated rats increased, although not significantly, in dorsomedial and lateral PAG. Thus, secretion of the endogenous opioids in the PAG was increased bilaterally by applying electrical stimulation to the amygdala. These results suggest that electrical stimulation to the amygdala facilitates secretion of endogenous opioids in the PAG and leads to the facilitation of descending antinociceptive system.

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PC67

Astrocytes use lactate to stimulate neuronal noradrenaline release in the locus coeruleus

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According to the hypothesis of "lactate shuttle", glycogen stored in astrocytes can be converted to lactate and exported to neurones as a preferred fuel (1). However, we employed optogenetics to investigate the signalling mechanism between astrocytes and noradrenergic (NAergic) neurones and here present data to suggest that lactate also plays a role in astrocyte-to-neurone communication.

To selectively stimulate G_q - and G_s -protein-mediated signalling cascades in astrocytes, we generated adenoviral vectors and expressed the opsin-GPCR chimeras, Opto α_1 - and Opto β_2 -adrenergic receptors (Opto α_1 AR; Opto β_2 AR) (2) under control of a transcriptionally enhanced GFAP promoter (3). We verified that these constructs activated the correct GPCR pathways in astrocytes (presented at the 2011 Physiological society meeting (4)).

To confirm that astrocytes break down glycogen to produce lactate, primary dissociated glial cultures expressing Opto α_1 AR and Opto β_2 AR and loaded with the pH indicator Snarf-5 were imaged ratiometrically using a Leica SP1 confocal microscope. Stimulation with 470nm light resulted in a significant change in the fluorescence ratio, indicative of acidification and consistent with the production of L-lactate (Opto α_1 AR: $+132.8\% \pm 3.4\%$, $n=44$ cells, $p<0.001$; Opto β_2 AR: $+147.4\% \pm 3.6\%$, $n=36$ cells, $p<0.001$; paired t-test). This effect could be blocked by pre-incubation with an inhibitor of glycogen breakdown, 1,4-dideoxy-1,4-imino-D-arabinitol (DAB; 500 μ M; Opto α_1 AR: $+101.9\% \pm 0.3\%$, $n=26$ cells, $p<0.001$; Opto β_2 AR: $+101.3\% \pm 0.7\%$, $n=34$ cells, $p<0.001$).

Fast scan cyclic voltammetry (FCV) in organotypic slices containing the locus coeruleus (LC) was used to measure NA release from NAergic neurones. To determine whether L-lactate released from astrocytes can stimulate NA release, astrocytes were transduced to express Opto α_1 AR or Opto β_2 AR. Stimulation of astrocytes with a 445nm PhoxX diode laser induced release of NA (Opto α_1 AR: $+85.2 \pm 12.2$ V*Sec, $n=24$ stimulations, $p<0.001$; Opto β_2 AR: $+88.7 \pm 11.1$ V*Sec, $n=20$, $p<0.001$; paired t-test). Pre-incubation with DAB blocked the effect of optogenetic stimulation of astrocytes on NA release (Opto α_1 AR: -11.16 ± 11.7 V*Sec, $n=6$, $p<0.001$; Opto β_2 AR: -14.74 ± 7.0 V*Sec, $n=5$, $p<0.001$). These experiments suggest that signalling mediated by $G_{q/s}$ PCRs in astrocytes could potentiate the release of NA from LC neurones via L-lactate. Consistent with this idea, application of L-lactate (400 μ M) resulted in the release of NA ($+127.6 \pm 14.6$ V*Sec, $n=15$, $p<0.001$; paired t-test).

In conclusion, activation of astrocytes results in powerful modulation of NA transmission via release of L-lactate, the cellular and molecular mechanisms of which are currently under investigation.

Pellerin L, Magistretti PJ. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci U S A* 1994 Oct 25;91(22):10625-9

Airan RD, Thompson KR, Fenno LE, Bernstein H, Deisseroth K. Temporally precise in vivo control of intracellular signalling. *Nature*. 2009 Apr 23;458(7241):1025-9

Liu B, Wang S, Brenner M, Paton JF, Kasparov S. Enhancement of cell-specific transgene expression from a Tet-Off regulatory system using a transcriptional amplification strategy in the rat brain. *J Gene Med* 2008 May;10(5):583-92.

Lane S, Marina N, Gourine AV, Teschemacher AG, Kasparov S. Are astroglial Gq-protein coupled receptors functionally relevant? *Proc Physiol Soc* 23 (2011).

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PC68

Effect of Neuronal Nitric Oxide Synthase inhibitor on the expression of cyclooxygenase-2 and glial cells in hemiparkinsonian rats with levodopa-induced dyskinesiasR. Cavalcanti-Kwiatkoski^{1,2}, M. Bortolanza², R. Raisman-Vozari³ and E.A. Del Bel²

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Dopaminergic replacement as symptomatic treatment of Parkinson's disease (PD) is effective and can be done through the administration of the dopamine precursor, L-3, 4-dihydroxyphenylalanine (L-DOPA). Continuous L-DOPA treatment is frequently associated with the development of dyskinesias. The inhibition of neuronal nitric oxide synthase (nNOS) were able to attenuate the L-DOPA-induced dyskinesias in 6-hydroxydopamine (6-OHDA; Sigma-Aldrich, St. Louis, MO, USA; 24 μ g in 3 μ l saline containing 0.05% ascorbic acid)-lesioned rats. We investigated the striatal expression of inflammatory markers (cyclooxygenase-2; glial fibrillary acid protein (GFAP), and the microglia (OX-42)) on hemiparkinsonian rats submitted to continuous treatment with L-DOPA or L-DOPA preceded by the nNOS inhibitor, 7-nitroindazol (7-NI). Methods: 6-OHDA-lesioned Wistar rats were treated daily with L-DOPA (30 mg/kg, orally by gavage) or L-DOPA plus 7-NI (30 mg/kg, ip.) over 21 days (Groups: 6-OHDA+vehicle+saline ($n=6$); 6-OHDA+7-NI+saline ($n=3$); 6-OHDA+vehicle+L-DOPA ($n=7$); and 6-OHDA+7-NI+L-DOPA ($n=7$)). Rats were examined periodically for the development of dyskinesias. The animals were anesthetized with ketamine and xylazine (100 and 10mg/kg, respectively; 1mL/kg, ip.) and submitted to transcardiac perfusion for removing the brain and carrying out the immunohistochemistry for COX-2, GFAP and OX-42. The statistical analysis was carried out by repeated two-way ANOVA followed by Tukey's multiple comparison tests ($p<0.05$). Results: The continuous treatment with L-DOPA induced the expression of COX-2 in neuronal-like cells in the striatum ipsilateral to lesion ($F_{3,22} = 130.510$; $P < 0.001$). We also observe that the COX-2 immunoreactivity in the striatum co-localize with cAMP-regulated phosphoprotein (DARPP-32), choline acetyltransferase (ChAT) and calbindin neurons but not with calretinin, parvalbumin and nNOS immunolabeling. Furthermore, L-DOPA also induced an increase in the density of nitric oxide synthase in neuronal cells ($F_{3,22} = 7.199$; $P < 0.001$), astrocytes ($F_{3,22} = 20.211$; $P < 0.001$), and microglial cells ($F_{3,22} = 20.620$; $P < 0.001$) compared to vehicle+saline group. In the same way as dyskinesias, all these cellular alterations were prevented by the pre-treatment of animals with the nNOS inhibitor 7-NI (COX-2 ($F_{3,22} = 130.510$; $P < 0.001$); nNOS ($F_{3,22} = 7.199$; P

= 0.001); GFAP (F3,22 = 20.211; $P < 0.001$) and OX-42, F3,22 = 20.620; $P < 0.001$). Conclusion: The major finding of this study was the parallel of L-DOPA-induced dyskinesias with expression of COX-2 in the striatum, as well astrocytic and microglial cells. Moreover, 7-NI prevented the striatal cellular changes, without harming its beneficial effects in dyskinetic rats. This outcome appears to involve striatal interneurons, projection neurons and the direct pathway.

Del-Bel E, Padovan-Neto FE, Raisman-Vozari R, Lazzarini M. Role of nitric oxide in motor control: implications for Parkinson's disease pathophysiology and treatment. *Curr Pharm Des*. 2011;17(5):471-88.

Del Bel EA, Guimarães FS, Bermúdez-Echeverry M, Gomes MZ, Schiaveto-de-souza A, Padovan-Neto FE, Tumas V, Barion-Cavalcanti AP, Lazzarini M, Nucci-da-Silva LP, de Paula-Souza D. Role of nitric oxide on motor behavior. *Cell Mol Neurobiol*. 2005 Mar;25(2):371-92.

Padovan-Neto FE, Echeverry MB, Chiavegatto S, Del-Bel E. Nitric Oxide Synthase Inhibitor Improves De Novo and Long-Term L-DOPA-Induced Dyskinesia in Hemiparkinsonian Rats. *Front Syst Neurosci*. 2011;5:40. Epub 2011 Jun 10.

Padovan-Neto FE, Echeverry MB, Tumas V, Del-Bel EA. Nitric oxide synthase inhibition attenuates L-DOPA-induced dyskinesias in a rodent model of Parkinson's disease. *Neuroscience*. 2009 Mar 31;159(3):927-35. Epub 2009 Jan 23.

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PC69

Immunohistochemical evidence for neuronal and glial expression of the inward rectifying potassium channel subtype Kir7.1 in the mouse brain

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The mammalian central nervous system (CNS) is comprised of two neural cell types - neurons and glia. The main types of glia are oligodendrocytes, which are the myelin-forming cells of the CNS, and astrocytes, which have a primary role in the extracellular K⁺ regulation during neuronal activity. A major feature of neural cells is their expression of inwardly rectifying K⁺ channels (Kir), which regulate excitability in neurons and maintain the strongly negative resting membrane potential of glia. A genome-wide microarray analysis of the mouse optic nerve identified prominent expression of the Kir7.1 subtype. Kir7.1 channels are expressed in epithelial tissues in the kidney, retina and gastrointestinal tract, where they are believed to be important for K⁺ recycling, but little is known about their expression or function in the CNS. Here, we have used immunohistochemistry to examine expression of Kir7.1 in the mouse brain. Mice aged postnatal day P0 to P40 (adult) were killed in accordance with the UK Animals Act (1986). Optic nerves were removed for tissue culture and brains immersion-fixed in 4% paraformaldehyde and sectioned using a vibratome (40-60µm). Immunolabelling was performed using antibodies to Kir7.1 and cell markers for neurons. Glial cells were identified by their expression of fluorescent reporters. Controls were treated with secondary antibody in the absence of primary antibody and exhibited no labeling. Immunolabelling was examined on an LSM710 confocal microscope (Zeiss) and analysed using Velocity software (PerkinElmer). In the adult mouse, there was prominent Kir7.1 immunolabelling in neuronal somata and primary dendrites, as well as on astrocytic and oligodendrocytic somata and processes, throughout the

cerebral cortex, corpus callosum hippocampus and cerebellum. Comparison of mice at different ages revealed a developmental increase in Kir7.1 immunolabelling in astrocytes and oligodendrocytes between P7 and P15, whereas neurons appeared to express Kir7.1 at all stages of development. Analysis of cultured neurons and glia confirmed expression of Kir7.1 in these cells, and showed the same developmental increase in expression in astrocytes and oligodendrocytes observed in vivo. The results demonstrate prominent expression of Kir7.1 in neurons and glia and suggest this channel may play an important role in neuronal excitability, the astrocytic function of potassium regulation and the oligodendrocyte function of myelination.

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PC70

Hypothalamic glucosensing neurons display defective electrical response to low glucose following antecedent ATP-sensitive K⁺ channel activation

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One of the major limiting factors in the successful management of type 1 diabetes is Recurrent Hypoglycaemia (RH) resulting from supraphysiological insulin supplementation, which augments the already impaired counterregulatory response to hypoglycaemia. The hypothalamus is essential for the maintenance of whole body glucose homeostasis, including glucose counterregulation (3). Glucosensing (GS) neurons of the hypothalamus use glucose not only as a fuel source but also as a signalling molecule, in order to modulate their electrical activity according to central glucose availability. The molecular mechanisms underlying the function of GS neurons still remain unclear but they are thought to contain the same glucose sensing cellular machinery as pancreatic β -cells, such as ATP-sensitive K⁺ channels (K_{ATP}), glucokinase, glucose transporter proteins and AMPK (5). It is well established that K_{ATP} channels regulate the release of insulin from pancreatic β -cells in response to blood glucose levels (1), however they also modulate the electrical activity of GS neurons and are vital for initiation of the counterregulatory response to hypoglycaemia (2,3). The aim of the current study was to determine whether the response to hypoglycaemia of GS neurons is altered following RH, and whether this defect is driven by prior K_{ATP} activation.

We have recently established that GT1-7 cells, a population of mouse hypothalamic GS neurons, display the characteristic features of a GS system as electrical activity of these cells is directly regulated by glucose metabolism. Patch-clamp electrophysiology was used to examine the electrical activity of GT1-7 cells after exposure to RH (3 hours of 0.1mM glucose for 3 days) or antecedent K_{ATP} opener NN414 (3 hours during the previous day) versus appropriate controls. Radiolabeled metabolic assays were used to investigate if any change occurred in glucose oxidation capacity or uptake kinetics. After exposing GT1-7 cells to RH, the magnitude of the ΔV_m hyperpolarising response to 0.5mM glucose was reduced by 66.7% compared to control ($\Delta V_{RH} = 4.9 \pm 1.0mV$ vs.

$\Delta V_{\text{Cont}} = 14.7 \pm 0.9 \text{ mV}$, $p < 0.0001$; $n = 7-10$). Antecedent NN414 exposure ($5 \mu\text{M}$) also produced a defect in the ability of the cells to hyperpolarise to 0.5 mM glucose by 73.1% compared to control ($\Delta V_{\text{NN414}} = 3.8 \pm 1.7 \text{ mV}$ vs. $\Delta V_{\text{Cont}} = 14.2 \pm 1.4 \text{ mV}$, $p < 0.01$; $n = 6$). Maximum K_{ATP} conductance and glucose uptake and oxidation were unaffected by RH and antecedent NN414 treatments. Values are means $\pm$ S.E.M., compared by ANOVA.

Thus exposure of GT1-7 cells to RH induces defective GS, which was mimicked by antecedent pharmacological activation of K_{ATP} channels, independently of any change in glucose uptake or oxidation. This suggests that defective GS, following RH, may be dependent on prior K_{ATP} activation.

Ashcroft FM, Harrison DE, Ashcroft SJ. (1984) Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. *Nature*. 1984. **312**:446-8.

Ashford ML, Boden PR, Treherne JM. (1990a) Glucose-induced excitation of hypothalamic neurones is mediated by ATP-sensitive K^+ channels. *Pflugers Arch*. **415**:479-83.

Borg WP, During MJ, Sherwin RS, Borg MA, Brines ML, Shulman GI. (1994) Ventromedial hypothalamic lesions in rats suppress counter-regulatory responses to hypoglycemia. *J Clin Invest*. **93**:1677-82

Evans ML, McCrimmon RJ, Flanagan DE, Keshavarz T, Fan X, McNay EC, Jacob RJ, Sherwin RS. (2004) Hypothalamic ATP-sensitive K^+ channels play a key role in sensing hypoglycemia and triggering counterregulatory epinephrine and glucagon responses. *Diabetes*. **53**:2542-51

Levin BE, Routh VH, Kang L, Sanders NM, Dunn-Meynell AA. (2004) Neuronal glucosensing: what do we know after 50 years? *Diabetes*. **53**:2521-8

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observed with expression of single subunits. Concatemers that contained Lys⁶⁹ in two of the three domains gave robust currents in response to ATP (KKA=KAK>AKK). Maximal currents were about 50% of those observed with KKK, but the effective concentrations of ATP were in the range of those required to activate receptors formed by expression of monomeric subunits. The activation of the currents (using concentrations of ATP that gave half-maximal current) was slower when concatemers contained one K69A mutation. Outside-out patch recording showed that these constructs had unitary conductances ($19 - 23 \text{ pS}$) not different from wild type P2X2 receptors formed from monomer expression, or the KKK construct. Concatemers with only one Lys-containing subunit also showed very small currents in two cases (KAA, AAK) and no current in the other (AKA). We can not exclude the possibility that these result from small amounts of degradation of the concatenated construct. Concatemers in which all three Lys residues at position 69 were replaced by Ala (AAA) did not respond to ATP. The results suggest that ATP can activate P2X receptors which have only two of the three binding sites intact, and that these channels open to a unitary conductance that is not different from that observed in wild type channels.

Jiang LH, Rassendren F, Surprenant A, and North RA (2000) Identification of amino acid residues contributing to the ATP-binding site of a purinergic receptor. *J Biol Chem* **275**:34190-34196.

Kawate T, Michel JC, Birdsong WT, and Gouaux E (2009) Crystal structure of the ATP-gated P2X4 ion channel in the closed state. *Nature* **460**:592-598.

North RA (2002) Molecular physiology of P2X receptors. *Physiol Rev* **82**:1013-1067.

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PC71

Activation of P2X2 receptors in a partially liganded state

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P2X receptors are trimers, in which each subunit has intracellular N and C termini, two membrane-spanning domains, and a large ectodomain (North, 2002; Kawate et al. 2009). The ATP binding sites are located at the interface between two subunits, and several key residues for agonist docking have been identified. These include Lys⁶⁹ in the P2X2 receptor, which is suggested to bind to oxygen atoms on the gamma phosphate of ATP. In the homotrimeric receptor, substitution by alanine at this position abolishes the response to ATP (Jiang et al., 2000).

We constructed eight concatenated cDNAs, each of which encoded three joined P2X2 subunits, where the mutation K69A was introduced into none, one, two or three of these subunits. All had similar plasma membrane expression when expressed in human embryonic kidney cells, as detected by biotinylation and Western blotting using an epitope tag present in each subunit. There was no evidence of significant degradation of the concatemers, except that a very faint band was sometimes observed corresponding in molecular weight to a dimer. We recorded membrane currents evoked by ATP. Cells expressing the concatemer with Lys at position 69 in each of the three subunits (KKK) had properties comparable to the wild type receptor, although maximal currents were about 50% of those

PC72

Get hot, get blocked – Temperature-dependence of allosteric modulators on nAChRs and GABAARs

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Nicotinic acetylcholine receptors (nAChRs) and γ -aminobutyric acid type-A receptors (GABAARs) belong to the superfamily of ligand-gated ion channels. nAChRs are activated by the neurotransmitter acetylcholine and nicotine, mediating excitation. GABAARs are activated by GABA, conducting chloride, which mainly hyperpolarizes the membrane, thus inhibiting neurotransmission.

The most abundant homomeric nAChR in the mammalian brain are the pentameric $\alpha 7$ nAChRs, which consist of five $\alpha 7$ subunits. Each subunit provides an orthosteric low affinity binding site for its endogenous ligand and a number of positive allosteric modulators (PAMs). Allosteric modulators of nAChRs have been implicated to be putative therapeutics in the pharmacological treatment of Alzheimer's disease, schizophrenia and Parkinson's disease. The GABAARs are also pentameric membrane proteins, possessing allosteric binding sites. PAMs of GABAARs are benzodiazepines and barbiturates, which are commonly used to treat the pathogenesis of epilepsy, neuropathic pain or developmental malfunctions. Hence, both, nAChRs and GABAARs have become potential targets for drugs, treating cognitive deficits, whereas PAMs get accredited a seminal role in the pharmacotherapy.

Here we show the results of the target screen of the PAM PNU-120596 on nAChRs, and the PAM Diazepam on GABAARs performed on high throughput automated patch-clamp devices. Fast and accurate solution exchange (<10ms) and exposure times (<200ms) given by stacking solutions inside the pipette and rapid application to the cell enables reliable and repetitive activation of the GABAARs and even of fast desensitizing receptors such as nAChRs. Recently, Sitzia et al. (2011) also observed that physiological 37°C modulate the effect of PNU-120596 on nAChRs. To test, whether different temperatures affect PAMs of nAChRs and PAMs of GABAARs, we used a heated pipette to increase the temperature of the added solution and then rapidly applied to the cell. For both nAChRs and GABAARs, the PAM evoked currents significantly decreased with increasing temperature, supporting the idea of strong temperature dependence the allosteric modulation by PNU-120596 on nAChRs and by Diazepam on GABAARs.

Taken together, we could reliably mimic the temperature dependence of PNU-120596. More importantly, we found for the first time an equal temperature dependence of GABAARs positive allosteric modulators.

Sitzia F, et al. (2011) Voltage- and Temperature-Dependent Allosteric Modulation of $\alpha 7$ Nicotinic Receptors by PNU120596. *Front Pharmacol.* 2011;2:81.

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PC73

The flux-coupling region for internal spermine block and its associated gating changes in the Kir2.1 channel

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The blocking effect of intracellular spermine (SPM) on the Kir2.1 channel is closely correlated with current flow, constituting one of the major bases of inward rectification. The flow dependence strongly suggests coupled movement of multiple ions in a single-file region of the pore. The location and the functional operation of this region, however, remain largely uncharacterized. Based on electrophysiological recordings in inside-out patches from *Xenopus* oocytes obtained by partial ovariectomy from frogs anesthetized with 0.1 % tricaine and pressure injected with wild-type or mutant Kir2.1 cRNA, we found that both the flux-coupling feature and the affinity of intracellular SPM block are decreased in the D172N but not S165L mutant channel. However, only the S165L mutant channel shows an evidently facilitated outward exit of the blocking SPM at strong depolarized potentials. On the other hand, the kinetics of SPM entry to and exit from the flow-dependent blocking site are markedly and correlatively slowed by specific E224 and E299 mutations, which always also disrupt the flux-coupling feature of SPM block. The slowed entry rates carry little voltage dependence, whereas the exit rates are always decelerated with more depolarization. Interestingly, the foregoing effect is present in the E299H mutant channel at pH 6.4 but not at pH 8.4, and the effect of E244/E299 mutations are mostly counteracted by concomitant mutations of R228/R260. Moreover, mutations involving residues I176 - A184 in the bundle crossing region markedly weaken the inward rectifying feature of SPM block of the Kir2.1 channel, and mutations of E224 and M183/A184 even alter the height

of the same asymmetrical barrier to SPM permeation. Also, the channel seems to be able to accommodate at least two SPM molecules simultaneously, as the unblocking of the blocking SPM is accelerated in higher intracellular SPM concentration. We conclude that E224 and E299, probably through electrostatic interactions with R228 and R260, play a pivotal role in the "gating" of the bundle crossing region of the Kir2.1 channel pore. This region, which probably contains an outmost site involving D172 for SPM binding and is demarcated externally by a large energy barrier for SPM to cross at S165, is also responsible for the flux-coupling feature and the inward rectification property of the channel. In this regard, SPM intriguingly serves as both a flow-dependent pore blocker and a "gating particle" capable of opening the bundle crossing of the pore.

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PC74

Expression and functional studies of the novel CNS protein CACHD1

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The putative protein CACHD1 was identified following a systematic search for novel ligand-binding proteins (Anantharaman & Aravind, 2000). CACHD1 has a predicted structure similar to that of the $\alpha 2\delta$ family of voltage-dependent calcium channel (VDCC) auxiliary subunits. The $\alpha 2\delta$ subunit isoforms are known to increase membrane expression and modulate the biophysical properties of VDCCs (Davies et al., 2007). Moreover, the $\alpha 2\delta$ -1 subunit is a binding protein of the anti-epileptic drug gabapentin, also commonly used in neuropathic pain therapy (Hendrich et al., 2008). Here, we examined the expression of CACHD1 within different tissues and tested if CACHD1 functionally interacts with VDCCs by comparing electrophysiological actions of CACHD1 with those of $\alpha 2\delta$ -1 on the Cav2.2 subunit.

Quantitative PCR of CACHD1 and $\alpha 2\delta$ -1 mRNA was performed on hippocampus, cortex, thalamus, cerebellum, dorsal root ganglia (DRG) and superior cervical ganglia (SCG) tissue from adult Wistar rats. Data were normalised to HPRT1 and expressed as fold difference between average tissue expression ($n=3$, means $\pm$ S.E.M., statistical analysis by ANOVA and Tukey post-hoc test). Immunocytochemistry (ICC) was performed on tsA201 cells transiently transfected with CACHD1, using rabbit anti-CACHD1 (1:250) and Alexa 488 IgG (1:1000) antibodies. Whole-cell patch clamp recordings were performed on tsA201 cells transiently transfected with Cav2.2/ $\beta 2a$ /GFP with or without $\alpha 2\delta$ -1 or CACHD1.

CACHD1 mRNA levels were significantly higher in the thalamus compared to cortex, DRG and SCG (3.7 ± 0.46 , 0.47 ± 0.13 , 0.33 ± 0.03 fold, respectively, both $p < 0.01$). $\alpha 2\delta$ -1 mRNA was significantly higher in the cortex compared to thalamus, cerebellum and DRG (2.13 ± 0.32 , 0.51 ± 0.11 , 0.19 ± 0.05 and 0.39 ± 0.02 fold, respectively, each $p < 0.001$). ICC showed CACHD1 protein expression at or near the membrane of transiently transfected tsA201 cells. Whole-cell patch clamp confirmed that $\alpha 2\delta$ -1 co-transfection with Cav2.2/ $\beta 2a$ /GFP significantly increase Ca^{2+} current amplitude (from -24.1 ± 4.6 pA/pF to -70.9 ± 11.1 pA/pF, $p < 0.0001$, $n=14-17$). However, when

CACHD1 was co-transfected with Cav2.2/ β 2a/GFP, Ca²⁺ currents amplitude remained unchanged (-23.5 ± 4.7 pA/pF and -24.1 ± 4.6 pA/pF, $n=14-17$, respectively) and other measured biophysical properties were also unaltered.

Results indicate that CACHD1 protein may have a differential, predominantly thalamic, expression within the CNS; such expression is largely complementary to that of $\alpha 2\delta$ -1. CACHD1 protein may be expressed at the membrane; however, initial data suggests that, unlike $\alpha 2\delta$ -1, CACHD1 protein does not functionally modulate electrophysiological properties of Cav2.2 channels. In the future, it will be of interest to investigate potential functional effects of CACHD1 on proteins, in particular other VDCCs, expressed at high levels within the thalamus.

Anantharaman & Aravind, 2000, Trends Biochem Sci., 25,535-7

Davies et al., 2007, Trends Pharmacol Sci, 28, 220-8

Hendrich et al., 2008, Proc Natl Acad Sci USA, 105, 3628-33

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PC75

Investigating the effect of REDOX agents on the structural re-arrangement of BK channel RCK1-RCK2 linker using fluorescence lifetime imaging microscopy

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Alternative splicing generates considerable functional diversity of large conductance calcium- and voltage-activated potassium (BK) channels. For example, inclusion of the STREX (STress-activated EXon) insert into the unstructured cytosolic linker between the two RCK domains of the channel generates a cysteine rich domain (CRD) that confers intrinsic hypoxia sensitivity to the channel [1].

We have utilized YFP-mCFP fluorescent fusion proteins of the CRD and exploited these to examine conformational rearrangements in the CRD in response to hypoxia and redox agents using fluorescence lifetime imaging microscopy (FLIM). The fusion protein, expressed in HEK293 cells, is targeted to the plasma membrane and responds to acute hypoxia (10 minute exposure at <5% oxygen) with a significant shift in fluorescence lifetime distribution. Pre-treatment with the oxidizing agent DTNB (1mM) appeared to cause a shortening in the lifetime distribution that did not block the hypoxia induced shift in fluorescence lifetimes ($n=4$ for each). Treatment with reducing agents NEM (1mM) or sodium sulfide (1mM) did not lead to significant changes in fluorescent lifetime nor did they block the hypoxia response ($n=3-5$ for each). The effects of redox manipulation on hypoxia sensitivity were recapitulated in patch clamp assays of channel activity. Together this data supports the hypothesis that the STREX channel hypoxia response can not be mimicked by redox agents and that FLIM, in conjunction with patch clamp electrophysiology, allows the monitoring of conformational rearrangements and investigation of the structure-function relationship of the linker region between the channel RCK domains.

[1] McCartney, C.E., McClaffert, H., Huibant, J.M., Rowan, E.G., Shipston, M.J., and Rowe, I.C. (2005) A cysteine-rich motif confers hypoxia sensitivity to mammalian large conductance voltage- and Ca-activated K (BK) channel alpha-subunits. Proc Natl Acad Sci USA 102:17870-17876

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PC76

Osmosensing in the PVN: A role for TRPV4

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Heart disease is the biggest killer in the UK. Elevated sympathetic activity plays a role in heart disease, possibly due to disturbed osmosensing. Body fluid osmolarity is kept within a narrow range ($\sim 290-300$ mOsm) controlled by the hypothalamus [1]. Hypertonic challenge leads to an increase in heart rate, blood pressure and renal sympathetic activity [2]. The paraventricular nucleus (PVN) of the hypothalamus has been implicated as having a role in osmoregulation. Treatment with hypertonic solutions has been shown to increase excitatory post synaptic potentials (EPSPs) within parvocellular neurons [3] and early fos expression increases in the PVN during water deprivation, causing hypertonic challenge in rats [4]. The transient receptor potential vanilloid channel TRPV4 is a possible candidate for volume sensing within the PVN as it has this role in other tissues [5].

We modelled the action of TRPV4 in Neuron (University of Yale) to determine if activity of this channel is likely to account for any changes in action potential (AP) frequency. We investigated the mechanism of osmoregulation within the parvocellular PVN by recording action currents as an indicator of underlying AP frequency in mouse brain slices using cell-attached patch clamp electrophysiology. Results given as a normalised mean $\pm$ SEM; significances assessed by paired t-test. Hypotonic challenge (280 mOsm) decreased AP frequency from 1.0 ± 0.1 Hz to 0.2 ± 0.1 Hz ($n=7$; $p<0.01$). The role of TRPV4 was investigated using the agonist 4- α -phorbol 12,13-didecanoate (1 μ M) which decreased AP frequency to 0.6 ± 0.5 Hz ($n=6$; $p<0.05$). Conversely, upon addition of the TRPV4 antagonist RN1734 (5 μ M) decreases in AP frequency during hypotonic challenge were not seen (0.6 ± 0.1 Hz) ($n=6$; $p>0.05$). Since activation of TRPV4 leads to an increase in intracellular Ca²⁺; we hypothesised activation of Ca²⁺-activated K⁺ channels may be responsible for the decreased APs seen during our experiments. In support of this hypothesis we found that the effects of hypotonic challenge on AP frequency did not occur when inhibiting SK channels using UCL-1684 (30 nM) as AP frequency increased from 0.2 ± 0.3 Hz to 0.7 ± 0.2 Hz ($n=4$; $p<0.01$).

These results suggest TRPV4 expressing neurones, along with those expressing SK channels, are involved in osmosensing within the PVN.

Bourque, C.W (2008). *Nat Rev Neurosci* 9(7): 519-31.

Chen, Q.H. and G.M. Toney (2001). *Am J Physiol Regul Integr Comp Physiol* 281(6): R1844-53.

Chu, C.P., H. Kannan, and D.L. Qiu (2010). *Neurosci Lett* 482(2): 142-5.

Stocker, S.D., J.T. Cunningham, and G.M. Toney (2004). *Am J Physiol Regul Integr Comp Physiol* 287(5): R1172-R1183

Gao, F. and D.H. Wang (2010). *J Hypertens* **28**(1): 102-10.

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PC77

TASK-1 channel expression and function in oligodendrocytes of the mouse brain: a role in white matter ischemic injury

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Tandem Pore Domain K⁺ channels (K2P) are accountable for K⁺ 'leak' currents in excitable cells and play a role in determining the excitability of neurons. In addition, the K2P subtype TASK-1 (KCNK3) is strongly pH-sensitive and may be a key regulator of neuronal damage during ischemia. Oligodendrocytes are the myelin-forming cells of the central nervous system (CNS) and are highly sensitive to ischemia. We have therefore examined whether oligodendrocytes express TASK-1 channels and if they have a function in ischemia. Mice were killed humanely in accordance with the UK Animals (Scientific Procedures) Act (1986) and brains or optic nerves removed for immunohistochemistry, tissue culture or electrophysiology. Immunohistochemistry of the cerebellum and optic nerve showed that TASK-1 is strongly expressed in oligodendrocyte somata and processes. Immunocytochemistry and whole cell patch-clamp of oligodendroglial cells cultured from mouse optic nerve explants demonstrated expression of TASK-1 channel protein and ionic currents with the properties of TASK-1 channels. Developing oligodendrocytes exhibit Kv and Kir currents, which when inhibited using a combination of extracellular (100 μ M BaCl₂, 3 mM tetraethylammonium chloride and 6 mM 4-aminopyridine) and intracellular (3 mM Na₂ATP and 250 μ M spermine) blockers revealed a residual outward K⁺ current that was sensitive to pH_o and the TASK-1 inhibitor, anandamide. Peak outward currents were significantly lowered by 10 μ M anandamide ($p < 0.05$, t-test at +50 mV, $n = 7$), and significantly increased at pH 8.4 compared to pH 6.4 ($p < 0.05$, t-test at +100 mV, $n = 5$). As a model for ischemic damage to oligodendrocytes, isolated intact optic nerves were subjected to oxygen-glucose deprivation (OGD) in an atmospheric chamber. Loss of oligodendrocyte processes was assessed by confocal microscope image analysis using transgenic fluorescent reporter mice (proteolipid protein (PLP) promoter driving dsRed or Sox10 promoter driving enhanced green fluorescence protein (EGFP) expression) and cell death assayed by propidium iodide staining. After 60 minutes of OGD there was a significant $36.12 \pm 4.74\%$ decrease in PLP⁺ oligodendrocyte processes compared to controls ($p < 0.05$, ANOVA and Newman-Keuls post-hoc test, $n = 4$) and a significant increase in oligodendrocyte cell death compared to controls ($238.6 \pm 44.1\%$, $p < 0.05$, t-test, $n = 4$). Treatment with methanandamide (10 μ M) to inhibit TASK-1 during OGD prevented the loss of oligodendrocyte processes ($p > 0.05$ vs control, $n = 4$, $p < 0.05$ vs OGD, $n = 4$) and significantly protected against cell death ($p > 0.05$ vs control, $n = 4$, $p < 0.05$ vs OGD, $n = 4$). These results provide evidence of functional expression of TASK-1 in oligodendrocytes and indicate that TASK-1 channels are key mediators of oligodendrocyte damage and cell death during ischemia.

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PC78

Kainate receptor activation involves a sequence of distinct conductance states

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Glutamate is the major excitatory neurotransmitter in the brain, activating NMDA-, AMPA-, and kainate- (KAR) selective families of ionotropic receptors. KARs rapidly desensitize in the continued presence of glutamate, a process thought to be mediated by the separation of subunits at the dimer interface of the ligand binding domain (LBD). Efforts to block desensitization through point mutations along this interface have produced macroscopic responses which are non-decaying. As of yet, the behaviour of these KAR dimer interface mutants has not been studied at the single channel level. Here, we investigated two such mutant subunits, GluK2 D776K, which forms an electrostatic bridge across the dimer interface, and GluK2 Y521C/L783C, which is thought to covalently crosslink dimer subunits. Through electrophysiological experiments performed on outside-out patches excised from transfected tsA201 cells, we found that D776K off-kinetics (2.4 ± 0.2 ms, $n = 11$) matched those of wild-type (WT) GluK2 receptors (2.3 ± 0.1 ms, $n = 7$) following brief agonist applications. In contrast, the off-kinetics of Y521C/L783C (10.5 ± 0.8 ms, $n = 9$) matched those of WT equilibrium responses (11.9 ± 1.3 ms, $n = 8$), observed after long agonist applications. Dose-response relationships also revealed glutamate potency was approximately ten-fold lower for D776K ($EC_{50} 520 \pm 91 \mu$ M, $n = 6$) and the WT peak response ($EC_{50} 652 \pm 47 \mu$ M, $n = 7$), compared to Y521C/L783C ($EC_{50} 52 \pm 1 \mu$ M, $n = 4$) and the WT equilibrium response ($EC_{50} 40 \pm 8 \mu$ M, $n = 4$). At saturating agonist concentrations, D776K single channels remained in an open state, except for brief closures, suggesting that this mutant is effectively non-desensitizing. In addition, the amplitude of the D776K unitary conductance was comparable with the main open state of the WT receptor. However, Y521C/L783C single channels accessed much lower conductance levels, and spent long periods in closed states. The difference between the desensitization we observed at the single channel level and the non-decaying macroscopic behaviour of the Y521C/L783C receptor can be explained if its activation process is desynchronized. In conclusion, our data suggests, unexpectedly, that KAR activation states are controlled by the LBD dimer interface, consistent with a sequential model of desensitization.

This work was supported by an operating grant to D.B. from the Canadian Institutes for Health Research (CIHR). G.B.D. is supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Alexander Graham Bell Canada Graduate Scholarship. D.B. is the recipient of the Canada Research Chair award in Receptor Pharmacology.

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PC79

The role of disulfide bond formation in a non-decaying kainate receptor

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Structural studies of kainate-type glutamate receptors (KARs) suggest that desensitization occurs by re-arrangement of the dimer interface. Consistent with this, crosslinking the dimer interface using disulfide bonds via double cysteine mutations renders responses non-decaying in the presence of glutamate. One such KAR mutation is GluK2 Y521C/L783C. Interestingly, single point mutations of the 521 residue greatly reduce KAR macroscopic decay suggesting that the non-decaying phenotype could arise without disulphide bond formation. We considered whether disulfide bond formation is necessary for the Y521C/L783C non-decaying phenotype.

Wildtype or mutant KARs were transfected into HEK cells and macroscopic responses were elicited by fast application of 10 mM glutamate (250 ms) to outside-out membrane patches. Wildtype receptors displayed an initial peak that decayed ($\tau = 5.5 \pm 0.3$ ms, $n=4$) to a steady state current. The Y521C mutant decayed with a τ of 69.4 ± 14.5 ms ($n=4$), consistent with the idea that the individual mutations might be cumulative. However, no functional currents could be obtained from L783C excised patches ($n=8$) or whole cells ($n=4$). The double cysteine mutation showed no macroscopic decay during the glutamate application ($n=6$) and this phenotypic response was unaffected by 30 mM dithiothreitol (DTT). If disulfide bond formation is necessary for this phenotype then reducing conditions (DTT) would break the bond formation and revert responses to a wildtype phenotype. Western blots of Y521C/L783C showed that dimers formed in control conditions and only monomers were formed in reducing conditions ($n=4$). However, this finding was identical to wildtype KARs ($n=4$) making it impossible to determine if the double cysteine mutation required disulfide bond formation. To decrease normal disulfide bond formation that occurs in KARs, wildtype and Y521C/L783C receptors that lacked the amino terminal domains (Δ ATD) were generated. Only monomers were revealed on western blots for both receptors in control and reducing conditions ($n=4$) strongly supporting that the introduction of two cysteines proposed to crosslink the dimer interface do not form disulfide bonds. Electrophysiologically, Δ ATD mutations were phenotypically identical to the full-length wildtype and Y521C/L783C receptors and the double cysteine Δ ATD was unchanged by DTT application.

Here we demonstrate that disulfide bond formation is likely not responsible for the non-decaying macroscopic current exhibited by the Y521C/L783C KAR mutation. This suggests that restriction of the dimer interface does not prevent desensitization. However it is clear that the dimer interface contains key amino acid residues that govern how a glutamate receptor responds to agonist binding.

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PC80

ELIC channel activation in response to agonist concentration jumps

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The best structural data available for nicotinic superfamily channels come from ELIC, GLIC and GluCl. Of these, ELIC, a cationic channel from the prokaryote *Erwinia chrysanthemi*, may be the best for electrophysiological studies aimed at characterizing channel activation, as it opens to a high single channel conductance (> 80 pS) in response to a variety of small molecule agonists such as propylamine, cysteamine and GABA (Zimmermann and Dutzler, 2011).

We have used the technique of rapid theta-tube application to elicit macroscopic agonist currents as an initial characterization of the channel activation process, to help us design subsequent single channel experiments. Outside-out patches were obtained from HEK293 cells expressing ELIC and held at -100 mV. Agonist concentration jumps (50 ms-1.5 s) had 20-80% exchange times faster than 500 μ s (measured with diluted extracellular solution after patch rupture). We tested propylamine (10- 50 mM), cysteamine (10-50 mM) and GABA (20-100 mM). Cysteamine was applied in the presence of 1 mM dithiotreitol.

For propylamine and cysteamine, the speed with which the current on-relaxation developed reached a clear maximum at 50 mM. The rising phase of the current response was well fitted by a single exponential, with time constants of 7 ± 1 ms ($n=9$ patches) and 10 ± 1 ms ($n=17$) at the saturating concentration of 50 mM for cysteamine and propylamine, respectively.

Agonist currents declined during sustained applications in a manner suggestive of desensitisation. This decline was well fitted by a single exponential (e.g. $\tau = 180 \pm 30$ ms, $n=9$ patches, for 50 mM 400 ms cysteamine pulse).

In contrast with the current onset, the off-relaxation of the macroscopic currents had a complex shape. A rebound current was observed at the end of the applications. This "off-current" does not disappear at positive potentials and therefore it is not due to the end of a voltage-dependent channel block by the agonist (a process well characterised in muscle nicotinic channels). Good fits to the decay phase of the agonist currents (from the peak of this rebound current) required two exponential components with amplitudes of opposite sign.

The most striking property of agonist-evoked ELIC responses was their slow time course. This is particularly obvious if we compare them with responses obtained from vertebrate Cys-loop channels. For instance, the onset speed of currents recorded from glycine channels in response to high glycine concentrations is very fast, indeed comparable to the of the solution exchange for the patch (e.g. time constant = 0.3 ± 0.03 ms, $n=11$ patches, 3 mM glycine and 40mM intracellular Cl⁻). Zimmermann, Dutzler, Plos Biol. 2011 Jun; 9 (6): e 1001101

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PC81

The expression & function of potassium channels in mouse N9 microglia

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Microglia are immunocompetent cells in the central nervous system that have many similarities with macrophages of peripheral tissues. Their activation protects local cells from foreign microbial infection in the CNS. However, “over-activated” microglia become a “Double-edged sword” which show neuronal toxicity and are implicated in a variety of neurodegenerative disease. Previous studies have suggested that potassium channels play a role in regulating microglial activation, migration and proliferation. However what kinds of potassium channel subunits are expressed in microglia, whether their expression changes after microglial activation and the functional role of most potassium channels expressed in microglia are poorly characterized.

To address these questions, we used the N9 mouse microglial cell line as a cell model for experiments in vitro. Lipopolysaccharide (LPS), which is the endotoxin of gram negative bacteria, was used to stimulate microglial activation that results in subsequent nitric oxide (NO) release. Using qRT-PCR, we analyzed mRNA expression of >80 potassium channel pore-forming subunits and their regulatory subunits in both LPS-treated (1 µg/ml for 24h) and untreated microglia. In resting microglia, $K_v3.3$ channel mRNA was most abundant with other mRNAs for other channels previously reported in microglia, such as the large (BK) and intermediate (IK) conductance calcium-activated potassium channels, and the voltage gated $K_v1.3$ and $K_v1.5$ channel mRNA, expressed at a lower level. The mRNA expression of some channel subunits changed significantly after LPS treatment. For example, mRNA expression of $K_{2p6.1}$ and IK was decreased by $34 \pm 4.1\%$ and $53 \pm 15.8\%$ respectively whereas $K_v1.3$ mRNA expression was increased by $194 \pm 18.3\%$. These data suggested that LPS-induced changes in channel mRNA expression may be involved in microglial activation. We examined whether pharmacological manipulation of these channels controlled LPS-induced NO release. We found that the IK selective blocker, Tram-34 (1 µM) and the $K_v1.5$ inhibitor propafenone (PPF) (10 µM) significantly decreased LPS-induced NO release by $9 \pm 1.2\%$ and $12 \pm 0.9\%$ respectively. Ba^{2+} (1 mM) that inhibits inwardly rectifying potassium channels as well as $K_{2p6.1}$ also significantly attenuated LPS-induced microglial activation by $13 \pm 1.7\%$. Inhibition of $K_v1.3$ channels using margatoxin had no significant effect on LPS-induced NO release. Inhibition of BK channels by paxilline had no significant effect alone however, paxilline attenuated the effect of Tram-34, PPF and Ba^{2+} to control LPS-induced NO release. In this study, we characterized the potassium channel mRNA expression pattern in N9 mouse microglia. IK, $K_v1.5$ channels but not $K_v1.3$ were implicated in regulating LPS-induced microglial NO release. In addition, BK channels may work as a modulator regulating the effect of other channels on NO release by activated microglia.

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PC82

Role of PKC α in adjusting of extrasynaptic AMPA receptors trafficking in dorsal horn neurons during persistent inflammatory painO. Kopach¹, V. Viatchenko-Karpinski¹, P. Belan¹, Y. Tao² and N. Voitenko¹

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Peripheral inflammation alters AMPA receptor (AMPA) subunit trafficking and increases AMPAR Ca^{2+} permeability at synapses of dorsal horn neurons by internalized GluR2-containing Ca^{2+} -impermeable AMPARs (Park et al., 2009). However, whether and how AMPAR trafficking at extrasynaptic sites of these neurons changed under persistent inflammation and which molecular mechanisms underlie these changes are still unclear.

Persistent peripheral inflammation was induced by complete Freund's adjuvant (CFA, 100 µl), injected subcutaneously into the plantar side of one hind paw of the rats, anesthetized with isoflurane (1.5–2% depending on age and verified by toe pinch). The animals were used in accordance with protocols that were approved by the Animal Care and Use Committee at the Bogomoletz Institute of Physiology and Johns Hopkins University and were consistent with the ethical guidelines of the National Institutes of Health and the International Association for the Study of Pain.

Using patch-clamp recording combined with Ca^{2+} imaging we showed that under normal conditions extrasynaptic AMPARs in rat substantia gelatinosa (SG) neurons consists predominantly of GluR2-containing Ca^{2+} -impermeable receptors. Peripheral inflammation induces a dramatic increase in functional expression of GluR1-containing Ca^{2+} -permeable AMPARs in the extrasynaptic plasma membrane of SG neurons, manifested as augmented AMPA-induced current and associated Ca^{2+} influx in neurons 1 d post-CFA, an increased sensitivity to selective inhibition of Ca^{2+} -permeable AMPARs and inward rectification of the currents (Kopach et al., 2011). These changes occurred only in SG neurons characterized by tonic firing properties, but not in those exhibiting a strong adaptation. By utilizing the oligonucleotides (ODNs) that specifically knockdown spinal cord protein kinase C α (PKC α), we found that a decrease in dorsal horn PKC α expression prevents CFA-induced upregulation of extrasynaptic Ca^{2+} -permeable AMPARs in tonically firing SG neurons, manifested as an abolishment of augmented AMPA-induced currents and associated $[Ca^{2+}]_i$ transients and as a reverse of the current rectification 1 d post-CFA. Finally, dorsal horn PKC α knockdown produced anti-nociceptive effect on CFA-induced thermal and mechanical hypersensitivity during the maintenance period of inflammatory pain.

Thus, persistent inflammation dramatically increases functional expression of extrasynaptic GluR1-containing Ca^{2+} -permeable AMPARs and their proportion in total AMPAR pool in tonically firing SG neurons that depends on PKC α . A significant anti-nociceptive effect of spinal cord PKC α knockdown may inspire the possible implications of PKC α gene-silencing therapy for preventing and/or treating persistent inflammatory pain.

Park JS, Voitenko N et al., J. Neurosci. 2009;29:3206-3219.

Kopach O et al., Pain. 2011 Apr;152(4):912-23.

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PC83

A comparison of neuronal properties in the ventral nucleus of the trapezoid body between two strains of inbred mice

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Age-related hearing loss (AHL) is the most prevalent sensory disorder in our population, reducing quality of life for millions of sufferers. The mechanisms underlying its development are not understood. There is some evidence that AHL may be preceded by a loss of function in the feedback loop which regulates auditory sensitivity via the medial olivocochlear system (MOC) (Zhu et al., 2007). These cells send a cholinergic projection to the outer hair cells of the cochlea, which regulate the gain and sensitivity of this sensory organ. In mice, the MOC neurons reside predominantly in the ventral nucleus of the trapezoid body (VNTB). This project compares VNTB neurons in two strains of mice: the C57BL/6 which demonstrates AHL from a young age and the CBA/Ca which maintains good auditory thresholds into old age. The VNTB expresses the slow delayed rectifier Kv2.2. It was postulated that differences in the expression of Kv2.2 over time may contribute to the differential progression of AHL between the strains. Mice (P8-190) were killed by decapitation in accordance with the UK Animals (Scientific Procedures) Act 1986. Immunohistochemistry (males, CBA n=3, C57 n=3) showed no difference in the expression of Kv2.2 between these strains by the age of 6 months. Whole cell voltage clamp from VNTB neurons (recorded from *in vitro* brainstem slices at 37°C) indicate that before hearing onset (P8 to P10) there was no significant difference (independent samples t-test) between C57 and CBA strains in the magnitude of peak outward currents (mean current $\pm$ S.E.M.). Peak currents (+60mV): CBA: 20.87 ± 5.84 nA (n=7), C57: 18.72 ± 5.58 nA (n=7). However, after hearing onset (P14 to P17) C57 VNTB neurons had greater low voltage-activated (LVA) currents than those of the CBA. Peak amplitude of outward currents in response to depolarising steps from -90mV to -30mV were: 14.73 ± 12.48 nA (CBA n=4) and 25.66 ± 8.25 nA (C57 n=5). These preliminary data indicate differences in VNTB neuronal excitability may develop rapidly following hearing onset, which may influence later development of hearing loss.

ZHU, X., VASILYEVA, O. N., KIM, S., JACOBSON, M., ROMNEY, J., WATERMAN, M. S., TUTTLE, D. & FRISINA, R. D. 2007. Auditory efferent feedback system deficits precede age-related hearing loss: contralateral suppression of otoacoustic emissions in mice. *J Comp Neurol*, 503, 593-604.

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PC84

Effects of high concentrations of lidocaine chloride on the recovery of the compound action potential depolarization time

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Introduction. The depolarization time of the compound action potential (CAP) represents a period between the onset and the peak of the CAP curve. The compound action potential is response of a peripheral nerve after the application of suitable stimulus.

Objective. The aim was to examine the effects of high concentrations of lidocaine chloride on blocked sodium channels recovery.

Method. This study was carried out at the Physiology Department, Faculty of Medicine, Motenegro, after National Ethic Committee approval was obtained. Isolated sciatic nerves (No20) from healthy adult frogs (*R. Dalmatina*) weighting 100-200 g were used in electrophysiological experiments. The animals were kept under ambient temperature (21-24 C) and natural light for 24 hours according with National Institute Health Guide for Care and Use of Laboratory Animals. Frogs were rapidly decapitated and then the sciatic nerves were dissected from the lumbar plexus to the level of the ankle and incubated in Ringer solution (2,0 mmol/l calcium solution) for two hours in order to achieve stable baseline and reproducible CAP. Afterward, control measurements of CAP depolarization time were taken. Then, the frog sciatic nerves were divided into two groups (10 nerves in each group), and were incubated for 15 minutes in the following solutions: Control group: Ringer solution; Experimental group: 100,0 mmol/l lidocaine solution. After that, nerves were washed and incubated in Ringer solution and the CAP depolarization time was measured at 30, 60, 120 and 180 minutes during recovery period. CAP was induced by stimulator with single electrical stimulus and recorded using digital oscilloscope. Data were collected and analysed using the statistical computer programme Grap-PadPrism 5.0.

Results. The depolarization time of all nerves in experimental group was blocked at 30 minute of recovery period. After 60 minutes the mean depolarization times were 308 ± 35.5 μ s and 431.1 ± 82.5 μ s for nerves in control and experimental group, respectively ($p < 0.01$). The difference in the mean amplitude values between groups after 120 and 180 minutes of the recovery period, was not statistically significant.

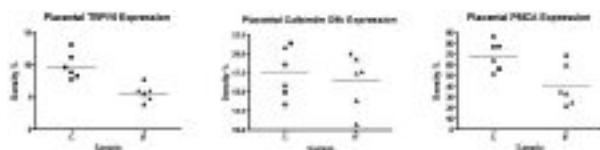
Conclusion. Knowing the fact that the CAP depolarization time shows the impact on sodium channel blockade, it may be concluded that lidocaine chloride in high concentrations reversibly blocks sodium channels, since the complete recovery of the depolarization time occurred 120 minutes after its application.

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PC85

The effect of intermittent fasting on the expression of calcium transporting proteins in the rat placentaL. Sansby¹, S. Alwasel², N. Ashton³ and H. Bond¹¹School of Applied Science, University of Huddersfield, Huddersfield, Yorkshire, UK, ²College of Science, King Saud University, Riyadh, Saudi Arabia and ³Faculty of Life Sciences, University of Manchester, Manchester, UK

During gestation the developing fetus is dependent upon nutrient transfer from the mother which is mediated by the placenta. Fetal and placental growth responds to maternal influences such as diet and therefore may be affected adversely by nutrient deprivation. During Ramadan, the month of Islamic fasting, participants must refrain from eating and drinking during daylight hours. Although exempt, many pregnant women in the Middle East participate in the period of fasting. In a human study conducted in Saudi Arabia, we observed that babies which were in utero in the 2nd or 3rd trimester of gestation during the fasting period had lower mean placental weights and placental weight to birth weight ratios compared to with those not in utero during Ramadan [1]. We have also recorded similar results in rats that were fasted intermittently during pregnancy [2]. The aim of this study was to determine the effect of intermittent fasting on placental calcium transporting protein expression as an indicator of fetal calcium accretion and thus skeletal growth. Pregnant Wistar rats (n=6) were subjected to intermittent fasting (IF); food was withdrawn between 17:00 and 9:00 each day for the duration of gestation. Pregnant control rats (n=6) had free access to food; all rats had unrestricted access to water. Rat placental tissue was collected on embryonic day 21 and calcium transporting protein expression was quantified by Western blotting. Calcium transport across the placenta occurs in 3 stages: diffusion into the trophoblast from maternal plasma through epithelial Ca²⁺ channels of the transient receptor potential (TRP) gene family, transfer across the trophoblast cytoplasm bound to the calcium binding protein calbindin-D9K and lastly active extrusion into the fetal compartment via the plasma membrane Ca²⁺-ATPase (PMCA) [3]. We have reported previously a significant decrease in PMCA expression in IF samples compared with control counterparts [4]. Here we show that placental Calbindin-calbindin-D9k expression is unchanged in placentas from intermittently fasted rats, whereas the epithelial calcium channel TRPV6 is significantly reduced in the IF placental tissue (P<0.01, Mann Whitney U, n=6, Figure 1). These data suggests that fetal calcium accretion may be diminished in the later stages of gestation when the mother is subjected to intermittent fasting. This implies that the increase in fetal weight associated with IF may not be due to greater bone mass.



Alwasel SH et al (2010). Placenta 31, 607-610

Wilkinson RJ et al (2010). Proc Physiol Soc 19, PC244

Bond H et al (2008). J Physiol 586, 2015-2025

Sansby LA et al (2011). Proc Physiol Soc 24, PC22

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PC86

Glutamate efflux across the microvillous membrane of the placental syncytiotrophoblast is mediated via the amino acid exchanger System Xc⁻ and a volume regulated mechanism

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Glutamate has a high concentration within the placental syncytiotrophoblast (5 mmol/l⁻¹) but its role is uncertain. Glutamate uptake is mediated by System X^{AG} but it remains unclear how it is released from the placental syncytiotrophoblast. The aim of this study was to investigate mechanisms by which glutamate might be released from the microvillous membrane (MVM) of the placental syncytiotrophoblast into the maternal circulation. Ethical approval for this study was granted by the Southampton and Southwest Hampshire Regional Ethics Committee. Placentas were collected following uncomplicated term pregnancies. Isolated perfused human placental cotyledons (n = 10) were perfused with modified Earle's bicarbonate buffer. ³H-proline, ¹⁴C-glutamate and 1.8 mM creatinine were perfused into the maternal arterial circulation. A bolus (16 μmol) of glutamate and a bolus of the System Xc⁻ substrate, N-acetylcysteine (0.1 mol), were injected into the circulation to stimulate glutamate transport by exchange. In addition, following the cessation of isotope perfusion, a bolus of 50 mmol urea was added to the maternal circulation to create an osmotic shock. Radioactivity in maternal and fetal venous samples were determined via liquid scintillation counting and creatinine levels were determined using an enzymatic assay. PCR was used to identify whether Xc⁻, OAT1, OAT2, OAT4, OAT5 and OAT8 were expressed in the placenta. Western blotting was used to determine whether Xc⁻ was localised to the MVM. The maternal N-acetylcysteine bolus stimulated release of glutamate, but not proline, into the maternal circulation (n = 5). PCR confirmed the expression of Xc⁻ in human cytotrophoblast tissue samples and western blotting localised this to the MVM. Following the maternal arterial urea bolus there was release of glutamate but not proline into the maternal circulation (n = 5). This study provides evidence for two routes of glutamate efflux from the placental syncytiotrophoblast into the maternal circulation. The first is via the amino acid exchanger System Xc⁻ which was localised to the MVM. Activity of System Xc⁻ is a rate limiting step in the provision of cystine to the cell for glutathione synthesis. As a result, high intracellular glutamate levels may create a gradient which mediates the uptake of cystine for glutathione production, protecting the fetus from oxidative stress. The release of glutamate in response to an osmotic shock is consistent with the volume regulated anion channel. This channel may be involved in regulating the volume of the syncytiotrophoblast. These data suggest that high intracellular glutamate concentrations generate a gradient which can be used to drive membrane transport and maintain cellular homeostasis.

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PC87

Impact of tyrosine phosphatase inhibition on CFTR function in human bronchial epitheliumC. Spearman¹, R. Muimo² and L. Robson¹¹Biomedical Science, University of Sheffield, Sheffield, UK and²Department of Infection and Immunity, University of Sheffield, Sheffield, UK

In human bronchial epithelial cells (HBE) CFTR plays a critical role in mediating Cl⁻ secretion. This important role is highlighted in cystic fibrosis, where CFTR mutations impact significantly leading to an increased risk of infection. Although CFTR is activated by a PKA dependent process, additional signalling pathways and a variety of accessory proteins also regulate its activity. In a previous patch clamp study pre-incubation with the tyrosine phosphatase inhibitor phenylarsine oxide (PAO) reduced the cAMP-activation of CFTR, suggesting that tyrosine phosphorylation is inhibitory (1). The aim of the current study was to determine whether this inhibitory action was also observed in epithelial monolayers. 16HBE14o- cells were grown on permeable supports until confluent and a transepithelial resistance of at least 200 Ω cm² was attained. Inserts were mounted in an Ussing chamber with standard Krebs solution (basolateral side) and low Cl⁻ Krebs (apical side, NaCl substituted with Na⁺ gluconate). Solutions were bubbled with 5% CO₂, and all potential and resistance measurements corrected. At steady-state control measurements were taken for 5 minutes, before 10 μ M forskolin (FSK) and 100 μ M IBMX were added to activate CFTR. 10 μ M CFTR_{inh172} was then added to the apical side of the insert to provide an indication of CFTR function. 10 μ A of current was injected each minute to allow for the calculation of the equivalent short circuit current (I_{SC}). In a second set of inserts cells were pre-incubated with 10 μ M PAO before exposure to FSK and IBMX. Statistical significance was tested using ANOVAs and Student's t-test as appropriate and assumed at the 5% level. In control inserts initial Vte was 6.40 $\pm$ 1.04 mV, and addition of FSK/IBMX increased this to 9.49 $\pm$ 1.81 mV. CFTR_{inh172} decreased Vte to 4.16 $\pm$ 0.97 mV (n=13). I_{SC} increased from 19.7 $\pm$ 1.66 μ S/cm² to 24.8 $\pm$ 2.70 μ S/cm² with FSK/IBMX, and then fell to 11.1 $\pm$ 1.37 μ S/cm² with CFTR_{inh172}. The CFTR_{inh172}-sensitive Vte and I_{SC} were 5.32 $\pm$ 1.12 mV and 13.8 $\pm$ 1.96 μ S/cm². In PAO incubated inserts the response to FSK/IBMX was absent. Vte was 5.54 $\pm$ 1.44 mV versus 3.68 $\pm$ 1.07 mV and I_{SC} 18.5 $\pm$ 2.23 μ S/cm² versus 18.1 $\pm$ 2.09 μ S/cm² in the absence and presence of FSK/IBMX, respectively (n=9). CFTR_{inh172} gave a decrease in both Vte and I_{SC} . However the magnitude of the response was attenuated compared to control. In PAO treated cells the CFTR_{inh172}-sensitive Vte was 1.80 $\pm$ 0.84 mV and I_{SC} was 5.56 $\pm$ 2.01 μ S/cm². These data support the hypothesis that tyrosine phosphorylation has an inhibitory action on CFTR function and highlight the complex nature of the regulation of CFTR. Further work is now investigating whether this inhibitory action is mediated via a change in the activity of the channels or whether there is an impact on channel trafficking to or removal from the membrane.

A England, R Muimo, L Robson. A potential role of tyrosine phosphorylation in the function of the cystic fibrosis transmembrane conductance regulator (CFTR) FASEB J March 17, 2011 25:659.5

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PC88

Rapid effect of 17 β -estradiol on airway surface liquid hydration in normal and cystic fibrosis bronchial epitheliaV. Saint-Criq¹, S. Kim², J.A. Katzenellenbogen² and B.J. Harvey¹¹Molecular Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland and²Department of Chemistry, University of Illinois, Urbana, IL, USA

Male cystic fibrosis (CF) patients survive 9 years longer than females ("CF gender gap") and lung exacerbations in CF females vary during the estrous cycle. Estrogen has been reported to reduce the height of the airway surface liquid (ASL) in female CF bronchial epithelium. Here we investigated the effect of estrogen on ASL height and ion transport in normal (NuLi-1) and CF (CuFi-1) bronchial epithelium monolayers grown on permeable filters in an air-liquid interface.

Confocal fluorescence microscopy experiments were performed to measure ASL height in normal and CF bronchial epithelial cells treated with 17 β -estradiol (E2 0.1 to 10nM). In order to determine the ion channel(s) involved in the effect of E2 on ASL height, different ion transporter modulators were used in ASL and Ussing chamber experiments. The nuclear-excluded Estrogen Dendrimer Conjugate and its negative control (empty dendrimer) were used in order to determine the involvement of the nuclear estrogen receptor pathway in the effect of E2 on ASL height.

Confocal experiments revealed that ASL height was significantly higher in the non-CF cell line compared to the CF cells (NuLi 6.82 $\pm$ 0.33 μ m vs. CuFi 5.58 $\pm$ 0.14 μ m, n=20, p<0.001). Physiological concentrations of E2 reduced the ASL height in both non-CF (25% decrease, n=5, p<0.05, ANOVA) and CF (20% decrease, n=5, p<0.05, ANOVA) cell lines after 30 min treatment. Treatment with the Cl⁻ transport inhibitor bumetanide or the KCNQ1 potassium channel blocker chromanol HMR1556 decreased ASL height significantly in both cell lines. However, E2 had no additive effect on ASL height in the presence of these ion transporter inhibitors. E2 decreased the bumetanide-sensitive Cl⁻ current in normal cells (E2: 6.47 $\pm$ 2.08 μ A/cm², Control: 9.52 $\pm$ 2.08 μ A/cm², n=3, p<0.05, paired t-test) and produced an increase in amiloride sensitive current in CF cells (E2: 11.097 $\pm$ 1.805 μ A/cm², Control: 8.801 $\pm$ 1.464 μ A/cm², n=7, p<0.05, paired t-test). Treatment with the nuclear-impaired Estrogen Dendrimer Conjugate (EDC 0.1 - 1nM E2 equivalent concentration) produced a significant reduction in ASL height in CF and non-CF cells (4.72 $\pm$ 0.25 μ m in NuLi-1 and 4.86 $\pm$ 0.42 μ m in CuFi-1, n=5, p<0.05 compared to control, ANOVA) whereas the empty dendrimer had no effect.

These results demonstrate that E2 dehydrates both CF and normal ASL and these rapid responses to E2 are membrane-initiated rather than via the classical nuclear estrogen receptor signal transduction pathway. The ion transporter inhibitor data indicate that E2 acts on ASL by inhibiting Cl⁻ secretion in non-CF cells and increasing Na⁺ absorption via ENaC in CF cells.

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PC89

Use of human nasal epithelial cells to study folliculin function in Birt-Hogg-Dubé syndrome

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Birt-Hogg-Dubé (BHD) syndrome is an autosomal dominant condition arising from germ line mutations in the Folliculin (Flcn) gene that cause mis-folding or truncation of the protein. Although the biological function of Flcn is not fully known its mutation effects are diverse, causing skin fibrofolliculoma, renal cancer, lung cysts and pneumothorax. Research into the lung-based aspects of BHD is hampered by the lack of suitable culture models and the risk of pneumothorax prevents collection of airway biopsy material. Human nasal epithelial (HNE) cell culture offers a solution as its collection avoids sites of lung pathology and yields viable epithelial tissue. The first purpose of this study (Part 1) was therefore to establish proof-of-principle for this model by determining if HNE cells express Flcn and its interacting partner, FNIP-1. Lung cyst formation has been linked to elevated matrix-metalloproteinase-9 (MMP-9) activity (Pimenta *et al.*, 2011) which disrupts epithelial turnover, therefore, our second purpose was to determine if MMP-9 inhibition recovers growth of human bronchial epithelial (HBE) cells carrying a stable knockdown of the Flcn gene (Part 2). For Part 1, HNE cells were obtained from four consenting donors by rubbing a cytology brush against the inferior turbinate. Cell material was cultured in Basal Epithelial Growth Medium and used for immunofluorescence and qPCR. For Part 2, HBE cells (16HBE14o-) stably transformed with either non-genomic or Flcn shRNA were treated with the MMP-9 inhibitor, Ilomastat (10 μ M), and ERK1/2 and retinoblastoma protein (Rb) phosphorylation were assessed to determine the effect on growth signalling pathways. Results for Part 1 demonstrated that mRNA and protein for Flcn and FNIP-1 were expressed in both ciliated HNE and also HBE cells. In HNE, immunofluorescence revealed that Flcn/FNIP1 localised to the ciliary base plate region and were associated with the ciliary markers, pericentrin and acetylated tubulin. Thus, Flcn/FNIP1 are apically polarised in HNE, suggesting that these cells are suitable for exploring Flcn function in airway epithelial organisation. Results for Part 2 revealed that Flcn knockdown in HBE attenuated cell growth (decreased S807/811 phosphorylation of Rb) relative to controls and that Ilomastat recovered this effect, together with elevated ERK1/2 T202/Y204 phosphorylation (n=4). Thus, an MMP-9-sensitive mechanism suppresses cell cycle activity in Flcn knockdown HBE cells, a phenomenon which could contribute to cyst formation in the BHD lung. Taking both parts of this study together, we conclude that Flcn and its partner protein, FNIP1, are expressed in airway epithelium and participate in the regulation of cell polarity and proliferation.

Pimenta SP, *et al.* (2011). *J Bras Pneumol.* **37**(4):424-30.

Antibodies to Flcn were supplied by A. Pause (Montreal) and FNIP1 from M. van Steensel (Maastricht). Supported by The Myrovlytis Trust.

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PC90

Loss of folliculin may promote lung cyst formation by altering VEGF/VEGFR2 signalling in human bronchial epithelial cells

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Birt-Hogg-Dubé (BHD) syndrome is associated with cyst formation in the lung but the link with the mutations in the folliculin gene which cause this disease remains unknown. Turnover of the airway epithelium is maintained by the autocrine action of epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) which induce clearance of aged cells and promote re-growth from progenitor cells. However, culture models lacking Flcn display slower growth and form poor cell junctions suggesting that the process of lung cyst formation could arise from a defect in this process. We therefore tested the hypothesis that Flcn alters growth responses to autocrine signals which regulate airway epithelial growth. Flcn expression levels were manipulated in an immortalised human bronchial epithelial cell line (16HBE14o- or "HBE"), by: i) Flcn over-expression, iii) transient siRNA or iii) stable shRNA knockdown. Comparisons were made with similarly treated control cells respectively transformed with empty or non-target constructs. Phosphorylation (T202/Y204) of ERK1/2 was used to indicate growth factor receptor activity and ELISA was used to assess growth factor secretion. Both transient and stable knock-down methods resulted in ~60% reduction in Flcn levels compared to controls and fluorescence-activated cell sorting (FACS) analysis revealed that this loss induced cell accumulation in G₀/G₁ phases of the cell cycle with a corresponding decline in pre-mitotic S phase cells. Recombinant EGF (10ng.ml⁻¹.6hrs) did not alter ERK1/2 phosphorylation on either Flcn over-expressing or knockdown HBE but raised VEGF secretion from 80.8 $\pm$ 6.0 to 184.4 $\pm$ 14.3pg.mgP⁻¹ suggesting that its major effect is to induce VEGF signalling. Addition of recombinant VEGF (10ng.ml⁻¹.6hrs) to control and shRNA Flcn knock-down cells raised ERK1/2 and PI3-kinase (phosphoAKT^{T308}) activity in both cell lines, however, the rate, magnitude and duration these effects were each suppressed in Flcn shRNA cells (P<0.05; n=4). We examined VEGF receptor 2 (VEGFR2) mRNA and protein expression and found that both were elevated by ~1.7 fold in Flcn shRNA cells. However, recombinant VEGF failed to induce receptor phosphorylation at Y1175 in these cells, a critical residue for receptor internalisation and activation (n=4). This was supported by immunofluorescence which revealed that VEGFR2 remained membrane-associated on VEGF treatment and failed to form active endosomal complexes. We conclude that Flcn knockdown suppresses autocrine signalling via the VEGF pathway by altering VEGFR2 signalling. Given the key role played by this factor in maintaining airway epithelial integrity, its dysregulation by Flcn mutation is likely to be a contributing factor for lung cyst formation in the BHD lung.

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PC91

NAADP- induced Ca^{2+} release in permeabilized rat hepatocytes

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Nicotinic acid adenine dinucleotide phosphate (NAADP) is an endogenous regulator of intracellular Ca^{2+} levels in a variety of eukaryotic cell types. Although, NAADP appears to be involved in direct Ca^{2+} release from the ER in different cells, evidence suggests that NAADP is an atypical messenger activating a novel two-pore channels (TPCs) channel located to the endo-lysosomal cellular compartment. Evidence suggests about NAADP-mediated Ca^{2+} release in liver microsomes and lysosomes (Mándi et al., 2006; Zhang et al., 2007). We assume that permeabilized hepatocytes as a good model representing a tool of different Ca^{2+} -stores (i.e. ER, mitochondria, Golgi apparatus, nucleus and lysosomes), could provide a more complex analysis of NAADP-mediated Ca^{2+} signaling events in these cells.

Experiments were carried out on isolated rat hepatocytes permeabilized with saponine (0.1 mg/ml). To estimate the changes of internal Ca^{2+} concentrations, hepatocytes were loaded with 100 μM chlorotetracycline (CTC) in the dark at room temperature for 20 min.

We showed that NAADP (7 μM) caused a significant decrease in CTC fluorescence intensity in permeabilized rat hepatocytes, which indicates about Ca^{2+} release from intracellular Ca^{2+} stores ($31.04 \pm 7.19\%$; $p < 0.01$, $n = 12$). The simultaneous application of NAADP (7 μM) and thapsigargin (1 μM) to the incubation medium decreased the CTC fluorescence to a slightly greater extent ($35.22 \pm 10.64\%$; $p < 0.05$, $n = 6$) compare to separate effects of NAADP and thapsigargin. Interestingly, NAADP did not affect fluorescence intensity after pre-incubation of hepatocytes with thapsigargin. This suggests that NAADP-mediated Ca^{2+} release in rat hepatocytes is thapsigargin-sensitive. Interestingly, a prior treatment of hepatocytes with thapsigargin followed by the application of nigericin led to a decrease in CTC fluorescence ($29.34 \pm 5.35\%$; $p < 0.01$; $n = 6$). Our observations revealed that in rat permeabilized hepatocytes the effect of thapsigargin on ER Ca^{2+} level is dependent on nigericin and mediated via Ca^{2+} release mechanism from acidic pools. In addition, application of NAADP in the presence of the mitochondrial Ca^{2+} uniporter inhibitor, Ruthenium red (10 μM), did not cause statistically significant changes of fluorescence intensity. Our findings suggest that NAADP-mediated Ca^{2+} release from acidic pools in permeabilized rat hepatocytes is sensitive to thapsigargin and Ruthenium red.

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PC92

Modulation of ion transport by bile acids in Calu-3 airway epithelial cellsS. Hendrick¹, C. Greene², S. Keely¹ and B. Harvey¹¹*Molecular Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland and* ²*Respiratory Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland*

Gastroesophageal reflux is common in Cystic Fibrosis (CF) patients and increases the risk of aspirating (duodeno)-gastric contents into the lungs (1). Bile acid aspiration often occurs after lung transplantation and can result in an exaggerated bronchial inflammatory reaction. Thus, bile acids have been identified as possible markers associated with the development of chronic rejection (bronchiolitis obliterans) (1,2). Depending on the concentration present, bile acids have been shown to acutely stimulate or chronically inhibit colonic epithelial Cl^- secretion (3).

We have investigated, using the short-circuit current technique, the effects of the unconjugated bile acid, deoxycholic acid (DCA, 25 μM), and the conjugated bile acid tauroDCA (TDCA, 25 μM) on basal transport and carbachol (100 μM) or forskolin (10 μM)-induced ion transport in Calu-3 airway epithelial cells. Cultured monolayers of Calu-3 cells were grown in an air-liquid interface and mounted in Ussing chambers. Electrogenic trans epithelial ion transport was measured as short-circuit current (Isc). Data given as Mean $\pm$ S.E.M where n = number of experiments performed on separate epithelia. Statistical analysis performed using student's paired t-test, where $p \leq 0.05$ is considered significant.

We found that acute (3 min) basolateral TDCA treatment of Calu-3 cells stimulated basal Isc by $35 \pm 7\%$ ($p = 0.0029$, $n = 9$) but had no effect on the Isc responses induced by carbachol or forskolin treatment. In contrast, acute apical treatment (30 min) of Calu-3 cells with DCA had no effect on basal Isc or on forskolin-stimulated Isc but did attenuate the Isc responses to carbachol by $43 \pm 8\%$ ($p = 0.001$, $n = 7$). In contrast, prolonged (24 hr) bilateral treatment with DCA or TDCA did not significantly alter either basal, forskolin- or carbachol-induced Isc. In order to determine if the increased Isc observed after TDCA treatment was as a result of local ATP release, experiments were repeated in the presence of hexokinase (24 U/ml) added either before or after TDCA. However, hexokinase did not significantly attenuate TDCA stimulated current indicating that ATP is unlikely to be a cellular mediator of bile acid action on airway ion transport. Western blotting showed that Calu-3 cells express the bile acid receptor, farnesoid X receptor (FXR). Chronic (24 hr) apical treatment of Calu-3 with an FXR agonist, GW4064 (5 μM), induced a $30 \pm 11\%$ increase in FXR expression ($p = 0.0306$, $n = 4$).

In summary, our data show that bile acid modulation of airway ion transport depends on the sidedness and duration of exposure, and the conjugated/unconjugated form of the bile acid. Further investigations are required to determine the role of the bile acid receptor FXR and the ion channels contributing to DCA and TDCA effects in modulating airway epithelium ion transport.

1. Pauwels A, et al. Bile acids in sputum and increased airway inflammation in patients with Cystic Fibrosis. *Chest* 2011 Dec 1, [Epub ahead of print]

2. Blondeau, K., V. Mertens, et al. (2008). "Gastro-oesophageal reflux and gastric aspiration in lung transplant patients with or without chronic rejection." *Eur Respir J* 31(4): 707-13

3. Keating, N, et al. Physiological concentrations of bile acids down-regulate agonist induced secretion in colonic epithelial cells." *J Cell Mol Med* 2009; 13(8B): 2293-303

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PC93

Temperature-dependent channel behaviour of wild-type and F508del-CFTR

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Cystic fibrosis (CF) is a common, lethal genetic disease caused by dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channel. The commonest mutation, F508del-CFTR disrupts CFTR processing and intracellular transport. It also impacts on channel stability and gating at the cell surface, with the result that F508del-CFTR Cl⁻ channels are slow to open, but rundown rapidly at 37 °C (1, 2). Here we investigate the effects of temperature on F508del-CFTR channel gating to understand better its instability. To address our aim, we studied CFTR Cl⁻ channels in excised inside-out membrane patches from C127 mouse mammary epithelial cells stably expressing wild-type and F508del-human CFTR at 23, 27, 30, 33 and 37 °C. The pipette (external) solution contained 10 mM Cl⁻, whereas the bath (internal) solution contained 147 mM Cl⁻, ATP (1 mM) and protein kinase A (PKA; 75 nM) at 23 - 37 °C; voltage was -50 mV.

At 23 °C, the single-channel current amplitudes of wild-type and F508del-CFTR were -0.54 ± 0.03 and -0.50 ± 0.01 pA (means $\pm$ SEM; $n \geq 4$; $p > 0.05$; student's unpaired t-test). As temperature was elevated to 37 °C, the current amplitude of wild-type and F508del-CFTR increased linearly to -0.75 ± 0.04 and -0.74 ± 0.02 pA ($n \geq 4$; $p > 0.05$; student's unpaired t-test). However, temperature had different effects on the open probability (P_o) of wild-type and F508del-CFTR. For wild-type CFTR, P_o increased exponentially from 0.24 ± 0.02 at 23 °C to 0.46 ± 0.04 at 37 °C ($n \geq 4$; student's unpaired t-test). By contrast, for F508del-CFTR, P_o increased from 0.06 ± 0.01 at 23 °C to 0.09 ± 0.01 at 30 °C before falling back to 0.06 ± 0.01 at 37 °C ($n \geq 4$). To understand better the temperature-dependence of P_o , we performed an analysis of bursts. For wild-type CFTR over the temperature range 23 - 37 °C, mean burst duration (MBD) decreased 2-fold and interburst interval (IBI) fell 7-fold ($n = 4$). However, for F508del-CFTR, MBD decreased 4-fold and IBI fell 7-fold over the temperature range 23 - 37 °C ($n = 4$).

In conclusion, our data suggest that temperature has similar effects on the single-channel current amplitudes of wild-type and F508del-CFTR. However, the gating behaviour of the two channels responds differently to temperature. For wild-type CFTR, maximal activity occurs at 37 °C, whereas for F508del-CFTR, it is around 30 °C. Thus, these data suggest that temperature might have different effects on the conduction and gating properties of CFTR.

Dalemans W, Barbry P, Champigny G, Jallat S, Dott K, Dreyer D, Crystal RG, Pavirani A, Lecocq JP, Lazdunski M. (1991) Altered chloride ion channel kinetics associated with the Δ F508 cystic fibrosis mutation. *Nature*. 354:526-8.

Aleksandrov AA, Kota P, Cui L, Jensen T, Alekseev AE, Reyes S, He L, Gentzsch M, Aleksandrov LA, Dokholyan NV, Riordan JR. (2012) Allosteric Modulation Balances Thermodynamic Stability and Restores Function of Δ F508 CFTR. *J Mol Biol*. [Epub ahead of print].

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PC94

Role of actin in haemolysis of red blood cells from patients with sickle cell disease in isosmotic non-electrolyte solutions

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Sickle cell disease (SCD) is one of the commonest severe inherited disorders with over 350,000 deaths yearly. It is caused by the presence of the abnormal haemoglobin HbS in patients' red blood cells (RBCs) instead of the normal adult HbA. When deoxygenated, HbS polymerises and distorts RBCs into sickles and other bizarre shapes. Clinical sequelae involve chronic anaemia with acute ischaemic episodes causing pain, organ damage and early death [1].

SCD RBCs have increased cation permeability which results in solute loss, shrinkage and increased [HbS]. On deoxygenation, the lag time to polymerisation is inversely proportional to [HbS]¹⁵⁻³⁰, so modest shrinkage markedly encourages sickling. Three main pathways are involved [2]: KCl cotransport, the Ca²⁺-activated K⁺ channel (Gardos channel) and a deoxygenation-induced non-selective cation pathway (P_{sickle}). P_{sickle} has a central role mediating Ca²⁺ entry, activating the Gardos channel and causing other sequelae such as Ca²⁺-induced phosphatidylserine exposure.

Deoxygenated RBCs from SCD patients also undergo haemolysis in isosmotic solutions of certain non-electrolytes [3]. Lysis shares a number of features in common with P_{sickle} activation, suggesting its involvement. We have used this phenomenon to design a novel diagnostic and prognostic test for SCD which makes use altered RBC permeability rather than presence of HbS per se [4]. Here we further characterise the lysis pathway. RBCs from SCD (HbSS) patients were washed in MOPS-buffered saline (in mM: NaCl 145, glucose 5, MOPS 10, pH 7.4, 290 $\pm$ 5mOsm.kg⁻¹, 37°C). The lysis solution was isosmotic sucrose solution (with sucrose replacing NaCl). At pH 7.4, RBCs lyse on deoxygenation. Pre-incubation of RBCs with cytochalasin B (10 μ M; Figure 1), latrunculin A (5 μ M) and mycalolide B (5 μ M) inhibited lysis. IC₅₀ for cytochalasin B was about 1 μ M. By contrast, incubating RBCs with phalloidin (10 μ M) increased haemolysis. With all inhibitors, O₂ saturation fell to values not significantly different from 0% within 20min (4 $\pm$ 3%, mean $\pm$ SEM, $n=3$, for cytochalasin B).

Reagents targeting actin therefore modulate the lysis pathway activated on deoxygenation of sickle RBCs in isosmotic sucrose. Effects were not due to alteration in O₂ saturation of Hb. We hypothesise that inhibiting cytoskeletal actin polymerisation with cytochalasin B, latrunculin A and mycalolide B prevents HbS aggregates from opening the P_{sickle} -like lytic pathway, whilst phalloidin stabilises actin polymers which may increase RBC permeability leading to greater haemolysis. Results are important for understanding the nature of the abnormal permeability of sickle cells, which is a pre-requisite to the rational design of inhibitory compounds to ameliorate the clinical features of SCD.

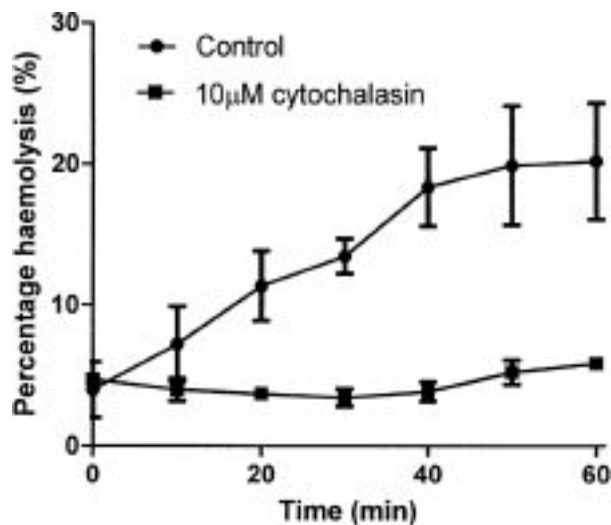


Figure 1. Effect of cytochalasin B (10 μM) on haemolysis of red blood cells from sickle cell disease patients incubated in deoxygenated isosmotic sucrose solution.

Steinberg, M. H. (1999). *New Eng. J. Med.* 340, 1021-1030.
 Lew, V. L. & Bookchin, R. B. (2005). *Physiol. Rev.* 85, 179-200.
 Browning, J. A., Robinson, H. C., Ellory, J. C. & Gibson, J. S. (2007). *Cell. Physiol. Biochem.* 19, 165-174.
 Milligan, C., Ellory, J. C., Rees, D. C., Osei, A. & Gibson, J. S. (2011). *Proc. Physiol. Soc.* 24, PC27.

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PC95

Acute inhibition of cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channels by thyroid hormones

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Cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channels are expressed in thyroid follicular cells where they play a role in transepithelial ion transport (1, 2). These findings together with the rapid, nongenomic effects of thyroid hormone on different ion channels and transporters (3) suggest that thyroid hormone might have direct effects on CFTR. To test this idea, we examined the acute effects of 3,3',5-triiodo-L-thyronine (T₃) and 3,5,3',5'-tetraiodothyronine (T₄) on CFTR Cl⁻ channels in excised inside-out membrane patches from C127 cells stably expressing wild-type human CFTR. The external solution contained 10 or 147 mM Cl⁻ and the internal solution 147 mM Cl⁻, 0.3 mM ATP and 75 nM PKA at 37 °C; voltage was -50 mV. When added to the internal solution facing the inside of CFTR, physiological concentrations or low micromolar concentrations of T₃ were without effect on phosphorylated or prephosphorylated CFTR (n = 10). However, higher concentrations of T₃ and T₄ (≥ 10 μM) inhibited phosphorylated CFTR. Single-channel studies demonstrated that T₃ (50 μM) inhibited CFTR Cl⁻ channels by a small decrease of single-channel amplitude (i) (control, i = -0.81 ± 0.03 pA; T₃, i = -0.73

± 0.03 pA; mean ± SEM; n = 6, p < 0.01; Student's paired t-test) and a great reduction of open probability (P_o) (control, P_o = 0.37 ± 0.07; T₃, P_o = 0.09 ± 0.01, n = 6, p < 0.01). Similar inhibitory effects on i and P_o were also observed with T₄ (50 μM) (n = 4). T₃ (50 μM) reduction of P_o was largely due to the prolongation of interburst interval (IBI) separating bursts of channel openings (control, IBI = 158 ± 14 ms; T₃, IBI = 667 ± 216 ms, n = 3, p < 0.01). In addition, channel block by T₃ was voltage independent (n = 5) and was relieved strongly when high concentrations of ATP (5 mM) were present in the internal solution (n = 4). By contrast, when added to the external solution facing the outside of CFTR, T₃ (50 μM) was without effect on phosphorylated CFTR (n = 6). We interpret our data to suggest that (i) physiological concentrations of T₃ were without direct acute effect on CFTR; (ii) micromolar non-physiological concentrations of T₃ inhibit CFTR primarily by an allosteric blocking mechanism (4) and (iii) the inhibitory binding sites of T₃ are located on the cytosolic side of CFTR and might share similarity with the ATP binding sites in the nucleotide-binding domain of CFTR.

O. Devuyst, et al. (1997). *Am. J. Physiol.* 272, C1299-C1308.

H. Li, et al. (2010). *Exp Physiol.* 952, 1132-1144.

Y. Shimoni. (1999). *Prog. Biophys. Mol. Biol.* 72, 67-108.

H. Li and D.N. Sheppard. (2009). *BioDrugs.* 23, 203-216.

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The anion transporter Slc26a6 (Putative Anion Transporter-1) regulates CO₂/HCO₃⁻ induced murine small intestinal fluid absorption

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Background: Slc26a6 (PAT-1), a multi anion transporter, is strongly expressed in the apical membrane of the upper small intestine. Data on its function are equivocal. Aim: To investigate if, under which conditions, and by what mechanisms, Slc26a6 contributes to intestinal fluid absorption *in vivo*. Methods: Small intestinal fluid absorption was assessed by single pass perfusion of 3 cm long, vascularly perfused jejunal segments in 2%-isoflurane anesthetized acid/base status controlled mice with prewarmed, isotonic solutions of different ionic compositions but constant pH of 7.4. The volume and pH of the inflowing and outflowing solution was measured. Results: WT jejunum absorbed fluid and acidified the lumen. Slc26a3 (DRA)-deficient jejunum absorbed less fluid than WT, and acidified the lumen more strongly, consistent with its action as a Cl⁻/HCO₃⁻ ex-changer. Slc26a6 (PAT-1)-deficient jejunum also absorbed less fluid, but resulted in less luminal acidification. Switching the luminal solution to a 5% CO₂/HCO₃⁻ buffer strongly augmented fluid absorption in a PAT-1- and NHE3- but not DRA-dependent manner. Removal of luminal

Na⁺ abolished fluid absorption in luminal NaCl but not in CO₂/HCO₃⁻, where the absorption became completely dependent on PAT-1 expression. In the absence of luminal Cl⁻, luminal CO₂/HCO₃⁻ also augmented fluid absorption, but in this case, the increase in absorption was dependent on both PAT-1 and NHE3 expression, and was markedly increased in DRA-deficient jejunum. Conclusions: The results suggest that Slc26a6 (PAT-11) strongly augments CO₂/HCO₃⁻-induced jejunal fluid absorption, likely operating in a 2 HCO₃⁻/1 Cl⁻ as well as 2 HCO₃⁻/1 HCO₃⁻ mode.

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PC97

Direct and indirect effects of Ca²⁺ on phosphatidylserine exposure in red blood cells from patients with sickle cell disease

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Phosphatidylserine (PS) is usually confined to the inner leaflet of red blood cells (RBCs) through inactivity of a Ca²⁺-dependent scramblase and high activity of the aminophospholipid translocase (APLT). Exposure occurs in a small, but variable, percentage of RBCs from sickle cell disease (SCD) patients (1). It may contribute to anaemia and ischaemia. PS exposure in RBCs from SCD patients is increased on deoxygenation in the presence of physiological levels of Ca²⁺_o and is prevented by Ca²⁺_i buffering (2). Ca²⁺ entry is via the deoxygenation-induced cation conductance pathway. Only low micromolar Ca²⁺_i levels are required (2 cf. 3). The mechanism(s) by which Ca²⁺ has its effect, however, remain(s) unclear. It may be direct via inhibition of APLT and scramblase activation. Alternatively Ca²⁺ may act indirectly via second messengers, which may involve Gardos channel activation and RBC shrinkage (4). These possibilities have been tested here.

Routine discarded blood samples were obtained from HbSS SCD patients, using EDTA as anticoagulant. RBCs were washed into high K⁺ (HK) Hepes-buffered saline (HBS) comprising (in mM): 90 KCl, 46 NaCl, 10 Hepes, 10 inosine, 0.15 MgCl₂ and in final step containing 2mM EGTA to remove contaminant Ca²⁺. To alter [Ca²⁺]_i, RBCs (0.5% Hct) were exposed to the ionophore Br-A23187 (2.5 or 6μM) and different [Ca²⁺]_o clamped with 2mM EGTA in low K⁺ (LK) (4mM KCl and 132mM NaCl) or HK HBS with 1mM vanadate (30min, 37°C). All solutions were pH 7.4 at 37°C, osmolality 290mOsm. Controls and inhibitors were tested in parallel. PS exposure was assessed using FITC-lactadherin (16nM, 10⁵ RBCs, Haematologic Technologies Inc.) and flow cytometry (2).

Incubation in HK saline significantly reduced PS exposure compared with LK HBS (p<0.0001, Figure 1a), using 2.5μM ionophore. When 6μM Br-A23187 was used, the difference between HK and LK was less apparent. Various inhibitors were tested: Z-VAD-FMK (to 60μM), a non-specific caspase inhibitor; quinacrine (to 100μM) a phospholipase A2 inhibitor; ABT491 (to 50μM) a PAF receptor antagonist; diclofenac (to 500μM) and acetylsalicylic acid (to 200μM), cyclooxygenase inhibitors to prevent PG synthesis; and GW4869 (to 10μM) and 3,4-

dichlorocoumarin (to 200μM), sphingomyelinase inhibitors to prevent ceramide production. None of these inhibitors resulted in a significant change in PS exposure (eg diclofenac, Figure 1b).

Findings indicate that Ca²⁺-dependent PS exposure is unlikely to involve PG, PLA2, ceramide or caspases. However, PS exposure was reduced in the absence of an outward K⁺ gradient. Possible mechanisms include prevention of K⁺ loss and cell shrinkage, loss of hyperpolarisation or sensitivity to K⁺_o *per se*. These possibilities are currently being tested.

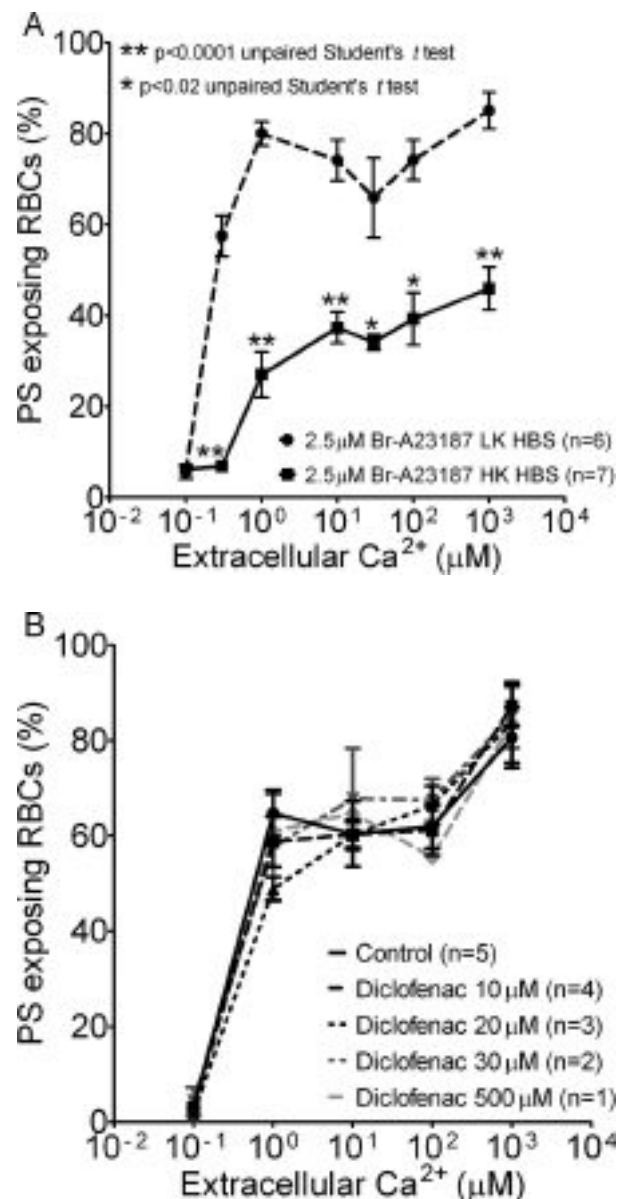


Figure 1. Effect of (a) potassium and (b) diclofenac, a cyclooxygenase inhibitor, on Ca²⁺ affinity of scrambling in RBCs from SCD patients, ** p<0.0001, * p<0.02, (unpaired Student's t test). Means±SEM (n<7).

Kuyper FA (2008). *Current Molecular Medicine* **8**, 633-638.

Weiss E, Rees DC & Gibson JS (2011). *Anemia* doi:10.1155/2011/379894.

Kamp D, Sieberg T & Haest CWM (2001). *Biochemistry* **40**, 9438-9446.

Lang F, Lang KS, Lang PA, Huber SM & Wieder T (2006). *Antioxidants and Redox Signalling* **8**, 1183-1192.

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PC98

EGF stimulates basolateral potassium channels in the Calu-3 cell line

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Epidermal growth factor (EGF) plays an important role in the regulation of cell growth, proliferation, and differentiation by binding to and activating the EGF receptor (EGFR). The EGFR is expressed by bronchial epithelial cells and regulates not only tissue repair, but also mucin production (1). EGFR signalling and potassium channel activity facilitate cell proliferation and migration. The inhibition of potassium channels by clofilium and glibenclamide reduced wound repair in NuLi (normal lung) and CuFi (CF) cells (2) and the EGFR is upregulated in the airways of asthma and COPD patients (3). While the effect of activation of EGF/EGFR in the gut is well documented (4), comparatively little is known about the role of the EGFR in ion transport in airway cells. Therefore, the aim of this study was to investigate the EGF signalling pathway and the regulation of potassium channels in the Calu-3 submucosal cell line.

Calu-3 cells were grown to confluency on permeable supports at an air-liquid interface (ALI). Supports were incorporated into an Ussing chamber 7-15 days after establishment of the ALI and short circuit current (I_{SC}) across the monolayer was measured. Data are presented as mean $\pm$ SEM (n = number of experiments) and were statistically analyzed using a one-way ANOVA with Tukey's post hoc test.

Pretreatment of confluent Calu-3 monolayers with 10ng/ml EGF to the basolateral side for 1 hour resulted in an increase in the starting I_{SC} ($24.8 \pm 3.0 \mu A cm^{-2}$, $n=10$, $P<0.001$) compared to $12.1 \pm 0.7 \mu A cm^{-2}$ ($n=98$) seen in untreated controls. Basolateral membrane permeabilisation abolished this effect. Pretreatment with 5 μM AG1478 (an EGFR inhibitor), resulted in a starting I_{SC} of $5.2 \pm 1.3 \mu A cm^{-2}$ ($n=5$, $P<0.001$) significantly lower than EGF alone ($24.8 \pm 3.0 \mu A cm^{-2}$, $n=10$). Preincubation with 50 μM wortmannin, a phosphoinositol-3-kinase (PI3K) inhibitor, resulted in a starting I_{SC} of $5.7 \pm 1.5 \mu A cm^{-2}$ ($n=4$, $P<0.001$), while staurosporine (0.1 μM), chelerythrine chloride (10 μM) and rottlerin (5 μM) resulted in starting I_{SC} of $5.3 \pm 0.8 \mu A cm^{-2}$ ($n=4$, $P<0.001$), $4.3 \pm 0.9 \mu A cm^{-2}$ ($n=4$, $P<0.001$), and $7.8 \pm 0.8 \mu A cm^{-2}$ ($n=5$, $P<0.001$), respectively, in all cases significantly lower than EGF alone ($24.8 \pm 3.0 \mu A cm^{-2}$, $n=10$). Pretreatment with charybdotoxin (1 μM), iberiotoxin (10 nM) and chromanol 293B (10 μM) resulted in a starting I_{SC} of $11.3 \pm 1.2 \mu A cm^{-2}$ ($n=5$, $P<0.01$), $4.3 \pm 0.6 \mu A cm^{-2}$ ($n=4$, $P<0.001$) and $10.1 \pm 3.2 \mu A cm^{-2}$ ($n=5$, $P<0.01$), respectively, significantly lower than with EGF alone ($24.8 \pm 3.0 \mu A cm^{-2}$, $n=8$).

In conclusion, these results show that pretreatment with EGF to the basolateral membrane increases chloride driving force through an increased ion transport via the $K_{Ca}3.1$ and $K_v7.1$ potassium channels through the EGFR and a PI3K/PKC δ dependent signalling mechanism.

Shute J *et al.* (2003). *Paediatr Respir Rev* **4**, 120-127.

Trinh NT *et al.* (2008). *Am J Physiol Lung Cell Mol Physiol* **295**, L866-880.

Lai H & Rogers DF (2010). *J Aerosol Med Pulm Drug Deliv* **23**, 219-231.

McCole DF & Barrett KE (2009). *Acta Physiol (Oxf)* **195**, 149-159.

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PC99

PDGFA mediates vasopressin-induced regulatory effects of myofibroblasts on the junctional permeability of cultured colonic epithelial cells

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Vasopressin (AVP) has trophic effects in the rat distal colon, increasing pericryptal myofibroblast growth and reducing the colonic crypt wall permeability. We have examined whether these effects of AVP can be reproduced in vitro, using the myofibroblast CCD-18Co cell line to study proliferation and the colonic epithelial cell line T84 to study the expression of tight junction proteins. The possible role of the platelet derived growth factor A (PDGFA) as mediator of myofibroblasts effects on the expression of junctional proteins was also studied. Cell proliferation was quantified from 5-Bromo-2'-deoxyuridine incorporation, the expression of tight-junction proteins β -catenin and claudin IV by Western blot and PDGFA expression by real time PCR. Results are expressed as means $\pm$ S.E.M. ($n=6$), compared by ANOVA. AVP (10 nM, 24 h) stimulated CCD-18Co proliferation by 60% ($p<0.05$) and increased PDGFA expression by 20%, and both effects were prevented when Manning peptide, a V1 receptor antagonist, and Tolvaptan, a V2 receptor antagonist, were present in the incubation medium. Similar effects were observed when exogenous PDGFA was added. In addition, when myofibroblasts were treated with vasopressin and pre-incubated with anti-PDGFA or anti-PDGFA receptor antibodies, the AVP effects were prevented. AVP had no direct effects on the expression of junctional proteins by the T84 cells; however, both β -catenin and claudin IV were increased when the cells were incubated for 24 h with conditioned medium (CM) from myofibroblasts stimulated by AVP. The CM increased T84 proliferation by 46% ($p<0.05$) and these effects were prevented by anti-PDGFA antibodies ($p<0.05$). The CM from CCD-18Co cells treated with AVP increased the expression of β -catenin ($p<0.05$) and claudin IV by 25% ($p<0.05$). These results indicate that changes in colonic permeability during dehydration are mediated by PDGFA secreted by myofibroblasts in response to raised AVP.

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PC100

17 β -estradiol rapidly increases amiloride-sensitive Na⁺ current and surface expression of the γ -ENaC subunit in renal collecting duct M1 cells

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17 β -estradiol (E2), regulates Cl⁻ secretion in distal colon in a gender-specific manner via inhibition of the basolateral K⁺ channel KCNQ1 (1). The fluid retention observed in females during periods of high circulating plasma E2 could be explained, in part, by the anti-secretory effect of E2 observed in the distal colon of female rats (1). The physiological role of fluid conservation in high E2 states may be to facilitate uterine expansion and restructuring in advance of embryo implantation and also to positively impact upon cardiovascular dynamics.

The effect of E2 on ion transport in other organs contributing to electrolyte and fluid homeostasis in females has not been characterised. For example, E2 modulation of Na⁺ re-absorption in the renal cortical collecting duct could contribute to fluid conservation observed during periods of high circulating plasma E2. The aim of this study was to determine whether E2 had an effect on Na⁺ re-absorption in the renal cortical collecting duct using the well-characterised M1 mouse renal cortical collecting duct line (M1 cells) and to reveal the molecular mechanisms underlying effects of E2 on ENaC.

Treatment with E2 (25 nM) for 15 min increased the amplitude of the amiloride-sensitive short-circuit current (I_{SC}) in M1 cells grown as polarized monolayers in Ussing chambers (Control 1.5 ± 0.4 μ A/cm², E2 2.8 ± 0.4 μ A/cm²; n=6). Experiments in amphotericin-B perforated epithelia, using ouabain, demonstrated that this response was not attributable to an increase in the Na/K -ATPase activity (Control 2.9 ± 0.2 μ A/cm², E2 3.3 ± 0.8 μ A/cm²; n=4). Moreover, there was no change either in total or basolateral surface abundance of the α -Na/K -ATPase subunit.

An explanation for the increase in the amplitude of the amiloride-sensitive I_{SC} observed above could come from changes in the total or apical surface expression of the ENaC subunits. Then, we looked at changes in the abundance of α -, β -, and γ -ENaC subunits using biotinylation, Western-blot and immunofluorescence. After 30 min treatment with E2 25 nM, there was not detectable change in either the total or apical surface expression of the α - and β -ENaC subunits. However, there was a significant increase in the apical membrane abundance and localization of the γ -ENaC subunit ($20 \pm 2\%$ compared with the control, n=4).

In conclusion, this work demonstrates that E2 can rapidly increase the amplitude of the amiloride sensitive I_{SC} in M1 cells. The results presented above rule out the involvement of the Na/K -ATPase in the process. However, E2 can induce an increase in the apical surface abundance of the γ -ENaC subunit, which could provide an explanation for the pro-absorptive effect observed for E2 in M1 cells.

F. O'Mahony, R. Alzamora, V. Betts, F. LaPaix, D. Carter, M. Irnaten, and B.J. Harvey. 2007. Female gender-specific inhibition of KCNQ1 channels and chloride secretion by 17 β -estradiol in rat distal colonic crypts. *J. Biol Chem*; 282 (34):24563-73

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PC101

Possible role of fibroblast growth factor-23 in the regulation of intestinal calcium absorption in male mice

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Normal plasma calcium (Ca²⁺) and phosphate (P_i) concentrations are controlled by the classic hormones, parathyroid hormone and 1,25(OH)₂D₃. Recently, fibroblast growth factor-23 (FGF-23) has been identified as a hypophosphatemic hormone which is synthesized by osteoblasts and osteocytes in bone tissues (1). Its main function is to increase urinary P_i excretion (2). However, whether FGF-23 regulates Ca²⁺ metabolism is not known. Since FGF receptors and co-receptor (α klotho) are also present in intestinal epithelial cells (3, 4), FGF-23 might effect intestinal function. Furthermore, because FGF-23 can antagonize 1,25(OH)₂D₃ action, it might compromise 1,25(OH)₂D₃-dependent Ca²⁺ absorption. Therefore, the objective of this study was to investigate the effects of FGF-23 on Ca²⁺ transport in the small intestine of male ICR (Imprinting Control Region) mice and the signaling pathways involved in vitro with Ussing chambers.

Mice were pretreated with vehicle or 1 μ g/kg 1,25(OH)₂D₃ (s.c.) for 3 days to elevate baseline Ca²⁺ absorption followed by i.v. FGF-23 injection. Mice were anesthetized (i.p.) with 70 mg/kg sodium pentobarbitone and euthanized (i.c.) by overdose with sodium pentobarbitone. Duodenal tissue was removed, mounted in Ussing chambers and directly exposed to FGF-23 and/or various inhibitors in the chambers. Net flux of Ca²⁺ and electrical parameters were measured throughout the 60-min experiment by ⁴⁵Ca kinetics and voltage clamp techniques, respectively. This study has been approved by the Institutional Animal Care and Use Committee of the Faculty of Science, Mahidol University.

Consistent with previous reports (5), pretreatment with 1,25(OH)₂D₃ significantly enhanced total active duodenal Ca²⁺ transport. Both injection of mice with FGF-23 and direct administration of FGF-23 into the Ussing chamber significantly decreased the 1,25(OH)₂D₃-enhanced duodenal Ca²⁺ absorption, indicating that FGF-23 might act directly on the intestinal epithelium. Inhibitors of FGF receptors (PD-173074), and the kinases; MAPK/ERK, p38 MAPK, and PKC, but not inhibitors of JNK, Src, JAK2, PI3K, or Akt significantly abolished the effect of FGF-23 on 1,25(OH)₂D₃-induced intestinal Ca²⁺ absorption. This study shows for the first time that, besides being a hypophosphatemic hormone, FGF-23 might also act as a hypocalcemic hormone through its inhibitory effect on intestinal Ca²⁺ absorption, via the intracellular signaling pathway involving MAPK/ERK, p38 MAPK and PKC.

Miramis M, Robinson BG, Mason RS, Nelson AE. Bone as a source of FGF23: regulation by phosphate? *Bone* 2004;35(5):1192-1199.

Shimada T, Urakawa I, Yamazaki Y, Hasegawa H, Hino R, Yoneya T, et al. FGF-23 transgenic mice demonstrate hypophosphatemic rickets with reduced expression of sodium phosphate cotransporter type IIa. *Biochem Biophys Res Commun* 2004;23(7):421-432.

Hagiwara A, Nakayama F, Motomura K, Asada M, Suzuki M, Imamura T, Akashi M. Comparison of expression profiles of several fibroblast growth factor receptors in the mouse jejunum: suggestive evidence for a differential radioprotective effect among major FGF family members and the potency of FGF1. *Radiat Res* 2009;172:58-65.

Wang Y, Sun Z. Current understanding of klotho. Ageing Res Rev 2009;8:43–51.

Christakos S. Mechanism of action of 1,25-dihydroxyvitamin D3 on intestinal calcium absorption. Rev Endocr Metab Disord 2011 (in press).

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PC102

The G Protein-coupled bile acid receptor, TGR5, is expressed on colonic epithelial cells and regulates ion transport

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Intestinal fluid movement is driven by osmotic gradients that are, in turn, established by active ion transport across the epithelium. Previously identified as a neuronal bile acid receptor in the gastrointestinal tract, TGR5 has been implicated in the regulation of intestinal motility. It has also been identified as a regulator of chloride (Cl⁻) secretion across gallbladder epithelium. However, there is still a paucity of information regarding the expression and role of TGR5 on colonic epithelial cells.

Aim: To investigate a potential role for TGR5 in regulation of colonic epithelial ion transport.

Methods: The semi-synthetic bile acid, 6 α -ethyl-23(S)-methylcholic acid (INT-777), was used to activate TGR5, and the muscarinic agonist, carbachol (CCh), was used to promote colonic epithelial Cl⁻ secretory responses. Ion transport was measured as changes in short circuit current (I_{sc}) across muscle-stripped segments of rat colon mounted in Ussing chambers. mRNA expression was measured by RT-PCR. Confocal microscopy was used to assess TGR5 expression and localisation in isolated rat colonic crypts.

Results: TGR5 was found to be highly expressed in colonic epithelial cells both at the mRNA and protein levels. Confocal imaging revealed the protein to be localised bilaterally in isolated rat colonic crypts. Acute bilateral addition of INT-777 (100 μ M) to voltage-clamped rat colonic mucosa caused a rapid and transient decrease in I_{sc} to 86.9 ± 5.2 % ($n = 8$, $p \leq 0.05$) of initial values, which returned to basal values by 15 minutes. An analysis of the sidedness of the effects of INT-777 revealed it to be effective from the basolateral, but not apical, side. Furthermore, treatment of rat colonic epithelial cells with INT-777 (100 μ M) significantly reduced subsequent secretory responses to CCh to 64.15 ± 1.73 % of those in control cells ($n = 3$, $p \leq 0.01$). The effects of TGR5 were not altered by pretreatment of the tissue with the neurotoxin, tetrodotoxin.

Conclusions: These studies reveal a novel role for TGR5 in regulating colonic epithelial ion transport. TGR5 activation rapidly reduces basal I_{sc} and inhibits agonist-induced Cl⁻ secretory responses. In contrast to its previously reported actions on gut motility, the effects of TGR5 on colonic epithelial secretory responses appear to be independent of neuronal activation. Our data have important implications for our understanding of how bile acids regulate colonic fluid and electrolyte transport in health and disease and suggest that TGR5 may provide a new target for development of drugs to treat intestinal dis-

orders associated with dysregulated fluid and electrolyte transport.

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PC103

Differential effects of hydroxyurea treatment on cation permeability of red blood cells from patients with sickle cell disease

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Red blood cells (RBCs) from patients with sickle cell disease (SCD) contain abnormal haemoglobin HbS instead of normal adult HbA. Deoxygenated HbS polymerises, distorts RBCs into bizarre shapes and alters rheology. RBCs from SCD patients show high cation permeability causing solute loss, shrinkage and increasing [HbS] thus greatly encouraging polymerisation. Several pathways participate in increased permeability [1]: KCl cotransport (KCC), the Gardos or Ca²⁺-activated K⁺ channel and a deoxygenation-induced non-selective cation pathway (P_{sickle}). The pathology of SCD is extensive but treatment remains largely supportive. Hydroxyurea has proven clinical efficacy, probably by encouraging expression of fetal Hb, HbF. However, not all patients respond equally to treatment [2]. Routine discarded blood samples were obtained from SCD patients using EDTA as an anticoagulant. RBCs were washed in saline comprising (in mM) NaCl 145, glucose 5, MOPS 10, pH 7.4, 290 ± 5 mOsm.kg⁻¹, 37°C. O₂ tension was controlled using Eschweiler tonometry and a Wösthoff gas mixer. Activities of the main transport pathways were measured using ⁸⁶Rb⁺ as a K⁺ congener (KCC as the Cl⁻-dependent flux replacing Cl⁻ with NO₃⁻, Gardos channel as the clotrimazole (CLT; 5 μ M)-sensitive flux, P_{sickle} as Cl⁻-independent, CLT-insensitive flux) [3]. Ouabain and bumetanide were present to inhibit the Na⁺/K⁺ pump and Na⁺-K⁺-2Cl⁻ cotransporter. Cell morphology was assessed by light microscopy after RBCs were fixed in 0.3% glutaraldehyde in saline.

On deoxygenation, 76 ± 2 % ($n=30$) of RBC from untreated SCD patients showed sickling. The majority of RBCs from treated patients could be put into two groups - showing either less than 65% ($n=11$) or more than 75% ($n=17$) sickling. After dividing patients into two groups on the basis of the extent of deoxygenation-induced sickling ('<70%': 57 ± 3 %, $n=12$; '>70%': 79 ± 1 %, $n=22$), we compared their HbF levels (14.4 ± 1.8 % vs. 8.3 ± 0.9 % for '<70%' vs. '>70%'; $p=0.001$) and transport activities. KCC, P_{sickle} and Gardos activities on deoxygenation were significantly reduced when the '<70%' group was compared with untreated patients ($p=0.009$, $p=0.019$, $p=0.018$, respectively), as was KCC activity at 100mmHg ($p=0.013$). RBC from untreated patients and the '>70%' sickling group showed no significant differences.

Whilst reduction in KCC activity following hydroxyurea therapy has been previously mentioned [4], albeit in a small number of patients ($n=3$), beneficial effects on deoxygenation-induced P_{sickle} and Gardos channel activity have not been hitherto described. These actions on RBC permeability may

therefore elucidate further the mechanism by which hydroxyurea has its therapeutic effect. They also imply an important role for HbF levels in regulation of RBC membrane permeability.

[1] Lew, V.L. & Bookchin, R.B. (2005). *Physiol. Rev.* 85, 179-200.

[2] Ferster, A. et al. (1996). *Blood* 88, 1960-1964.

[3] Hannemann, A. et al. (2011). *Anemia* 248527, 1-8.

[4] Bridges et al. (1996). *Blood* 88, 4701-4710.

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PC104

Evidence that central respiratory-sympathetic coupling drives Traube-Hering Waves in man

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Arterial pressure exhibits rhythmic fluctuations with the respiratory cycle (Traube-Hering Waves; THW). These have been attributed to cyclical alterations in intrathoracic pressure and venous return affecting cardiac output. An alternative view posits that they are due to central coupling between the sympathetic and respiratory pattern generators, as sympathetic vasomotor activity is respiratory-modulated. This is pertinent, as increased respiratory-sympathetic coupling has been linked with hypertension (Simms et al 2009). We used microneurography to explore this hypothesis in man.

Methods: 6 healthy, normotensive males (age 21-30) had muscle sympathetic nerve activity (MSNA) recordings from the peroneal nerve alongside continuous arterial pressure, ECG and respiratory thoracic excursion. Recordings were made at rest, during fixed frequency (ff-) breathing and with 5cmH₂O positive end-expiratory pressure (PEEP). Respiratory-triggered averages of MSNA and mean arterial pressure (MAP) defined the magnitude and phase relationships of the THW and MSNA bursts. This was used to collect a breath-by-breath time series of thoracic excursion, Δ MAP and integrated MSNA. Data are expressed as means $\pm$ SEM, significance tested using ANOVA or t-tests ($p < 0.05$) and relationships explored with linear regression and cross correlation.

Results: All 6 subjects showed THW (1.9 ± 0.3 mmHg), with a latency of 2.7 ± 0.2 s after end inspiration (resting conditions, $p < 0.005$). THW amplitude was not changed by ff-breathing but was increased to 4.7 ± 0.2 mmHg ($p = 0.04$, $n = 6$, ANOVA) by addition of PEEP. Significant respiratory-related bursts of MSNA were observed in all subjects. The sympathetic burst started 1.2 ± 0.3 s prior to end inspiration and end 0.9 ± 0.4 s after. The addition of PEEP tended to increase MSNA bursts amplitude and shift its phase. The magnitude of the respiratory-related MSNA correlated positively ($p < 0.01$) with the amplitude of the following THW in 1 subject at baseline, in 3 subjects during paced breathing and this was strengthened by the addition of PEEP. In contrast, amplitude of the preceding THW did not predict the magnitude of the MSNA burst. There were also positive correlations ($p < 0.05$; $n = 4$) between the amplitude of thoracic excursion and that of the following THW, most prominent at baseline. Thoracic excursion did not correlate with MSNA amplitude.

Conclusion: Amplitude domain analysis of MSNA in man reveals respiratory-sympathetic coupling. Time domain analysis

shows predictive correlations between sympathetic burst strength and the following THW amplitude (not vice versa) suggesting a causal relationship that can be strengthened by application of PEEP, a manoeuvre that may increase central respiratory drive. This lends weight to the hypothesis that THW are the product of central respiratory-sympathetic coupling. Simms AE, Paton JF, Pickering AE, Allen AM (2009) Amplified respiratory-sympathetic coupling in the spontaneously hypertensive rat: does it contribute to hypertension? *J Physiol* 587:597-610

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PC105

Impact of mild traumatic brain injury on the cerebrovascular function

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The majority of research into the assessment of mild traumatic brain injury (mTBI) has been focussed on the neuropsychological aspect of the injury, with neuropsychological tests forming the cornerstone of concussion management (Ross et al., 1987). The impact that mTBI has on the cerebrovascular system still remains relatively unclear (Wilberger et al., 2006, McCrory et al., 2009). With advancing techniques such as transcranial Doppler (TCD) assessing the impact on the cerebrovascular system has become a key aspect of recent research (Len and Neary, 2011). In the study we tested the hypothesis that cerebral function would show the greatest impairment i.e. reduced vasomotor reactivity in individuals who had the highest exposure to repetitive head trauma.

Twelve currently active professional boxers aged 27 (mean) $\pm$ 4 (SD) years and 10 physically trained concussed individuals (28 ± 8 years), with a history of knockouts 2 ± 3 & 4 ± 5 (respectively) were compared to 13 physically trained non-concussed individuals (22 ± 3 years) and 11 sedentary individuals (25 ± 5 years). Haemodynamic function was assessed following 3-minute of hypocapnea, with continuous recording of the middle cerebral artery velocity (MCAv), mean arterial pressure (MAP) and end-tidal partial pressure of carbon dioxide (PETCO₂). Cerebrovascular resistance (CVR) was calculated as $MAP/MCAv$ and cerebrovascular conductance (CVC) as $MCAv/MAP$. Vasomotor reactivity (VMR) was calculated as the percentage change in MCAv from hypocapnea to baseline/ Δ change in PETCO₂. Data were analysed using a two-way repeated measures ANOVA and Bonferroni corrected independent and paired samples t-test. Significance was set at $P < 0.05$.

As shown in table 1, there was no visible impairment of the cerebrovascular function following the hypocapnea challenge across all groups.

These findings demonstrate that cerebrovascular function remains preserved following a mTBI. By incorporating techniques such as TCD to assess cerebral function with neuropsychological tests will help improve further the understanding of the injury.

Table 1: Cerebral haemodynamic function

	ICA _{av} (cm/s)	MAP (mmHg)	CVR (mmHg/cm/s)	CVC (cm/s/mmHg)	PETCO ₂ (mmHg)	VMR (%/mmHg)
Boxers	Baseline	51 ± 13	89 ± 18	1.88 ± 0.66	0.59 ± 0.39	48 ± 3
	Hypocapnia	39 ± 12	86 ± 23	2.38 ± 0.89	0.47 ± 0.15	28 ± 4
	Δ (%)	-12 ± 6	-3 ± 13	0.50 ± 0.38	-0.12 ± 0.09	-12 ± 4
Trained Concussed	Baseline	54 ± 13	91 ± 13	1.76 ± 0.48	0.61 ± 0.18	36 ± 3 *
	Hypocapnia	36 ± 8	90 ± 12	2.61 ± 0.67	0.41 ± 0.11	22 ± 4 *
	Δ (%)	-19 ± 8	-1 ± 7	0.85 ± 0.27	-0.20 ± 0.08	-12 ± 8
Trained Non-concussed	Baseline	49 ± 10	92 ± 13	2.00 ± 0.68	0.53 ± 0.13	38 ± 2
	Hypocapnia	33 ± 9	91 ± 15	2.88 ± 1.02	0.37 ± 0.11	31 ± 4 *
	Δ (%)	-16 ± 9	-4 ± 5	0.88 ± 0.58	-0.16 ± 0.08	-14 ± 5
Sedentary	Baseline	51 ± 9	87 ± 12	1.75 ± 0.39	0.60 ± 0.14	37 ± 45 *
	Hypocapnia	32 ± 9	82 ± 12	2.75 ± 0.97	0.41 ± 0.14	22 ± 5
	Δ (%)	-19 ± 8	-5 ± 8	1.00 ± 0.72	-0.19 ± 0.08	-13 ± 6

Values are mean ± SD; * P < 0.05 vs. boxers.

LEN, T. K. & NEARY, J. P. 2011. Cerebrovascular pathophysiology following mild traumatic brain injury. *Clin Physiol Funct Imaging*, 31, 85-93.

MCCRORY, P., MEEUWISSE, W., JOHNSTON, K., DVORAK, J., AUBRY, M., MOLLOY, M. & CANTU, R. 2009. Consensus statement on concussion in sport - The 3rd international conference on concussion in sport held in Zurich, November 2008. *PM R*, 1, 406-20.

ROSS, R. J., CASSON, I. R., SIEGEL, O. & COLE, M. 1987. Boxing injuries: neurologic, radiologic, and neuropsychologic evaluation. *Clin Sports Med*, 6, 41-51.

WILBERGER, J., ORTEGA, J. & SLOBOUNOV, S. 2006. Concussion Mechanisms and Pathophysiology

Foundations of Sport-Related Brain Injuries. In: SLOBOUNOV, S. & SEBASTIANELLI, W. (eds.). Springer US.

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PC106

Ethnic differences in autonomic tone and heat adaptation during intensive exercise in English Premiership Footballers training in extreme heat

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Athletic performance may be compromised in extreme heat; this was relevant for the Athens and Beijing Olympics and may be for the 2014 and 2022 FIFA World cups in Brazil and Qatar respectively; under those conditions prior determination of required fluid intake is vital for athlete safety. Thompson (1954) described lower sweating rates in Bantus compared with Caucasians. The three aims of the study are: first, to determine the ethnic mix of the English Football Premiership. Second, to establish the fluid intake, sweat production and sweat evaporation during exercise in extreme heat. Third, to determine whether there are ethnic differences in the above and to establish the cardiorespiratory and other physiological factors contributing to any putative ethnic differences.

Using Scout 7 software, we analysed all 270 English Football Premiership footballers in 2009; 44% players were of African origin (AO) and 56% of European origin (EO).

Heart rate variability (HRV) was calculated from a five minute collection of resting ECG using bespoke frequency domain analysis software using American Heart Association guidelines (Omega Wave; Oregon USA). The ratio of Low frequency power (LF; 0.04 to 0.15 Hz) to High frequency power (HF; 0.15 to 0.40 Hz) i.e. LF/HF ratio, an index of sympatho-vagal tone balance, was calculated for all players. Mean + SD LF/HF ratio was 1.07

+ 0.48 in AO, significantly lower than in EO (2.0 + 1.27; p = 0.03; unpaired t-test).

Studies were performed in United Arab Emirates after four days heat acclimation, during 80 minutes training at 40 ± 2°C ambient temperature; 25 ± 8% relative humidity on 21 professional Premiership footballers from a single team with Ethics clearance. 12 were AO and 9 EO. Players could drink ad libitum throughout.

AO and EO subjects exercised to a similar degree as evidenced by heart rates (Polar Electro). There was no difference between AO and EO in height, weight, body surface area, skin fold thickness, resting or average heart rate, electrocardiographically measured QT interval, echocardiographically measured cardiac chamber dimensions, left ventricular ejection fraction or maximum VO₂. During exercise, fluid intake in AO ranged from 1.2- 3.0L (1.8 + 0.63L, median + SD) and in EO ranged from 0.5-1.9L (1.2 + 0.51L; p=0.055; unpaired t-test). Sweat production in AO ranged from 1.8 – 3.9L (2.3 + 0.56L) compared with 1.3 – 2.7L in EO (2.09 + 0.48L; p = 0.09).

We conclude that fluid intake during exercise in extreme heat may be up to 2.25 L/hr. There may be an increased sweat production in AO players which would confer a performance advantage to exercising in the heat and that this appears to be associated with an increased sympathetic tone, which has also been related to an increased risk of cardiac events (Tsuji H et al, 1996).

Thompson, M.L. (1954) A comparison between the number and distribution of functioning eccrine sweat glands in Europeans and Africans. *J. Physiol.*, 123, 225-233

Tsuji H, Larson M G, Venditti F J, Manders E S, Evans J C, Feldman, C L and Levy D (1996) Impact of Reduced Heart Rate Variability on Risk for Cardiac Events. The Framingham Heart Study. *Circulation*, 94, 2850-2855

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PC107

Influence of pregnancy stage on heart rate variability

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There remains uncertainty regarding the magnitude and patterns of variability in some aspects of physiology in normal pregnancy. Previous work has indicated an influence of gestation on the autonomic nervous system (ANS) control of heart rate but a definitive study of this is beneficial (Moertl et al., 2009). Characterising and understanding such changes during normal pregnancy could benefit in early identification of abnormal physiological function and identify patients at risk for adverse responses to interventions such as vasodilator therapy (Walther et al., 2005). Longitudinal study designs involving cardiovascular reflex tests during antenatal and post-partum periods are likely to yield information on the dynamic adaptations to pregnancy. We sought to obtain a longitudinal characterisation of the influence of normal pregnancy on ANS function using heart rate variability (HRV) analysis. Continuous ECG monitoring (Reynolds Holter; Spacelabs Medical UK) assessed autonomic function in a group of 51 healthy non-pregnant (NP) females (20-40 yrs). Longitudinal assessment was then performed on healthy pregnant women (20-40 yrs) at 14.8±0.4 weeks (T1, n=32), 25.9±0.2 weeks (T2, n=20),

35.9±0.7 weeks (T3, n=14) and at 12.3±0.7 weeks post partum (PP, n=7). Participants performed a protocol that consisted of posture (supine 6 min, standing 6 min), step exercise (6 min), seated recovery (6 min), mental arithmetic (3 min), metronomic breathing (3 min) and spontaneous breathing (3 min). R-R interval (RRI), RMSSDRR, SDRR, LF, HF and Total Power were investigated via repeated measures ANOVA which assessed the influence of trimester, protocol stage and their interaction (pregnancy status x stage). One-way ANOVA additionally compared NP and trimester at each specific stage of the protocol. Significant difference in trimester and stage interaction were found in LFn RR ($P=0.25$) and RRI ($P=0.03$). Comparing responses over the whole protocol, there was a significant influence of pregnancy-stage on HFn ($P=0.03$), HR ($P=0.04$) and RRI ($P=0.01$), attributable to T1 vs T3 differences. Significant changes were also found in RMSSDRR ($P=0.03$) and RRI ($P=0.03$), between T3 vs PP. A significant influence of trimester ($P<0.05$) was evident in the responses to 'postural change' (supine-to-standing) and respiratory frequency change (spontaneous-to-metronomic breathing) between the paired groups NP vs T3, T1 vs T3 and T3 vs PP. Our protocol was designed to provoke autonomic nervous system responses and was assessed via changes in HRV. Non-pregnant responses were similar to those during T1 and response trends were independent of pregnancy status. However, we observed a significant influence of advanced gestation on the postural and respiratory-induced responses compared with NP and T1 pregnancy groups. We will extend this pilot work to use this protocol to developing a screening tool for antenatal disorders.

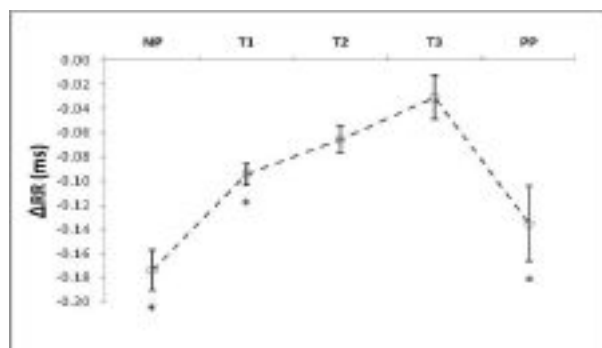


Figure 1 Influence of pregnancy on the change in R-R during the supine-to-standing postural manoeuvre. * Denotes significant differences compared with T1. Similar changes were found in RMSSDRR, SDRR, Total Power, LF and HF. Unlabeled values. Dotted values are shown with $\pm$ SE.

Moertl M.G., Ulrich D., Karoline I. Pickel K.I., Klaritsch P., Schaffer M., Flotzinger D., Alkan I., Lang U., and Schlembach D (2009). Changes in haemodynamic and autonomic nervous system parameters measured non-invasively throughout normal pregnancy. *European Journal of Obstetrics & Gynecology and Reproductive Biology* 144 179–183

Walther T, Wessel N, Baumert M, (2005). Longitudinal analysis of heart rate variability in chronic hypertensive pregnancy. *Hypertens Res*; 28: 113–8.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC108

Heart rate variability following high intensity interval training and endurance training in obese individuals

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Low heart rate variability (HRV), indicative of reduced cardiac parasympathetic efferent activity, has been reported in obese individuals and is associated with increased cardiovascular risk [1]. Endurance exercise training (ET) has been shown to improve HRV in a range of populations. High intensity interval training (HIT, 9 minutes per week) can elicit remarkable metabolic adaptations and improvements in maximal aerobic capacity (VO_{2max}), equivalent to traditional moderate intensity ET (~5 hours per week) [2]. However, it is presently unclear whether HIT training and ET elicit comparable changes in HRV in obese individuals.

To begin to examine this, 11 sedentary, obese, male subjects were assessed before and after either 6 weeks of ET (n=5, 27±2 years) or 4 weeks of HIT (n=6, 26±3 years). ET consisted of cycling at 65% of VO_{2peak} for 40-60 minutes, 5 times a week. HIT consisted of 30 second intervals (4-7 repetitions) at constant load of 200% maximal aerobic power (W_{max}) with 2 minutes recovery between intervals, 3 times a week. At baseline and post-training, subjects underwent a maximal incremental exercise test on an electrically braked cycle ergometer to determine VO_{2max} , W_{max} , maximal heart rate (HR_{max}), and heart rate recovery (HR_{rec}) at 1 and 3 min post-exercise. HRV was determined on a separate day from 5 min electrocardiogram recordings obtained with subjects resting quietly in the supine position. HRV parameters were determined using time and frequency domain methods (Kubios HRV software). Statistical analysis was performed using two-way repeated measures ANOVA and post hoc Holm Sidak tests.

At baseline, body mass index (BMI; 33±2 vs. 35±1 kg.m²) and % body fat (31±1 vs. 31±3 %) were similar in the ET and HIT groups ($P>0.05$). % body fat decreased with training similarly in both groups (by ~1%, $P<0.05$), while BMI was unchanged. VO_{2max} and W_{max} were similar in ET and HIT groups at baseline ($P>0.05$) and were increased similarly post-training (VO_{2max} ; ET, 36±2 to 40±2; HIT, 37±3 to 39±2 ml.kg.min⁻¹; baseline vs. post training $P<0.01$). HR_{max} was slightly, but significantly reduced with exercise training (ET, ~2 bpm; HIT, ~6 bpm, baseline vs. post-training $P<0.05$). HR_{rec} and HRV were not different between groups, and not significantly altered following either ET or HIT training ($P>0.05$).

Our preliminary findings indicate that 6 weeks of HIT or ET in obese individuals elicits similar increases in aerobic exercise capacity (VO_{2max}) and reductions in % body fat, whereas HRV remains unchanged. Further studies are required to fully elucidate how exercise training modality may differentially affect HR regulation in obese individuals.

Dekker JM et al. (2000). *Circulation* 102, 1239-44

Burgomaster KA et al. (2008). *J Physiol* 586, 151-60

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PC109

 Ω -3 polyunsaturated fatty acids (PUFA) attenuate Transforming Growth Factor- β 1 (TGF- β 1) induced myofibroblast differentiation via modulation of the sphingosine kinase pathway but not peroxisome proliferator-activated receptor- γ (PPAR γ)J.K. Whittingham-Dowd² and J.T. Allen²¹University of Manchester, Manchester, UK and ²Biosciences Research Institute, The University of Salford, Salford, UK

TGF- β 1 is an important pro-fibrogenic growth factor. In skeletal muscle it has been identified as an inhibitor of myogenesis [1]. It has previously been determined that TGF- β 1-induced transdifferentiation of C2C12 myoblasts involves Sphingosine kinase-1 (SK1) and altered Sphingosine-1-phosphate (S1P) receptor expression [2]. Ω -3 PUFA can attenuate TGF- β 1 activities against myogenesis and reportedly interact with other antimyogenic pathways, such as TNF α -induced NF κ B activation, via PPAR γ [3]. It was therefore hypothesized that Ω -3 PUFA may prevent TGF- β 1 induced transdifferentiation of C2C12 myoblasts by modulation of the SK1/S1P axis and/or via PPAR γ .

C2C12 myoblasts were differentiated by culture in growth medium containing 2% horse serum (DM). Cells were treated with 50 μ M eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), oleic acid (OA) or Linoleic acid (LA) and TGF- β 1 (1ng/ml) either independently or as a co-treatment for 96 hours. PPAR γ was specifically antagonized with GW9662 for 2hrs prior to withdrawal and replacement with treatment medium. Myogenesis was evaluated morphologically by a myogenic index, by myotube metrics and by myosin heavy chain (MyHC) expression and by qPCR of the myogenic markers MyoD and Myogenin. Fibrotic biomarkers α SMA and transgelin were evaluated by immunocytochemistry and qPCR. S1Pr1, S1Pr3 SK1 and PPAR γ were examined by qPCR at 6hrs and 18hrs post-induction of differentiation.

TGF- β 1-induced decreases in MyHC protein expression/myotube morphometrics, increased α SMA/ transgelin gene and protein expression and altered MyoD/ Myogenin gene expression were attenuated by co-treatment with EPA or DHA but not OA or LA. Furthermore, TGF- β 1-induced increases in SK1 and S1Pr3:S1Pr1 were prevented by co-treatment with EPA or DHA in conjunction with TGF- β 1. Both PUFAs separately reversed the TGF- β 1-mediated decrease in PPAR γ gene expression and significantly increased its gene expression above control levels. However, specific blockade of PPAR γ did not prevent EPA or DHA blocking the effects of TGF- β 1 on myogenesis. In conclusion the Ω -3 PUFAs, EPA and DHA, attenuate TGF- β 1 induced transdifferentiation of C2C12 myoblasts by a mechanism involving the sphingosine kinase pathway but independently of PPAR γ . Additionally this effect is considered unique to fish oils as it is not achieved by the non Ω -3 PUFAs, OA and LA.

[1] Murakami, M., Ohkuma, M., Nakamura, M. (2008). Molecular mechanism of transforming growth factor-beta- mediated inhibition of growth arrest and differentiation in a myoblast cell line. *Development, Growth and Differentiation*. 50: 121-130

[2] Cencetti, F., Bernacchioni, C., Nincheri, P., Donati, C., Bruni, P. (2010) Transforming growth factor-beta1 induces transdifferentiation of myoblasts into myofibroblasts via up-regulation of sphingosine kinase-1/S1P3 axis. *Molecular Biology of the Cell*. 21(6):1111-24

[3] Magee, P., Pearson, S., Whittingham-Dowd, J., Allen, J. (2012) PPAR γ as a molecular target of EPA anti-inflammatory activity during TNF- α -impaired skeletal muscle cell differentiation. *Journal of Nutritional Biochemistry*. ePub ahead of print

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC110

Ibuprofen effect on experimental peri-implantitisP. Alina Elena¹, A. Sandu Florin², C. Simona³, A. Camelia⁴, T. Marian Aurel⁵, B. Andreea⁶, C. Cornel⁵, M. Aurel⁶ and P. Alina⁷

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Peri-implant diseases are site-specific plaque-induced inflammatory reactions in the soft tissues surrounding implants. The treatment alternatives and prognosis depend on the host-parasite-implant interactions in the peri-implant tissues. In periodontitis ibuprofen significantly reduced induced nitric oxide synthase (iNOS) dependent nitric oxide (NO) synthesis and bone loss. The aim of the present study was to evaluate the effect of systemic short-term treatment with low ibuprofen dose, on nitric oxide synthesis and oxidative stress in an experimental peri-implant disease at dogs. Six adult, systemically healthy mixed breed male dogs, about 2 years old, weighing 20-25 kg, were used. In the first step the second premolar was extracted and an OT dental implant 3,2/8mm (OT medical GmbH, Konsul-Smidt-Str. 8b, 28217 Bremen) was placed. The flaps were adjusted and sutured around the neck of all implants. The sutures were removed after 2 weeks. In the second step, five weeks after implant installation, experimental peri-implantitis was initiated by placing a cotton ligatures in a submarginal position around the neck portion of the implants. In the third step animals were randomly treated, three with ibuprofen (5mg/kg b.w.) and three with placebo, for one week. All surgical procedures were performed under general anesthesia accomplished by sodium pentobarbital (30-40 mg/kg b.w. i.v. in the lateral saphenous vein). At baseline, 1, 3, 5, 7, 9 and 10 weeks from implant placement blood was collected from the lateral saphenous vein to assay total nitrites and nitrates (NOx), total oxidative status (TOS), total antioxidant reactivity (TAR) and oxidative stress index (OSI) (fig.1). Values are means $\pm$ S.E.M., compared by ANOVA. At ibuprofen treated animals NOx, TOS and OSI decreased significantly at completion the treatment ($p < 0,001$). Ibuprofen had no significant influence on TAR ($p > 0,05$). These results suggest that in dog experimental peri-implant disease, short term low ibuprofen dose administration had an anti-inflammatory effect by reducing NO synthesis and oxidative stress, without an important change of the total antioxidants.

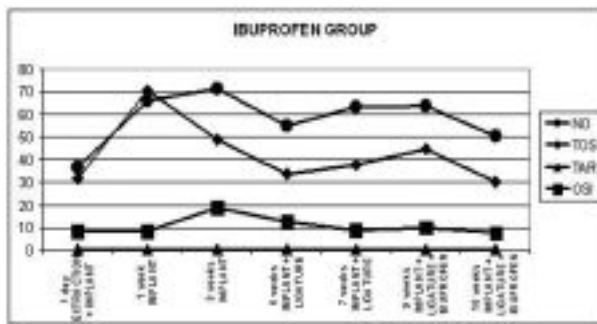


Figure 1: Progression of NOx, TOS, TAR and OSI in the group with experimental peri-implant disease and ibuprofen treatment.

Albouy J-P., Abrahamsson L., Persson L.G., Berglundh T. Spontaneous progression of ligature induced periimplantitis at implants with different surface characteristics. An experimental study in dogs: histological observations. Clin. Oral Impl. Res. 2009, 20, 366–371.

Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005, 38, 1103-1111.

Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004, 37, 112-119.

Miranda K.M., Espey M.G., Wink D.A. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide, 2001, 5, 62-71.

Shin J.S., Baek S.R., Sohn S.I., Cho Y.W., Lee K.T. Anti-inflammatory effect of pelubipropfen, 2-[4-(oxocyclohexylidenemethyl)-phenyl]propionic acid, mediated by dual suppression of COX activity and LPS-induced inflammatory gene expression via NF- κ B inactivation. J Cell Biochem. 2011, 112(12), 3594-3603.

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PC111

Myostatin and its downstream signalling pathways are well maintained between Mus musculus and Homo sapiens

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Skeletal muscle is key in the maintenance of homeostasis in humans; atrophy of muscle has negative health effects. A significant regulator of muscle mass is the myostatin signalling pathway (McPherron et al., 1997), which inhibits anabolism and myoblast proliferation, and promotes catabolism via multiple signalling pathways (reviewed by Elliott et al., 2012). In muscle research, the mouse C2C12 myoblast line is widely used, as derived by Yaffe and Saxel (1977). A frequent critique of research utilizing animal cell lines is the relevance of findings to human physiology in vivo.

We therefore aimed to examine the relevance of results arising from use of the C2C12 cell line to translational work. Specifically, we chose to examine the evidence of protein homology of myostatin, its receptors, and its downstream intracellular signalling pathways between mouse and human. Protein basic local alignment search tool (pBLAST) was used to assess homology (NCBI; Altschul et al., 1997). As well as myostatin and its receptor pair, activin receptor 2B (ARIB) and

activin receptor 1B (Alk 4), the targeted proteins were the smad2, smad3, smad4 of the myoblast proliferation inhibition pathway, FoxO1, atrogin and murf of the proteasomal pathway and Akt, mTOR and, GSK-3b, p70s6k and 4EBP-1 of the pro-anabolic pathway.

BLAST protein-protein analysis revealed significant percentage identity between human and mouse myostatin proteins (96% similarity) and significantly, 100% conservation of myostatin's bioactive C-terminus. This similarity is decreased in other common research models. For example, myostatin from Danio rerio (Zebrafish) shows only 68% similarity when compared to human, with an 86% similarity in the C-terminus region.

Homology is conserved in both receptor components, ARIB (94%) and Alk 4 (98%). Smad signalling is well conserved, with smad2, smad3 and smad4 sharing 99%, 99% and 97% identity to the compared human proteins, respectively. The FoxO1-atrogin-murf signalling pathway is similarly well conserved, with 91%, 95% and 93% identity, respectively to the human homologues. Akt and mTOR share 88% and 99% sequence identity respectively, while p70S6k, GSK-3B and 4E-BP1 show 99%, 99% and 91% sequence identity, respectively.

ALTSCHUL, S. F., MADDEN, T. L., SCHAFER, A. A., ZHANG, J., ZHANG, Z., MILLER, W. & LIPMAN, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research, 25, 3389-402.

ELLIOTT, B., RENSHAW, D., GETTING, S. & MACKENZIE, R. 2012. The Central Role of Myostatin in Skeletal Muscle and Whole Body Homeostasis. Acta physiologica.

MCPHERRON, A. C., LAWLER, A. M. & LEE, S. J. 1997. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature, 387, 83-90.

YAFFE, D. & SAXEL, O. 1977. Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. Nature, 270, 725-7.

Dr. Paul Curley of the University of Westminster for assistance with bioinformatics.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC112

Reduced fibrinolytic activity in women after delivery

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Fifty pairs of mother and Cord blood from women with normal delivery were studied. Haemorrhagic and fibrinolytic indices comprising of Packed Cell Volume (PCV), Relative Plasma Whole Blood Viscosities (RPV and RWBV), Hemoglobin Lysis Time and Plasma Fibrinogen (PFC) were analysed with standard techniques. Student t- test was used for statistical analysis. We observed significant increases in cord blood values of RWBV and PFC when compared with that of controls ($P < 0.05$ respectively), while there were no significant difference in PCV, RPV and ELT ($P < 0.05$ respectively). The maternal blood samples showed a statistically significant decrease in PCV, RPV and Fibrinolytic index (increased ELT) ($P < 0.05$), while PFC showed a significant increase ($P < 0.05$) when compared with control. Also, there were significant decreases in maternal values of PCV, RWBV and Fibrinolytic index ($P < 0.05$ respectively) when compared with cord values but RPV and PFC exhibited statistical increases in ($P < 0.05$). We concluded that

increased plasma values of PFC and RPV are consistent physiological changes in pregnant women progressive term while the increase in cord blood could also be associated with fetal neonatal physiology. The decreased fibrinolytic activity in mothers could be regarded as a protective mechanism against post partum haemorrhage.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC113

Effects of a 4 weeks exercise intervention on body composition, energy balance and metabolism in lean and overweight/obese women

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According to the World Health Organisation, in 2011, 1.5 billion adults were estimated to be overweight worldwide with serious consequences for health. Exercise is often prescribed for weight loss in overweight/obese population. The aim of this study was to investigate the effects of a 4 weeks circuit training intervention (3d/wk, 60min/d, 70-80%HRmax) in sedentary overweight/obese and lean females on body weight control and metabolism. Importantly, subjects had been naïve to the purpose of the intervention and had been selected from population based on not being interested in weight loss. Sedentary lean (L) (n = 10, age: 22.4±4.6, BMI: 22.7±2.1kg.m⁻², VO₂ peak: 32.5±8.3ml.kg⁻¹.min⁻¹) and overweight/obese (O) (n = 7, age: 26.9±3.9, BMI: 31.1±5.6kg.m⁻², VO₂ peak: 26.6±7.2ml.kg⁻¹.min⁻¹) females finished the study. All participants completed a 3 day diet diary each week during the study including 2 weeks prior to the exercise intervention and 2 weeks post intervention which was analysed for energy and macronutrient intake. Pre and post intervention measures included peak oxygen uptake (VO₂ peak), weight, body composition analyzed by Dual-Energy X-ray Absorptiometry (DEXA) and capillary blood samples for plasma leptin and adiponectin levels. Exercise dependent energy expenditure over the 4 weeks circuit training was matched between groups. Post intervention, no significant alterations (analysed by ANOVA) were observed in weight and body composition (fat and lean mass) in both groups suggesting an intact body weight set-point regulation in lean and overweight/obese. Additionally no time effects for VO₂ peak were found in both groups; however, at 25 Watts overweight/ obese subjects showed a significant alteration in energy expenditure post intervention (1.15±0.38 to 1.32±0.53kcal.min⁻¹.m⁻², P<0.05) suggesting an improvement in aerobic metabolism. Respiratory exchange ratio (RER) during 50-110 watts of VO₂ peak assessment was significantly higher in the overweight/obese subjects compared to the lean group (All P's<0.05), although no changes were seen post intervention within groups. Overweight/obese participants were found to have significantly higher plasma leptin concentrations and significantly lower plasma adiponectin levels than their lean counterparts (All P's<0.05) but no time effects were observed. Energy balance showed a trend (P<0.1) towards better regulation in both groups during and after the exercise intervention. In summary, 4 weeks exercise training of overweight/obese and lean females did not lead to weight/fat loss in subjects naïve to the purpose of the study suggesting that set point regulation is not impaired in overweight/obese female. Weight loss seen in other exercise studies might be

more related to subconscious or conscious dieting than to the exercise energy expenditure.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC114

Limited range of motion lumbar extension resistance exercise in chronic low back pain participants

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Non-specific chronic low back pain (CLBP) is a prevalent multifactorial condition causing great economic costs. Numerous symptoms and dysfunctions are associated with CLBP including limited sagittal range of motion (ROM). Pain can also intensify in the extremes of ROM. This study examined whether progressive lumbar extension resistance exercise through a limited ROM can produce full ROM strength changes. An RCT of three groups was performed; FullROM, LimROM and Control. Participants (n=15 males, n=12 females) were randomised to the study groups (n=11 FullROM, n=8 LimROM, n=8 Control) and demographics recorded (Age 44.4±14.2yrs, Stature 175.8±6.8cm, Body Mass 80.4±13.8kg, BMI 25.8±3.0, Symptom Duration 14.0±11.7yrs). Pre/post full ROM strength testing was measured on a MedX Lumbar Extension Machine. Pre/post schober's tests of flexion and extension, visual analogue pain scale (VAS) and Oswestry questionnaire were completed. Intervention was 12 weeks progressive resistance exercise of the isolated lumbar extensors (1 set, 8-12 reps to momentary muscular failure). FullROM trained through 100% of their ROM, LimROM trained through the mid 50% of their ROM, the Control group did not train. One way ANOVA and Tukey post hoc tests compared between group relative changes (mean±SD). Strength data was averaged over quarters of ROM (i.e. Q1, Q2, Q3, and Q4). Groups did not differ (p>0.05) in age, stature, body mass, BMI, symptom duration, strength, VAS or attendance (84.6±21.8%). ANOVA on initial preliminary data (n=8 FullROM, n=5 LimROM, and n=6 Control; Full data set to be presented at conference) show significant changes in VAS (p=0.002) and Oswestry (p=0.004). No changes were seen in schober's test measures. Trends toward improvement in lumbar extension strength at each quarter (Q1 p=0.058; Q2 p=0.081; Q3 p=0.057; Q4 p=0.051) were found. Post hoc tukey show between Controls and FullROM significant improvement in VAS (p= 0.003, -59.0±36.8%), Oswestry (p=0.01, -46.7±19.9%) and significant strength changes in Q3 and Q4 (Q3 p=0.049, 44.5±53.8%; Q4 p=0.043, 31.4±27.3%); but improvements failed to achieve significance in Q1 or Q2. Between Controls and LimROM were significant changes in VAS (p=0.007, -61.7±18.8%) and Oswestry (p=0.008, -56.4±24.3%), but not in any of the strength variables (Q1-4). No significant differences between training groups were found in any variables. Initial data suggest limited ROM lumbar extension exercise may produce full ROM strength changes as indicated by the absence of difference between training groups, with pain and disability improvement also. This may have important application to the rehabilitation of CLBP as many suffer from end ROM pain increases. Avoiding these positions in exercise may still be effective and may also provide less pain during exercise for participants.

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PC115

Calprotectin as a marker in pharyngeal mucosa and serum, in relation to training in athletes, illness symptoms and cytokines

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Calprotectin, a leucocyte cytosolic protein but with bactericidal properties when released on cell activation, is documented as a marker of systemic inflammation. It crosses mucosal walls to be detectable in faeces and urine in inflammatory conditions but its presence on pharyngeal mucosa is unknown. Its possible use as a non-invasive marker of local pharyngeal inflammation was investigated and compared with serum calprotectin. Monthly serum and throat swab samples were taken from trained male endurance athletes (N=16, 22-54yrs) who gave informed consent. They were studied over a period of 4 months of increasing training volume and intensity, with concurrent recording of hours of sleep, sleep quality (Pittsburgh Score), psychological mood scores (POMS), training logs and symptoms (Wisconsin). Samples were taken (using Becton Dickinson ProbeTec®CT/GC swabs) from the posterior pharyngeal wall after a cleaning procedure to exclude salivary calprotectin. Serum samples were tested for C-reactive protein (CRP; high sensitivity assay); calprotectin, IL-6, IL1ra, IL-10, IL-17, TNF α via ELISA. Values for samples and questionnaire scores were compared over time by repeated measures ANOVA and against each other by Pearson's product-moment correlation coefficient. Calprotectin was successfully measured in all throat swab samples. No correlation was found between serum and pharyngeal calprotectin ($r=-0.2$, $P<0.19$). Serum calprotectin was positively correlated with CRP ($r=0.42$, $P<0.006$), IL-6 ($r=0.47$, $P<0.0024$) and training intensity ($r=0.53$ $P<0.0003$); no correlations were seen with the other cytokines measured. However, IL-6 correlated markedly with CRP ($r=0.90$ $P<0.00001$). No correlation of POMS was seen with time or training intensity. Respiratory symptoms in some athletes appeared to be linked to increased training intensity. The serum calprotectin results provide further evidence that this may be a useful marker of exercise induced immunodepression.

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PC116

Follicles in gut-associated lymphoid tissues create preferential survival niches for follicular T helper cells escaping T-cell depleting treatment in mice

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INTRODUCTION: Depletion of T cells by monoclonal antibodies is a widely used therapeutic approach for inducing tolerance and preventing graft versus host disease in patients with organ transplants (1). Although a substantial number of CD4 T cells can escape depletion, there are no data on their specific tissue location and phenotypic characteristics in different peripheral lymphoid tissues (2). In the present study we investigated survival, phenotype and location of CD4-positive T cells immediately following anti-Thy-1 monoclonal antibody treatment in mouse.

METHODS: BALB/c mice at 6-10 weeks of age were injected i.v. 300 μ g of IBL-1 (anti-Thy-1/CD90) monoclonal antibody on two consecutive days and sacrificed 36 hours after first injection. In a separate set of experiments, prior to IBL-1 treatment, mice were immunized by human RBC (100 μ l/recipient of a 10% suspension in PBS i.p.) or treated by lymphotoxin beta-receptor fusion protein (100 μ g/recipient i.v.), for induction of germinal centre reaction or acute disruption of follicular structure, respectively. For the purpose of evaluating the effect of acute IL-7 withdrawal, mice received simultaneously both anti-Thy-1 (600 μ g/recipient) and polyclonal rabbit anti-mouse IL-7 (160 μ g/recipient) blocking Abs. Their spleen, lymph nodes and Peyer's patches (PP) were collected and further processed for the flow cytometric and immunofluorescent microscopic assessment using CD62L, CD44, CD4, CD8, Bcl-2, CXCR5 and PD1 markers. T-cell proliferation was monitored by using BrdU incorporation assay.

RESULTS: We found a preferential survival of CD4 T cells in the Peyer's patches and, to a lesser degree, in the mesenteric lymph nodes of BALB/c mice, where majority of the surviving CD4-positive T cells displayed CD44hi/CD62Lneg phenotype corresponding to effector memory T cell features. In addition, these CD4-positive cells also expressed CXCR5 and PD-1 markers characteristic for follicular TH cells (TFH), and were enriched in the Peyer's patch follicles. Using BrdU-incorporation we demonstrate that the immediate survival of these cells does not involve proliferation. Furthermore, simultaneous blockade of IL-7 did not enhance the efficiency of anti-Thy-1 depletion. Induction of germinal center formation during T-dependent immune response in spleen resulted in enhanced survival of splenic TFH cells, while the dissolution of follicular architecture by lymphotoxin beta-receptor antagonist treatment slightly reduced TFH survival.

CONCLUSION: Our results thus raise the possibility that the natural germinal center microenvironment in the GALT may be favorable for the survival of T-follicular helper cells of memory phenotype.

Neujahr DC, Chen C, Huang X, Markmann JF, Cobbold S, Waldmann H, Sayegh MH, Hancock WW, Turka LA. Accelerated memory cell homeostasis during T cell depletion and approaches to overcome it. J Immunol. 2006 Apr 15;176(8):4632-9.

Pearl JP, Parris J, Hale DA, Hoffmann SC, Bernstein WB, McCoy KL, Swanson SJ, Mannon RB, Roederer M, Kirk AD. Immunocompetent T-cells with a memory-like phenotype are the dominant cell type following antibody-mediated T-cell depletion. *Am J Transplant*. 2005 Mar;5(3):465-74.

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PC117

Hearing loss is more prevalent in Rheumatoid arthritis patients. Results from a case controlled study

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Introduction - Rheumatoid arthritis (RA) is well known to affect many different organ systems. Some previous work suggests that this includes the auditory system. The aim of this study was to evaluate whether patients with rheumatoid arthritis (RA) are more likely to have hearing loss compared with people without RA a.

Materials and methods - Case-control cross-sectional study; 2 groups: 22 RA patients (20 women and 2 men, mean age of 44.2 years, disease duration median of 41 months (21, 141)) and 17 healthy subjects (13 women and 4 men, mean age of 41 years) appaired for age and sex (no difference between the two groups regarding age and sex).

No subject of the 2 groups has had any abnormalities at otoscopic examination. Standard frequency pure tone audiometric tests, impedance audiometry (tympanometry and acoustic reflex test) were performed and compared between the 2 groups.

Results: Hypoacusis was more common symptom in RA patients (50 vs 35 %; $p=0.06$). RA patients have worse hearing at low and middle frequencies (250 Hz to 2 kHz) and more elevated Air-bone gaps (45.5 vs 5.9; $p=0.01$).

Furthermore, Stapedial reflex abonormalities were significantly more frequent in RA patients (27.2 vs 0%; $p=0.02$).

Conclusion: This study suggests that hearing loss risk is higher in RA patients . Audiological evaluation should must be performed periodically to identify possible audiological damage. Nevertheless, larger studies are needed to confirm those results.

Relationship between hearing loss and RA remission

Parameters	RA remission		p
	Yes	No	
Mean hearing threshold	12.9 ± 4.6	18.4 ± 6.4	0.04
Hearing loss	12.8 ± 5.2	17.7 ± 6.2	0.07
Stapedial reflex absence (%)	0	35.7	0.06

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PC118

Influence of phase I duration and age on pulmonary oxygen uptake kinetics during heavy intensity exercise

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Pulmonary oxygen uptake ($\dot{V}O_2$) kinetics are slowed and phase I duration prolonged in older adults during moderate intensity exercise; whether the same is true during heavy exercise is unknown. Conventionally, this slowing has largely been attributed to an age-related reduction in the capacity to both deliver and utilise O_2 . However, the recent observation of a prolonged phase I duration in older people suggests that slower $\dot{V}O_2$ kinetics might be an artefact of the inappropriate exclusion of phase I. Furthermore, slower $\dot{V}O_2$ kinetics in older people has always been reported in combination with a lower peak $\dot{V}O_2$. Therefore, it remains to be elucidated whether the slowing of $\dot{V}O_2$ kinetics is attributable to age *per se*, to the concomitant reduction in aerobic fitness or to a methodological artefact.

Fifty younger (24 ± 4 yrs, 28 male) and 15 older (54 ± 3 yrs, 6 male) healthy participants completed repeated bouts of moderate (Mod; 70% gas exchange threshold, GET) and heavy (Hvy; 30% of the difference between the GET and peak $\dot{V}O_2$) intensity exercise. Pulmonary gas exchange variables were measured throughout. Parameters describing the dynamic $\dot{V}O_2$ response to the step change in work rate were derived using a mono-exponential model with a time delay, constrained to exclude the visually identified slow component during heavy exercise. The phase I-phase II transition was visually identified (VI) and compared to fixed exclusion time periods ranging from 15s to 40s.

Irrespective of age, Phase I duration did not affect the phase II τ during Mod. During Hvy, the phase II τ was significantly slower when fitting started at 15s compared to at the VI phase I duration (Y: 31 ± 9 vs. 29 ± 9 ; O: 35 ± 11 vs. 30 ± 8 s; $P < 0.05$) but no difference was evident when fitting started at any other phase I duration. Neither the phase II τ (irrespective of phase I duration) nor peak $\dot{V}O_2$ were influenced by age during Mod or Hvy. The $\dot{V}O_2$ slow component was significantly smaller in older participants (O: 9 ± 4 vs. Y: 15 ± 11 %; $P < 0.05$).

The age-independence of $\dot{V}O_2$ kinetics observed here in participants with similar peak $\dot{V}O_2$ refutes the notion that slowing of $\dot{V}O_2$ kinetics in older people is attributable to age *per se*. The apparent age-related slowing in previous studies may be attributable to concomitant reductions in aerobic fitness. Our results do not support a role of Phase I duration in explaining apparent age-related differences: provided a minimum period of 20s was excluded, the phase I duration did not influence phase II τ in young or older people. Finally, the smaller slow component amplitude observed here in older participants agrees with a preferential loss of Type II motor units with age (1), the additional recruitment of which might be linked to development of the slow component (2, 3).

Bell et al. (1998) *Adv Exp Med Biol*, 84, 747-59

Krustup et al. (2004) *EJAP*, 447, 855-66

Rossiter et al. (2002) *JAP*, 93, 2059-69

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PC119

Validity of stoichiometric equations to estimate carbohydrate and fat oxidation rate vs. $^{13}\text{C}/^{12}\text{C}$ breathing technique in endurance athletes

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Indirect calorimetry is used routinely to estimate energy expenditure in athletes. By means stoichiometric equations, carbohydrates (CHO_{OXR}) and fat (FAT_{OXR}) are estimated from VO_2 and VCO_2 (1). This methodology is useful to understand exercise-induced metabolic adaptations and athletes' fuel needs. There are different stoichiometry equations in literature (2), but no studies had evaluated the validity of all these equations respect to a gold standard for whole intensities of aerobic exercise. The present study was designed to assess the agreement between the different stoichiometric equations vs. the breath $^{13}\text{C}/^{12}\text{C}$ ratio technique as a gold standard. 16 male endurance well-trained athletes ($n=1$ Olympic level in Triathlon) (BMI: $22.3 \pm 6.2 \text{ kg/m}^2$, $\text{VO}_{2\text{Peak}}$: $4.05 \pm 0.50 \text{ L/min}$) followed a glycogen depletion-repletion protocol to increase the ^{13}C enrichment of endogenous CHO (3). The day after, subjects performed a laboratory test on cycloergometer: 10min warm-up at 2.0 W/kg and increments of $0.5 \text{ W/kg}/10 \text{ min}$ until exhaustion. VO_2 and VCO_2 were measured during the test (Oxycon-Pro®). CHO_{OXR} and FAT_{OXR} were estimated for all different equations (2) at resting and at the end of stages. Isotope infusion, ^{13}C plasma enrichment of CHO, fats, and proteins were measured; as well as CHO_{OXR} and FAT_{OXR} from $^{13}\text{C}/^{12}\text{C}$ breathing (3). The agreement between stoichiometric equations vs. the gold standard was assessed by Bland-Altman (4). In resting ($6\text{-}16\% \text{VO}_{2\text{Peak}}$) all FAT_{OXR} equations had the same agreement ($-0.03 \pm 0.18 \text{ g/min}$) and all CHO_{OXR} equation showed similar agreements being the Brouwer the best one ($0.08 \pm 0.46 \text{ g/min}$). All FAT_{OXR} equations presented good agreements at low-moderate intensities of exercise ($19\text{-}80\% \text{VO}_{2\text{Peak}}$), to moderate-high intensities ($81\text{-}100\% \text{VO}_{2\text{Peak}}$) all FAT_{OXR} equations presented moderate errors, being the lowest one for the Brouwer's equation (Lower limit agreement (LLA): -0.46 to 0.10 , Bias: -0.22 to 0.26 and Upper limit agreement (ULA): 0.02 to 0.43 (g/min)). For CHO_{OXR} equations at low-moderate intensities the Frayn's and the Ferranini's (for glucose) equations showed the lowest error (LLA: -0.09 to 0.01 , Bias: -0.01 to 0.06 and ULA: -0.02 to 0.21 (g/min)), and the Ferranini (for Glycogen) one at moderate-high intensities (LLA: -0.96 to -0.20 , Bias: -0.48 to 0.77 and ULA: 0.00 to 1.75 (g/min)). In summary, from resting to medium intensities of exercise all FAT_{OXR} equations can be used indistinctly but at moderate-high intensities the Brouwer one is the most suitable to use. The best CHO_{OXR} equations were the Brouwer one for resting, the Frayn and the Ferranini (for Glucose) ones at low-moderate intensities, and the Ferranini (for Glycogen) one at moderate-high intensity. Ferrannini E (1988) *Metabolism* **137**,287-301.

Jeukendrup AE & Wallis GA (2005) *Int J Sports Med* **26**,S28-S37.

Romijn JA et al. (1992) *Am J Physiol* **263**,E64-E71.

Bland JM & Altman DG (1999) *Stat Methods Med Res* **8**,135-160.

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PC120

Hypoxia induces myotube atrophy in a time-dependent manner via NF- κ B

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Skeletal muscle is key in the maintenance of homeostasis in humans. Atrophy of muscle has negative health effects. Atrophy is due to decreased synthesis, increased degradation, or impaired proliferation of precursor myoblasts. One poorly understood inducer of muscle atrophy is hypoxia. Exposure to altitude has been linked to atrophy and a catabolic state in humans and rats (Hoppeler et al., 1990, Preedy et al., 1985) and increases in pro-inflammatory cytokine regulator NF- κ B (Rius et al., 2008). In humans exposed to 2 hours of 12% O_2 , basal muscle protein synthesis is not altered (Etheridge et al., 2011), suggesting that elevated degradation or impaired myoblast proliferation and migration underlies atrophy. Myotubes treated with cobalt chloride (a hypoxia mimicking agent) show elevated myostatin expression (Hayot et al., 2010), key as myostatin has been shown to increase degradation and decrease proliferation. We therefore aimed to explore the result of hypoxic exposure on migration of immature myoblasts and atrophy of mature myotubes. We also aimed to examine the role of inflammation in hypoxia effects. C2C12 myoblasts were grown to confluence in 6-well plates or 10cm dishes. 6-well plates were scratched, photographed (2.5X zoom) and exposed to control (21% O_2) or hypoxic (1% O_2) conditions in the presence of the NF- κ B inhibitor PS1145 (10 μM in PBS) or blank (PBS only). After 16 hours, cells were photographed and scratch closure quantified using ImageJ. 10cm dishes were differentiated as standard (DMEM with 2% horse serum, pen/strep) into mature myotubes, then treated with control (21% O_2) or hypoxia (1% O_2). Hypoxic myotubes were further treated with PS-1145 (10 μM in PBS) or hypoxic control (PBS only). Myotubes were stimulated for 2, 24 or 48 hours. Cells were photographed by light microscopy (10X zoom, phase contrast), lysed and supernatant frozen for future analysis.

Control plates showed significant closure of 63.7% 16 hours post scratch ($p < 0.05$), PS1145 had no further effect on closure. Hypoxic plates showed reduced closure (39.6%) relative to control conditions. Stimulation of hypoxic myoblasts with PS1145 showed trends towards increased closure.

Incubation of myotubes in 1% hypoxia reduced myotube diameter at 2 hours that was further reduced at 24 and 48 hours (14.2%, 36.9% and 34.2%, respectively). Atrophy in response to hypoxia was prevented by co-incubation with PS1145 at 2 hours ($p < 0.05$) and showed trends towards offsetting atrophy at 24 and 48 hours. Expression of myostatin was unaltered by hypoxia or PS1145 exposure.

Here we report that hypoxia directly regulates both myotube size and myoblast migration. These effects appear in part to be via NF- κ B. Counter to previous reports (Hayot et al., 2010), we did not see increased myostatin expression in response to hypoxic exposure.

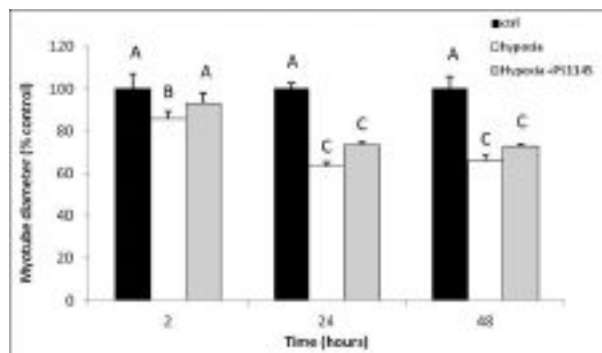


Figure 2: Hypoxia reduces myotube diameter in a time dependent manner, NF- κ B inhibition delays this. Myotube diameter as a function of time in C2C12 myotubes, expressed relative to control group. Control cells exposed to 23%O₂, hypoxic cells 1%O₂ +/- PS1145 (10 μ M). N=3 per group. Error bars indicates se, letters (A,B,C) indicates significance.

ETHERIDGE, T., ATHERTON, P. J., WILKINSON, D., SELBY, A., RANKIN, D., WEBBORN, N., SMITH, K. & WATT, P. W. 2011. Effects of hypoxia on muscle protein synthesis (MPS) and anabolic signaling at rest and in response to acute resistance exercise. *American journal of physiology. Endocrinology and metabolism*.

HAYOT, M., RODRIGUEZ, J., VERNUS, B., CARNAC, G., JEAN, E., ALLEN, D., GORET, L., OBERT, P., CANDAU, R. & BONNIEU, A. 2010. Myostatin up-regulation is associated with the skeletal muscle response to hypoxic stimuli. *Mol Cell Endocrinol*.

HOPPELER, H., KLEINERT, E., SCHLEGEL, C., CLAASSEN, H., HOWALD, H., KAYAR, S. R. & CERRETELLI, P. 1990. Morphological adaptations of human skeletal muscle to chronic hypoxia. *Int J Sports Med*, 11 Suppl 1, S3-9.

PREEDY, V. R., SMITH, D. M. & SUGDEN, P. H. 1985. The effects of 6 hours of hypoxia on protein synthesis in rat tissues in vivo and in vitro. *The Biochemical journal*, 228, 179-85.

RIUS, J., GUMA, M., SCHACHTRUP, C., AKASSOGLU, K., ZINKERNAGEL, A. S., NIZET, V., JOHNSON, R. S., HADDAD, G. G. & KARIN, M. 2008. NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. *Nature*, 453, 807-11.

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PC121

Acute ingestion of dietary nitrate does not enhance running performance in highly-trained endurance athletes

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Dietary nitrate supplementation has been shown to reduce the oxygen cost of submaximal exercise and improve tolerance of high-intensity exercise in man. Limited data are available regarding the effects of nitrate ingestion during simulated competition, and it remains to be determined whether nitrate supplementation has performance-enhancing effects in highly-trained athletes. The present study examined the effects of acute dietary nitrate supplementation on the oxygen cost of submaximal exercise and endurance running time-trial performance in highly-trained athletes. Ten Norwegian male junior-elite cross-country skiers (maximum oxygen uptake $\approx$ 70

mL/kg/min) each completed two trials in a randomised, double-blind, cross-over design. Participants received dietary supplementation with potassium nitrate (614 mg nitrate) or a nitrate-free placebo 2.5 h before performing two 5-min submaximal exercise tests on a treadmill at 10 km/h ($\approx$ 55% of maximum oxygen uptake) and 14 km/h ($\approx$ 75% of maximum oxygen uptake), followed by a 5-km running time-trial on an indoor track. Ozone-based chemiluminescence was employed for plasma detection of nitric oxide metabolites. A paired two-tailed t-test was used to identify difference between means and a two-way general linear model for repeated measures (treatment x time) was used to identify differences over time. Data are means $\pm$ standard deviation and statistical significance was set at the 0.05 level. Plasma nitrite concentrations were higher after nitrate supplementation (325 ± 95 nmol/L) compared with placebo (143 ± 59 nmol/L, $p < 0.001$). There was no significant difference in 5-km time-trial performance between nitrate (1005 ± 53 s) and placebo treatments (996 ± 49 s, $p = 0.124$). The oxygen cost of submaximal running was not significantly different between placebo and nitrate trials at 10 km/h (both 2.84 ± 0.34 L/min) and 14 km/h (3.89 ± 0.39 L/min versus 3.77 ± 0.62 L/min). These results suggest that acute ingestion of dietary nitrate may not represent an effective strategy for reducing the oxygen cost of submaximal exercise or for enhancing endurance running performance compared to a nitrate-free placebo in this group of highly trained athletes.

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Physical performance profile of sub-elite juvenile Gaelic Games players and the prevalence of a Relative Age Effect (RAE)

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Gaelic Games are the indigenous sports played in Ireland, the most popular being Gaelic football and hurling. The games are contact sports and the physical demands are thought to be similar to those of Australian Rules football, rugby union, rugby league, field hockey, and lacrosse (Delahunt et al., 2011). The difference in chronological age between children in a single age group is known as relative age and its consequences as the RAE, whereby younger players are disadvantaged (Del Campo et al., 2010). The purpose of this study was to describe the physical and performance profile of sub-elite juvenile Gaelic Games players and to establish if a RAE is present in this cohort and any influence physiological moderator variables may have on this. Following receipt of ethical approval (EHSREC11-45), six sub-elite county development squads (Under-14/15/16 age groups, male, $n = 115$) volunteered to partake in the study. Anthropometric data including skin folds and girths were collected. A number of field tests of physical performance including 5 and 20m speed, vertical and broad jump distance, and an estimate of $\dot{V}O_{2\max}$ were carried out. Descriptive data are

presented as Mean $\pm$ SD. Juvenile sub-elite Gaelic Games players aged 14.53 ± 0.82 y were 172.87 ± 7.63 cm tall, had a mass of 64.74 ± 11.06 kg, a BMI of 21.57 ± 2.82 kg.m⁻² and 9.22 ± 4.78 % body fat. Flexibility, measured by sit and reach was 33.62 ± 6.86 cm and lower limb power measured by vertical and broad jump were 42.19 ± 5.73 and 191.16 ± 25.26 cm, respectively. Participant time to complete 5m, 20m and an agility test (T-Test) was 1.12 ± 0.07 , 3.31 ± 0.30 and 9.31 ± 0.55 s respectively. Participant's estimated VO₂max was 48.23 ± 5.05 ml.kg.min⁻¹. Chi-Square analysis of birth month by quartile (Q1 = January-March) revealed that a RAE was present in this cohort, whereby an over-representation of players born in Q1 compared with Q2, Q3 and Q4 was evident ($\chi^2 = 14.078$, df = 3, p = 0.003). Kruskal-Wallis analysis of the data revealed no significant difference in any of the performance parameters based on quartile of birth (Alpha level = 0.05). This study provides a physical performance profile of juvenile sub-elite Gaelic Games players, comparable with those of other sports such as soccer and rugby. This novel data can inform us of the physical requirements of the sport. The evidence of a RAE is similar to that observed in other contact sports such as soccer and rugby league (Carling et al, 2009; Till et al, 2010). Although a RAE exists in this cohort, this cannot be explained by any physical/physiological moderator variables.

Carling C et al. (2009). *Scandinavian Journal of Medicine and Science in Sport* **19**, 3-9.

Delahunt E et al. (2011). *Journal of Athletic Training* **46**, 241-5.

Del Campo DG et al. (2010). *Journal of Sport Science and Medicine* **9**, 190-198.

Delorme N et al. (2010). *European Journal of Sport Science* **10**, 91-96.

Till K et al. (2010). *Scandinavian Journal of Medicine and Science in Sports* **20**, 320-329.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC123

Using a model gut system to assess if alginate delivered in a bread vehicle is effective in inhibiting pancreatic lipase activity

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Problem. Despite current anti-obesity treatments the number of overweight and obese people is expected to rise to 2.3 billion and 700 million worldwide, respectively. Dietary fibre, specifically alginates are possible treatments for numerous health related conditions, including obesity [1]. We have developed a system which simulates digestion in the mouth, stomach and small intestine, and have used this to measure pancreatic lipase inhibition. Here we observed the release of alginate from bread in the gut system, and extracted the alginate from the gut system and assessed pancreatic lipase inhibition. Method. Alginate release – 5.2g of alginate bread (4% alginate by weight) and control bread were added in separate experiments. The model gut system consists of mastication in the mouth (30 seconds), digestion in the stomach (60 minutes) and small intestine (120 minutes). 1ml samples taken every 15 minutes were analysed using a Periodic acid-Schiff

method modified to remove interference from digestive components to determine the release of alginate. Isolation and Inhibition – Upon completion of the gut system 4 and 8ml samples were taken from alginate and control bread digestions (n=3) and analysed after precipitation and freeze drying. Samples were then re-suspended to make 4, 3, 2 and 1mg/ml in an olive oil substrate buffer, and lipase inhibition was assessed as described previously [2]. To calculate percent inhibition Orlistat (0.025mg/ml) was used as 100% inhibition to compare against alginate. To compare the level of inhibition from the freeze dried samples alginate that had not gone through the model gut system or freeze drying process was made up to 3 and 2mg/ml concentration and analysed using the same lipase inhibition method as mentioned previously. Data are presented as mean $\pm$ standard deviation (SD). Results. Alginate release – There was 9+3% alginate release from the bread between 0-60 minutes and 91+45% alginate release between 60-180 minutes. Isolation and Inhibition – The 4, 3, 2 and 1mg/ml had 56+5%, 39+15%, 36+16% and 8+12% lipase inhibition. The level of inhibition from normal alginate at 3 and 2mg/ml was 31+8% and 29+7% compared with 39+15% and 36+15% with the model gut samples at the same concentration of 3 and 2mg/ml respectively. Conclusion. The findings demonstrate that over 90% of the alginate is released in the small intestinal phase of the model gut system, where the majority of fat is digested. Furthermore these results suggest that the cooking process of the bread and transit through the model gut system does not prevent inhibition, and results in similar levels of inhibition as normal DM alginate. The data here suggest that bread may be a good vehicle to deliver an anti-obesity treatment based on alginate.

Brownlee, I.A., The physiological roles of dietary fibre Dietary Fibre and Bioactive Polysaccharides 2009. 25(2): p. 238-250.

Vogel, W.C. and L. Zieve, A Rapid and Sensitive Turbidimetric Method for Serum Lipase Based Upon Differences Between the Lipase of Normal and Pancreatitis Serum. *Clinical Chemistry*, 1961. 9(2): p. 168-181.

Peter Chater, Matthew Wilcox, Iain Brownlee, Chris J. Seal and Jeff P. Pearson.

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PC124

The acute effect of an 8 week exercise programme on cardiovascular risk factors in patients newly diagnosed with a transient ischaemic attack (TIA)

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Stroke is a common cause of death and is the leading cause of disability in New Zealand (NZ), with approximately 7,000 people suffering an initial or recurrent stroke each year.¹ When symptoms of stroke resolve within 24 hours it is known as a Transient Ischaemic Attack (TIA). Many individuals presenting with a TIA have predisposing coronary artery disease (CAD) and cerebrovascular disease (CVD) risk factors such as hypertension, diabetes mellitus, hyperlipidaemia, obesity and physical inactivity.² It has been suggested that 80% of recurrent vascular events could be prevented through a comprehensive

multi-factorial strategy.³ Preliminary evidence suggests that TIA patients in the non-acute phase can reduce vascular risk factors and improve their cardiovascular fitness following exercise training.^{3,4} The purpose of the present study was to examine whether an 8 week exercise intervention reduces risk factors aligned with CAD and CVD in TIA patients.

Sixty newly diagnosed TIA patients (68.1 ± 10.4 y), identified within 7 days of symptom onset, participated in the trial. All participants completed a baseline assessment which included a CAD risk factor assessment, including: systolic and diastolic blood pressure (SBP & DBP, respectively), total blood lipid profile (total cholesterol [TC], high-density lipoproteins [HDL], TC:HDL ratio), fasting blood-glucose (diabetes risk), smoking status, family history of CAD, body mass index (BMI) and waist-to-hip ratio. Participants were randomised to either an 8-week exercise programme or to a control group (usual care). Identical CAD risk factors were assessments at a follow-up assessment on completion of the intervention. Values are reported as means $\pm$ SD, as analysed by ANOVA. Participants randomised to the exercise group elicited a significantly greater reduction from baseline to follow-up in SBP (141 ± 15 & 131 ± 15 mmHg, respectively) and TC (4.1 ± 1.2 & 3.6 ± 0.7 m.mol⁻¹, respectively) than the control group (138 ± 12 & 136 ± 17 mmHg, and 4.1 ± 1.0 & 3.8 ± 1.0 m.mol⁻¹; $P < .05$). However, no differences were observed between the exercise and control group for BMI, waist-to-hip ratio, DBP or blood glucose ($P > .05$). This study has demonstrated that a short-duration (8-week) exercise based intervention may elicit favourable changes in blood lipid profile (TC) and resting SBP for TIA patients. Further research is necessary to examine the long-term effect of exercise on reducing CAD and CVD risk factors and recurrent vascular events in this population.

Gommans J, Barber A, Hanger C, Bennett P. Rehabilitation after stroke: changes between 2001 and 2007 in services provided by district health boards in New Zealand. *N Z Med J*. 2008;121:26-33.

Wolf PA, Clagett GP, Easton JD, Goldstein LB, Gorelick PB, Kelly-Hayes M. Preventing ischemic stroke in patients with prior stroke and transient ischemic attack: A statement for healthcare professionals from the Stroke Council of the American Heart Association. *Stroke*. 1999;30:1991-1994.

Prior PL, Hachinski V, Unsworth K, et al. Comprehensive cardiac rehabilitation for secondary prevention after transient ischemic attack or mild stroke: I: Feasibility and risk factors. *Stroke*. 2011;42:3207-3213

Lennon O, Carey A, Gaffney N, Stephenson J. A pilot randomized controlled trial to evaluate the benefit of the cardiac rehabilitation paradigm for the non-acute ischaemic stroke population. *Clin Rehabil*. 2008;22:125-133.

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Ergogenic effect of bicarbonate ingestion on a specific test based in field conditions during BMX competitions

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The results about the influence of bicarbonate ingestion on performance are contradictory (1). Several studies have demonstrated no ergogenic effect of bicarbonate ingestion on high-intensity exercise performance, despite significant blood alkalosis (2), although there are not studies in field conditions during bicycle motocross (BMX) competitions. Therefore, the aim of the study was to examine the effect of sodium bicarbonate ingestion on a specific test based in field conditions, analyzing the acid-base status and the cardiorespiratory response and its influence on performance. Ten elite BMX riders participated in this study which consisted in two trials performed in separated days. Each trial included three consecutive BMX races in field conditions separated by 15 min recovery. The experiment was carried out in a BMX track to simulate a real competition. Ninety minutes prior to exercise subjects ingested either NaHCO₃ (0.3 g.kg⁻¹ body weight) or placebo. The two treatments conditions were administered in a counterbalanced, crossover, randomly assigned and double-blind manner. Expired air and blood samples were collected for the assessment of cardiorespiratory and blood acid-base response, respectively. Oxygen uptake (VO₂), ventilation (V_E), pH and blood lactate concentration ([La⁻]) were measured. Performance variable of race time was measured for each BMX race (R) repetition. Two way ANOVA with repeated measures were used to determine differences between treatments at each exercise moment. Both conditions (bicarbonate and placebo) showed a similar response ($p > 0.05$) of VO₂ (1999 ± 361 vs. 2090 ± 467 mL.min⁻¹ after R1; 2142 ± 490 vs. 2229 ± 428 mL.min⁻¹ after R2; 2143 ± 440 vs. 2171 ± 359 mL.min⁻¹ after R3, respectively) and V_E (70.8 ± 28.2 vs. 72.1 ± 27.6 L.min⁻¹ after R1; 72.9 ± 26.5 vs. 73.7 ± 29.4 L.min⁻¹ after R2; 76.9 ± 23.9 vs. 79.3 ± 28.7 L.min⁻¹ after R3, respectively). The ingestion of NaHCO₃ had the expected effect on blood pH, since remained reduced ($p < 0.05$) across experimental protocol in the placebo condition (7.18 ± 0.05 vs. 7.12 ± 0.05 3 min after R1; 7.14 ± 0.07 vs. 7.06 ± 0.03 3 min after R2, bicarbonate vs. placebo conditions, respectively). No significant differences were found in the [La⁻] between both conditions, with higher values in the bicarbonate condition (13.5 ± 2.9 vs. 12.1 ± 2 mmol.L⁻¹ 3 min after R1; 15.5 ± 2.8 vs. 14.03 ± 0.8 mmol.L⁻¹ 3 min after R2; 16.8 ± 2.5 vs. 16.5 ± 1.8 mmol.L⁻¹ 3 min after R3, respectively). Race time was similar in each race between conditions ($p > 0.05$) (31.4 ± 1.3 vs. 31.1 ± 1.05 s R1; 31.2 ± 1.3 vs. 31 ± 1.04 s R2; 31.3 ± 1.2 vs. 31.05 ± 1.04 s R3, bicarbonate vs. placebo conditions, respectively). Sodium bicarbonate ingestion modified significantly the blood acid-base balance, although the attenuated acidosis did not improve the performance across three consecutive races in field conditions in elite BMX cyclists.

1. Carr AJ, Hopkins WG, Gore CJ. Effects of acute alkalosis and acidosis on performance: a meta-analysis. *Sports Med*. 2011 Oct 1;41(10):801-14.

2. Zabala M, Peinado AB, Calderon FJ, Sampedro J, Castillo MJ, Benito PJ. Bicarbonate ingestion has no ergogenic effect on consecutive all out sprint tests in BMX elite cyclists. *Eur J Appl Physiol*. 2011 Dec;111(12):3127-34.

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PC126

Influence of eccentric exercise on vascular function

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Eccentric exercise (i.e. the lengthening of a muscle under tension) can induce muscle damage, inflammation and oxidative stress. Inflammation and oxidative stress are related to vascular dysfunction. It has previously been shown that 48 hours following bilateral eccentric leg press exercise there is an increase in central pulse wave velocity, indicative of an increase in large artery stiffness [1]. Given that the microvasculature is reportedly more susceptible than the large arteries to the deleterious effects of inflammation [2], we hypothesised that along with unfavourable changes in macrovascular function, eccentric exercise would elicit impairments in microvasculature function. To examine this, twelve healthy, non-weight trained males (age 22 ± 4 years; mean $\pm$ SE) were recruited, and participated in either: 1) an eccentric exercise group ($n=7$) or, 2) a no exercise time control group ($n=5$). Participants in the exercise condition performed 50 eccentric repetitions of bilateral leg press exercise at 110% of their concentric 1 repetition maximum. The eccentric exercise group were assessed pre-exercise (baseline), 24 and 48 hours post-exercise, and the control group were assessed on 3 separate time-matched days. At each experimental session cutaneous microvasculature responses to iontophoresis of acetylcholine (1%, endothelial-dependent vasodilator) and sodium nitroprusside (1%, endothelial-independent vasodilator) were assessed using laser Doppler flowmetry. Measures of large artery stiffness (augmentation index), heart rate (ECG), blood pressure (brachial artery), arm and leg blood flow (venous occlusion plethysmography) and leg muscle pain (McGill Pain Questionnaire) were also made. Statistical analyses were performed using one-way repeated-measures ANOVA. Post-exercise muscle damage was verified by elevated ratings of leg muscle pain at 24 and 48 hours following eccentric exercise. Arterial stiffness (augmentation index -16.3 ± 4.2 , -9.7 ± 4.8 , $-3.7 \pm 3.1\%$ at baseline, 24 hours and 48 hours respectively) was significantly increased 48 hours following eccentric exercise ($P < 0.05$ vs. baseline). Increases in cutaneous perfusion in response to iontophoresis of endothelial-dependent (843 ± 91 , 730 ± 100 , $733 \pm 182\%$ at baseline, 24 and 48 hours, respectively) and -independent (1176 ± 148 , 1066 ± 140 , $1219 \pm 251\%$ at baseline, 24 and 48 hours, respectively) vasodilators were not different following eccentric exercise ($P > 0.05$). Similarly, arm and leg blood flow, heart rate and blood pressure were similar before and after eccentric exercise ($P > 0.05$). In the time control group, arterial stiffness, cutaneous microvasculature responsiveness, leg and arm blood flow, heart rate and blood pressure were similar at all time points studied ($P > 0.05$). Our preliminary data suggest that bilateral eccentric leg press exercise elicits increases in large artery stiffness, while cutaneous microvascular function appears unaltered.

Barnes JN et al. (2010). *J Appl Physiol* 109(4), 1102-8.

Wagenmakers AJ et al. (2006) *Essays Biochem* 42, 193-210

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PC127

The influence of changes in plasma osmolality on the measurement of haemoglobin concentration and haematocrit in whole blood

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Measurements of haemoglobin concentration (Hb) and haematocrit (Hct) are often used to estimate changes in blood and plasma volume, most commonly employing the calculations described by Dill and Costill (1974). Since red cell volume is highly responsive to changes in extracellular osmolality, the measurement of Hct using automated analysers that dilute the sample in a medium of fixed osmolality may not be valid when repeat measures of Hct are made after interventions that change osmolality. This investigation quantified the effect of changes in plasma osmolality on the measurement of Hct by centrifugation and using an automated analyser. With ethics committee approval, eight healthy male volunteers visited the laboratory after an overnight fast. After 15 minutes of seated rest, a 20mL blood sample was collected from a superficial antecubital vein by venepuncture. Whole blood was treated with K_2EDTA (1.5mg/mL) before being aliquoted into collection tubes containing 100 μ L of saline of varying concentrations to alter the sample osmolality. Samples were thoroughly mixed and analysed for haemoglobin concentration using the cyanmethaemoglobin method, and haematocrit, by spun packed cell volume (PCV). Hb and Hct were also determined using an automated haematology analyser (Beckman-Coulter AC.T 5diff). Plasma osmolality was measured using freezing point depression. Changes in blood, plasma and red cell volumes were calculated as outlined by Dill and Costill (1974). The addition of saline to the samples resulted in a plasma osmolality range between 263 ± 4 and 335 ± 3 mosmol/kg. Plasma osmolality did not influence Hb measurements made using the cyanmethaemoglobin method ($P = 0.239$) or the haematology analyser ($P = 0.306$). While Hct values from the haematology analyser were not different ($P = 0.652$), PCV was progressively lower as the osmolality of the sample increased ($P < 0.001$). When using the PCV data, plasma osmolality did not influence calculated changes in blood volume ($P = 0.299$), but a significant increase in plasma volume was apparent when osmolality increased ($P < 0.001$). Regression analysis revealed that a 10 mosmol/kg change in plasma osmolality would result in an apparent 1% change in plasma volume. With the auto-analyser data, however, it appeared that no change in red cell ($P = 0.525$) or plasma volume ($P = 0.291$) occurred. Conditions or interventions which result in a marked change to plasma osmolality may therefore produce a discrepancy in Hct measured using an automated analyser. This may invalidate the use of these data to determine changes in plasma volume.

Dill DB & Costill DL (1974). *J Appl Physiol* 37, 247-248.

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PC128

Exergaming (Wii Fit Plus cycling) elicits similar cardiovascular and respiratory responses to its traditional counterpart (static cycling) in women

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A disproportionate decline in physical activity is evident when young women enter early adulthood (Kwan *et al* 2012). Traditional modes of exercise, such as cycling, can have unfavourable enjoyment ratings and are rated as 'hard work' by untrained adults (Graves *et al* 2012). Interactive video games (exergames) have the potential to meet current guidelines for minimal daily activity in a novel way. Our aim was to compare the cardiovascular and respiratory responses to exergame cycling with its traditional counterpart in young women. This study is unique in focusing on the responses to traditional exercise and exergaming in the female population.

Seven female subjects (aged 20-22 years) completed three exercise sessions. Work capacity and maximum oxygen consumption (VO_2max) were first determined by an incremental test on the static bike. Subsequent sessions comprised 30 mins cycling on the static bike or Wii Fit Plus game (3 x 10 min bouts, with 2 min rest periods). Heart rate (HR), oxygen consumption (VO_2) and minute ventilation (V_E) were measured at rest and during the two exercise modes. Subjects also scored their enjoyment of the exercise and rated their perceived exertion. Both modes of cycling qualified as moderate-intensity exercise, since HR increased to more than 64% of HRmax during the sessions. The increase in heart rate from rest was significantly greater during traditional cycling (TC) compared to exergame cycling (EC), to $152 \pm 8\text{bpm}$ and $128 \pm 8\text{bpm}$ respectively (mean $\pm$ SE; $n=7$; $p<0.005$ by ANOVA and post-hoc Bonferroni tests). However, both VO_2 and V_E showed similar increases from rest. VO_2 increased by $298 \pm 83\%$ (mean $\pm$ SE; $n=7$) during the TC session and $211 \pm 72\%$ (mean $\pm$ SE; $n=7$) during EC; V_E increased by $220 \pm 53\%$ (mean $\pm$ SE) during TC and $164 \pm 54\%$ (mean $\pm$ SE; $n=7$) during EC. These increases were not significantly different between the two exercise modalities ($p>0.05$). The menstrual cycle had no significant effect on exercise performance in this study. HR, V_E and VO_2 under all conditions were similar regardless of the menstrual cycle phase, as were VO_2max values ($p>0.05$). Although both exercises generated a similar profile of physiological responses, subjects perceived the exergaming to require significantly less work than traditional cycling ($p<0.05$; paired t-test) and the level of enjoyment was also greater ($p<0.05$; paired t-test).

Our data show that a 30 minute Wii Fit Plus cycling session represents a moderate intensity exercise, similar to a traditional bike ride. However, young women may be more motivated to engage in this form of exercise as it is perceived to be more enjoyable and less effort. Our results favour the use of this exergame to maintain physical activity in young women. Graves, L.E.F. *et al* (2010) *Journal of Physical Activity and Health* 7; 393-401

Kwan M.Y *et al* (2012). *American Journal of Preventive Medicine*. 42(1), 14-20.

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PC129

Trace metal ranges in elite international athletes

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Many authors have suggested the importance of trace elements such as Zinc (Zn), Copper (Cu) and Selenium (Se); as well as Magnesium (Mg) in the function of different physiological systems for physical performance in sport including energy metabolism; nervous, muscle, function, and immune function; blood and bone health; and fluid electrolyte balance. These metals should be maintained within normal ranges as either deficiency or toxicity, have adverse effects on both general health and athletic performance (1-3). Suggested normal ranges for these elements are for Cu: 11.0-25.0 $\mu\text{mol/L}$, Zn: 10.7-24.5 $\mu\text{mol/L}$, Se: 0.89-1.65 $\mu\text{mol/L}$ (4) and Mg: 0.66-1.00 mmol/L (5). There are very few articles in the literature that examine trace metal and Mg status of elite international athletes. Thus, the aim of this study is to evaluate the ranges of serum trace elements and Mg in elite international athletes from different sports. 60 male (27.4 ± 3.6 yrs) and 29 female (25.5 ± 4.0 yrs) elite international athletes (Football, Athletics, Canoeing, Sailing, Modern Pentathlon), the cohort which included a number of World and Olympic champions, from whom were drawn an average of 3 blood tests over the course of two seasons of International competition (2010-2011). All athletes were following their own nutritional programs as advised by the teams. Se, Cu, Zn and Mg were measured in plasma. Results were expressed as mean $\pm$ SD and as 95% CI. Differences between male and female were compared by an equal or unequal variance Student's *t*-test for unpaired data after a *F*-test. Statistical significance was set at $P<0.05$. Plasma Cu, Se, Zn and Mg levels can be observed in the table placed below. It is interesting to note that the 95% CI for each element measured was narrower than the reference ranges suggested in literature for healthy adult populations, with the exception of plasma Zn level for female athletes, amongst whom a higher range was observed. Furthermore, there were no differences in Se and Zn between genders, although Mg was significant higher for male and Cu for female. The average serum level of trace elements and Mg is within the normal range of normality although the 95% CI for the elite international athletes of this study is narrower than the reference ranges suggested in the literature for healthy adult population. Further research into the trace metal status of elite athletes from a range of modalities is warranted and that sport specific reference ranges may prove useful when assessing the specific needs for individual athletes.

Element	Reference Range	Male			Female		
		n	mean $\pm$ SD	95% CI	n	mean $\pm$ SD	95% CI
Mg (mmol/L)	(0.66-1.00)	272	0.89 $\pm$ 0.09	0.85-0.93	222	0.85 $\pm$ 0.08	0.84-0.86
Cu (mmol/L)	(11.8-15.0)	128	13.1 $\pm$ 2.2	12.6-13.7	88	14.4 $\pm$ 1.2	13.6-15.2
Se (mmol/L)	(0.89-1.65)	114	1.40 $\pm$ 0.16	1.30-1.52	76	1.52 $\pm$ 0.24	1.34-1.69
Zn (mmol/L)	(10.7-24.5)	56	15.4 $\pm$ 30.9	17.2-31.6	58	21.8 $\pm$ 14.3	20.6-26.7

* $P<0.05$, Significant differences between male and female.

González-Haro C *et al.* (2010) *J Trace Elem Med Biol* 25,S54-S58.

Lanham SA *et al.* (2011) Wiley-Blackwell, London (UK).

Soria M *et al.* (2011) *Magnes Res* 24,36-44.

Taylor AW (2006) Department of Clinical Biochemistry, Guildford (UK).

Immanuel S & Iriani A (2006) *Med J Indones* 15,229-235

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PC130

Circulating parathyroid hormone concentrations are reduced following acute, treadmill running

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Transient increases in circulating parathyroid hormone (PTH) concentrations produce anabolic effects on bone. It has been suggested that the most favourable effects of PTH on the skeleton with exercise would be achieved with low basal levels and large increases during exercise itself (1). PTH concentrations are transiently increased with acute, endurance exercise (2–4) and several recent studies suggest they might also be decreased in the first hours post-exercise (2,3), but this has not been confirmed with a non-exercising control group. Ten, physically-active men (mean±1SD age: 26±5 y; height: 1.79±0.05 m; body mass: 78.3±5.8 kg; $\text{VO}_{2\text{max}}$: 57.3±6.9 ml/kg/min) arrived at the laboratory after an overnight fast, having refrained from exercise and followed a controlled diet for at least 18 h. A standardised breakfast was provided at 0815 h after which they completed either a 60 min bout of treadmill running at 65% $\text{VO}_{2\text{max}}$ starting at 1030 h (EX) or remained semi-recumbent (CON). Blood samples were obtained immediately before (1015 h) and after (1130 h) exercise, and at 1230 h, 1330 h and 1415 h. Samples were analysed for PTH, albumin-adjusted calcium (ACa), and phosphate (PO_4), corrected for changes in plasma volume (5). Data were analysed using linear mixed model (LMM) ANOVA, with *post hoc* analysis using Dunnett's (within each condition, with 1015 h as 'Control') and Student-Newman-Keuls (between conditions) tests. LLM ANOVA revealed significant *Condition* × *Time* interactions for PTH ($p<0.01$), ACa ($p<0.05$) and PO_4 ($p<0.001$). PTH concentrations were increased (+64%, $p<0.05$) with running and higher than in CON immediately at the end of exercise (4.3 ± 1.8 vs 2.6 ± 0.8 pmol/L, $p<0.05$). Compared with pre-exercise, from 1230 h to 1415 h, PTH was 3–10% lower and 8–18% higher in EX and CON, resulting in lower concentrations in EX compared with CON at 1330 h (2.3 ± 0.6 vs 2.9 ± 0.8 pmol/L, $p<0.01$) and 1415 h (2.5 ± 0.8 vs 2.9 ± 0.6 pmol/L, $p<0.05$). ACa was lower in EX than in CON immediately after exercise (2.22 ± 0.08 vs 2.35 ± 0.16 mmol/L, $p<0.05$) and tended to be higher at 1230 h, but this did not reach the assigned level of significance (2.44 ± 0.10 vs 2.34 ± 0.15 mmol/L, $p=0.058$). PO_4 was higher than in CON immediately after exercise (1.39 ± 0.08 vs 1.03 ± 0.16 pmol/L, $p<0.001$) but not different thereafter. PTH concentrations are significantly increased during, and reduced following, acute endurance running. The post-exercise decrease accentuates the transient nature of the prior increase in PTH, possibly promoting a stronger osteogenic effect. The decrease is not fully explained by increased ACa, or decreased PO_4 , concentrations.

Brahm H, Piehl-Aulin K, Ljunghall S (1997). *Calcif Tissue Int* **61**, 192–8.

Scott JP, Sale C, Greeves JP, Casey A, Dutton J, Fraser WD (2011). *J Appl Physiol* **110**, 423–32.

Scott JP, Sale C, Greeves JP, Casey A, Dutton J, Fraser WD (2010). *J Clin Endocrinol Metab* **95**, 3918–25.

Guillemand J, Accarie C, Peres G, Guillemand S (2004). *Calcif Tissue Int* **74**, 407–14.

Dill DB, Costill DL (1974). *J Appl Physiol* **37**, 247–8.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC131

Efficacy of an 8-week exercise programme on short- and long-term cardiovascular fitness in newly-diagnosed Transient Ischaemic Attack patients

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Transient ischaemic attack (TIA) patients are often at high risk for a recurrent stroke or cardiovascular event due to an elevated cardiovascular disease risk (CVD) profile.¹ Physical inactivity is one such risk factor that contributes to the increased risk of a recurrent vascular event. Preliminary evidence suggests that TIA patients in the non-acute phase can improve cardiovascular fitness and reduce vascular risk following exercise training.^{1,2} The purpose of the present study was to examine the efficacy of a short-term exercise programme on physical fitness in TIA patients.

Sixty newly diagnosed TIA patients were randomised into one of two conditions: i) Health-Enhancing Physical Activity Programme (HEPAP; $n = 30$) or ii) Control group ($n = 30$). All participants underwent baseline assessment (BL) comprising standard measures of CVD risk and a graded-exercise test (GXT) using a modified Bruce treadmill protocol. Heart rate (HR), blood pressure (systolic: SBP) and the ratings of perceived exertion (RPE; Borg 6–20 scale³) were recorded at the end of each GXT stage. The HEPAP group then underwent an 8-week exercise programme, whereas, the Control group remained as 'usual care', before all participants completed a post-intervention assessment (PI) comprising the aforementioned measures, which was repeated 3-months subsequently (3PI). Data presented is based upon the first two GXT stages from BL, PI and 3PI.

ANOVA revealed a greater change in HR between BL and PI for HEPAP (105 ± 19 cf. 91 ± 16 b·min⁻¹, respectively) than Control (107 ± 19 cf. 102 ± 16 b·min⁻¹, respectively; $P<0.05$). A greater decrease in SBP was also observed between BL and PI for HEPAP (167 ± 18 cf. 150 ± 16 mmHg, respectively) than Control (159 ± 19 cf. 156 ± 17 mmHg, respectively; $P<0.05$). An RPE interaction signified that the rate of change in RPE between stages 1 and 2 of the GXT was significantly lower for HEPAP (8.5 ± 1.8 & 10.5 ± 1.6 , respectively) than for Control (9.0 ± 1.7 & 11.6 ± 1.8 , respectively; $P<0.05$). Despite similarities in BL exercise duration for HEPAP and Control (7.0 ± 2.9 cf. 6.6 ± 2.7

min, respectively), PI exercise duration was significantly increased for HEPAP (10.5±3.0 cf. 7.5±3.1 min, respectively; $P<0.05$). ANOVA revealed no changes in HR, SBP, RPE or exercise duration between PI and 3PI ($P>0.05$).

TIA patients exercised longer, for a lower physiological and psychological cost following a HEPAP than those who did not perform structured physical activity. Of particular importance, systolic blood pressure (a key cerebrovascular risk factor) decreased to a greater extent for HEPAP than for Control. As HEPAP participants maintained their physical fitness level 3-months following the completion of the exercise intervention, physical activity programmes should be encouraged as a rehabilitative strategy for newly-diagnosed TIA patients.

Prior PL, Hachinski V, Unsworth K, Chan R, Mytka S, O'Callaghan C, et al. Comprehensive cardiac rehabilitation for secondary prevention after transient ischemic attack or mild stroke: Feasibility and risk factors. *Stroke*. 2011;42:3207-3213.

Lennon O, Carey A, Gaffney N, Stephenson J. A pilot randomized controlled trial to evaluate the benefit of the cardiac rehabilitation paradigm for the non-acute ischaemic stroke population. *Clinical Rehabilitation*. 2008;22:125-133.

Borg G. Borg's Perceived exertion and pain scales. Champaign, IL: Human Kinetics; 1998.

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PC132

Effects of sodium bicarbonate ingestion on Heart Rate Variability before and after a simulated BMX cycling competition

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Effects of sodium bicarbonate (NaHCO_3) ingestion on improving performance or recovery are not clear. Several studies have demonstrated both ergogenic and non ergogenic effects of NaHCO_3 ingestion on high-intensity exercise performance, or improvements in perceptions of effort and recovery (1). Despite the use of Heart Rate Variability (HRV) as a marker of recovery or even precompetitive anxiety (2), there are not studies focused on the possible effects of NaHCO_3 on HRV during BMX races. The aim of the study was to examine the possible effects of NaHCO_3 on HRV indexes among a field test simulating a BMX race. Ten elite BMX riders (19.2±3.4 years, 72.4±8.4 kg, 174.2±5.4 cm) participated in this study consisting in two trials performed in separated days. Each trial included three consecutive BMX heats in an Olympic track separated by 15 min recovery. Ninety minutes prior to exercise subjects ingested either NaHCO_3 (0.3 g.kg⁻¹ body weight) or placebo. The two treatments conditions were administered in a counterbalanced, crossover, randomly assigned and double-blind manner. Heart Rate Variability was measured each day at rest using a Polar RS800 monitor during 15 min, just before ingesting the substance (PRE) and also after finishing the third heat (POS). Polar Pro Trainer 5 software (Polar Electro, Kempele, Finland) and each downloaded R-R interval file was then further analyzed by means of Kubios HRV Analysis Software 2.0 (The Biomedical Signal and Medical Imaging Analysis

Group, University of Kuopio, Finland). Two way ANOVA with repeated measures were used to determine differences between treatments at each exercise moment. The descriptive results in PRE for placebo vs. NaHCO_3 conditions respectively were (mean±SD): MeanRR 882±122 vs. 830±123; SDNN 70±27 vs. 57±24; rMSSD 65±31 vs. 50±31; LnLF 7.7±0.7 vs. 7.2±0.6; LnHF 7.2±1 vs. 6.7±1.2; LF/HF 3.2±5.7 vs. 2.7±2.7; SD1 46.6±22.5 vs. 36.3±22.6; SampEn 1.4±0.2 vs. 1.4±0.3; DFA1 1.1±0.2 vs. 1.2±0.3. In POS, the results for placebo vs. NaHCO_3 conditions respectively were (mean±SD): MeanRR 524±43 vs. 539±38; SDNN 6.4±2.4 vs. 8.8±2.0; rMSSD 4.1±1.9 vs. 4.0±1.1; LnLF 2.8±1.2 vs. 3.0±1.0; LnHF 0.9±0.8 vs. 1.2±1.0; LF/HF 8.3±4.1 vs. 10.9±9.5; SD1 2.9±1.4 vs. 2.9±0.8; SampEn 0.8±0.2 vs. 0.8±0.3; DFA1 1.4±0.3 vs. 1.5±0.1. Despite in POS no significant differences were found between both conditions ($p>0.05$), after the NaHCO_3 ingestion before the tests started (PRE) significant differences were found for some important HRV indexes as MeanRR ($p=0.043$), SDNN ($p=0.005$), rMSSD ($p=0.027$) and SD1 ($p=0.027$), all of them in favor of the placebo condition (higher HRV). So, besides any HRV indexes were not improved in NaHCO_3 condition, some important indexes got worse may be because of subjects' feeling and effects related to NaHCO_3 ingestion.

Zabala M, Peinado AB, Calderon FJ, Sampedro J, Castillo MJ, Benito PJ. Bicarbonate ingestion has no ergogenic effect on consecutive all out sprint tests in BMX elite cyclists. *Eur J Appl Physiol*. 2011 Dec;111(12):3127-34.

Mateo-March M, Blasco-Lafarga C, Martínez-Navarro I, Guzmán JF, Zabala M. Heart Rate Variability and pre-competitive anxiety in BMX discipline. *Eur J Appl Physiol*. 2012 Jan;112(1):113-23.

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PC133

Coronary risk, lipid and lipoprotein profile of staff members of Jimma University, Jimma, Ethiopia

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The incidence of Atherosclerosis has been increasing lately in developing countries where the health system heavily burdened by the conventional infectious diseases (Uma and Ishwarlal, 2006). Extensive clinical and statistical studies have identified several factors that increase the risk of coronary heart disease and heart attack, of which dyslipoproteinemia, tobacco smoking, having high blood pressure and obesity are the most important and yet modifiable (Loscalzo, 2005). The WHO Step wise approach to Surveillance (STEPS) instrument was employed among three hundred and seventeen staff members of Jimma University. Data was collected using structured questionnaire, physical examination and Laboratory tests. Written Informed consent was obtained for blood sampling and voluntary participation in the study prior to data collection. Data was analysed by using SPSS version 16 and risk was appraised by using Framingham risk scoring method. Prevalence of current cigarette smoking status was 15(6.9%), binge drinking of alcohol 23(12.63 %), 3(8.57%) for male and female, respectively, physical inactivity 13.82% and personal history of hypertension 9.2%. The mean (sd) SBP (mmHg) was 118.3(11.15) in males and 113.83 (12.59) in females. Systolic BP shows a significant correlation with level of LDL and TC both

in males ($p < 0.01$) and females ($p < 0.05$). BMI was found as the most significant determinants of mean SBP and DBP. Obesity was 10 (4.6%) and 5 (14.28) among male and females, respectively. Majority of the study subject (70.5%) has HDL level of above 40mg/dl. Bivariate correlation between HDL and BMI shows a negative correlation coefficient ($r = -0.164$, $p < 0.05$). The Mean (sd) LDL level was 98.94(2.29) for males and 94.95(3.35) among females. Bivariate correlation between LDL and BMI, Income, Blood glucose and age shows a positive correlation ($P < 0.05$) while recreational hours involving exercise shows a negative correlation coefficient ($r = -0.234$, $p < 0.05$). The mean (sd) of TCL level was 161.35(39.69) for males and 149.45(45.44) among females. Hypercholesterolemia was found to be 15 (6.2%). Bivariate correlation between TCL and BMI, income, blood glucose, age and heavy recreational hours shows a positive correlation ($P < 0.05$) while heavy recreational hours shows a negative correlation coefficient with level of LDL ($r = -0.213$, $p < 0.05$). Among the subjects 16.2% and 8.29% has borderline high to high TG level for male and female, respectively. The Framingham total coronary risk score ranged from -3 to 11, with a mean (sd) score of 2.47 (1.49). The overall study showed the need for effective strategies to promote primary and secondary levels of prevention of coronary atherosclerosis directed towards the identified risk factors and also demands further investigation at a larger scale across the country.

Uma S. and Ishwarlal J. Oxidative stress and atherosclerosis. Pathophysiology Elsevier Ireland Ltd. 2006; 13: 129–142

Loscalzo J. Molecular mechanisms of atherosclerosis, Taylor & Francis Group .2005, (1)1-2

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PC134

Preproglucagon neurons innervate spinal autonomic neurons

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Within the brain, the satiety peptide GLP-1 is produced by preproglucagon (PPG) neurons, located primarily in the nucleus tractus solitarius. PPG axons are widely distributed throughout brain, mainly to autonomic control areas^{1,2}. The PPG innervation pattern corresponds well with regional GLP-1 receptor expression³. GLP-1 receptor mRNA is also found throughout the rat spinal cord³; but to date, nothing is known about the spinal distribution of PPG axons. Here, we analysed the distribution of PPG promoter-driven YFP expression in coronal and longitudinal sections of seven mouse spinal cords between thoracic segment 1 (T1) and sacral segment 3 (S3). We also explored whether PPG axons form close appositions with cholinergic neurons; including preganglionic autonomic neurons and somatic motor neurons. YFP and choline acetyltransferase (ChAT) immunoreactivity was visualised with two colour immunoperoxidase labelling using black (YFP) and

brown (ChAT) reaction products. Many YFP-immunoreactive (IR) non-varicose axons travelled rostrocaudally in white matter tracts. The greatest density of these axons occurred in the ventral white commissure and around the ventral median fissure. They were also distributed throughout the lateral and ventral funiculi in rostral thoracic segments. Their density declined from rostral to caudal segments. In spinal segments T1-L2, where sympathetic preganglionic neurons are located, many varicose, YFP-IR axons travelled between the intermediolateral cell column and the intercalated nucleus. Many of these axons closely apposed ChAT-IR cell bodies and proximal dendrites. There were also many varicose mediolaterally-oriented axons containing YFP-IR in lamina X; these formed close appositions on ChAT-positive lamina X perikarya. In segments S1 and S2, rare YFP-IR terminals closely apposed ChAT-IR somata in the parasympathetic nucleus, which contains the parasympathetic preganglionic neurons.

Varicose YFP-IR axons projected ventrolaterally through lamina VII into laminae VIII and IX; areas containing limb motor neurons. Here, close appositions between YFP-IR axons and ChAT-IR motor neurons were infrequent. A few dorsolaterally-oriented axons occurred in lamina V. No YFP-IR fibres occurred more dorsally. In addition to YFP-positive axons, a small numbers of YFP-IR cell bodies were present in lower lumbar and upper sacral segments, mostly within laminae V and VI. These results demonstrate that PPG neurons innervate cholinergic neurons in both autonomic and somatic motor regions of the spinal cord, with sympathetic areas receiving the densest innervation. Like in other parts of the CNS, there is a good correlation between the distribution of spinal GLP-1 receptors and the distribution of spinal PPG axons. These observations indicate that PPG neurones could directly modulate sympathetic outflow through their inputs to sympathetic preganglionic neurons.

Llewellyn-Smith, I.J., Reimann, F., Gribble, F.M. & Trapp, S. Preproglucagon neurons project widely to autonomic control areas in the mouse brain. Neuroscience 180, 111-121 (2011).

Larsen, P.J., Tang-Christensen, M., Holst, J.J. & Orskov, C. Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. Neuroscience 77, 257-270 (1997).

Merchenthaler, I., Lane, M. & Shughrue, P. Distribution of pre-proglucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. J Comp Neurol 403, 261-280 (1999).

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PC135

The role of nuclear factor E2-related factor 2 in diabetes development in mice

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Aims: Increased oxidative stress has been proposed as an initiating factor in the development of type 2 diabetes. The transcription factor nuclear factor E2-related factor 2 (Nrf2) is a master regulator of cellular oxidative stress. Thus the aim of

the study was to investigate the effect of removal of Nrf2 on the phenotype of mice fed a high fat diet.

Methods: Age matched C57BL/6 wild type (WT) and Nrf2 KO mice¹ (n = 10-11) were fed regular chow (RC, 4% fat) or high fat diet (HFD, 45% fat) for 20 weeks. During the study body weight, food intake, glucose tolerance and insulin sensitivity were measured to monitor changes in metabolic phenotype. Intraperitoneal glucose (2 mg/kg) and insulin (0.75 U/kg) tolerance tests were performed following an overnight or 4 hour fast respectively. Blood glucose measurements were monitored, from blood taken from the tail vein (~5 l), at regular intervals for 2 hours post administration. All data are expressed as mean ± standard error of mean, and statistical significance determined by one-sample or Student's t-test.

Results: Nrf2 KO mice on the RC diet were phenotypically comparable to WT mice on the same diet. After 20 weeks on HFD, Nrf2 KO mice exhibited protection against diet-induced obesity along with improved glucose tolerance, as measured by glucose tolerance test, with a significant reduction in the area under the curve compared to HFD WT littermates (WT 2008 ± 138.6, KO 1491 ± 133.1; p < 0.05). Peripheral insulin sensitivity of Nrf2 KO mice was also significantly greater than WT controls following HFD (p < 0.05).

Conclusions: Removal of Nrf2 significantly improves glucose homeostasis and obesity in mice. These data suggest that global loss of Nrf2 precludes development of a HFD-induced diabetic phenotype. As Nrf2 is a therapeutic target for cancer prevention, Nrf2 inhibitors may also be useful for anti-diabetic therapy.

Itoh K et al. (1997). *Biochem Biophys Res Commun.* 236(2):313-22.

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PC136

Effect of hypoxic signalling on acid-extrusion in cancer cell-lines

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Acid-extrusion by membrane-bound transporter-proteins is an essential pH-regulatory mechanism that protects cells from excessive acidification. This is particularly important in cancer cells, which produce large quantities of acid by virtue of their high metabolic rate. Signaling cascades can regulate acid-extruding transporters through gene expression, post-translational modifications or changes in the chemical milieu, which then affect transport rate. Hypoxia is a major trigger of adaptive responses in cancer and plays an important role in disease progression [1]. However, the effect of hypoxic signaling on acid-extrusion remains unclear. Acid-extrusion was measured in eight cancer-derived cell-lines (HCT116, RT112, MDA-MB-468, MCF10A, HT29, HT1080, MiaPaca2, HeLa) using the pH-sensitive dye, cSNARF-1 [2]. Hypoxic signaling was triggered by pre-incubation in low (1-2%) O₂ or with the 2-oxoglutarate-dependent dioxygenase inhibitor dimethyl-oxalylglycine (DMOG). By means of selective pharmacological inhibition (30 μM dimethylamiloride to block NHE1 activity) or transport-substrate removal (Na⁺, HCO₃⁻), acid-extrusion flux was dis-

sected into components due mainly to Na⁺/H⁺ exchange (NHE) and HCO₃⁻ transport. In HCT116 cells, hypoxia-triggered signaling reduced NHE-flux reversibly (by 51±8% with a time-constant of 1.8 hrs for 1% O₂; by 64±4% with a time-constant of 1.1 hrs for 1mM DMOG). This was associated with a change in NHE1 protein phosphorylation (consensus sequence substrates for protein kinases A and C) but not NHE1 expression or internalization (studied with monoclonal NHE1 antibody) or ATP levels (luciferase assay). NHE-flux remained reduced (by ~53%) following a 48-hour activation of hypoxic pathways (2% O₂ or 1mM DMOG), but the effect was associated with reduced expression of the mature form of NHE1 and was slowly reversible. These hypoxic responses of acid-extrusion in HCT116 cells were also observed in RT112 and MCF10A cells. Acid-extrusion by NHE was not hypoxia-sensitive in MDA-MB-468 cells, whereas in the remaining cell-lines, normoxic NHE activity was too low to resolve for hypoxic-response. HCO₃⁻ transport, in contrast, was not significantly affected by low O₂ or DMOG pre-incubation in any of the cell-lines studied. Thus, HCO₃⁻ transport emerges as a constitutive and stable element of pH regulation, whereas NHE flux can vary widely in magnitude between different cell-lines and their hypoxic status. Heterogeneity of O₂ tension in tumors can produce complex and important effects on the ability of cells to regulate pH. Different degrees of hypoxia-sensitivity may underlie adaptive responses in cancer, and be of significance to therapeutic approaches targeting pH-regulating proteins.

Semenza G (2003) *Nat Rev Cancer* 3(10): 721-32.

Hulikova A et al. (2011) *J Biol Chem* 286(16):13815-26.

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PC137

Oxidant/antioxidant balance and the ubiquitin-proteasome system during hypothermic perfusion in the isolated rat liver (IPRL)

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Hypothermia appears to be useful in the prevention of tissue damage. Recent reports⁽²⁾ suggest that the use of hypothermic perfusion media (20°C) can improve the preservation of liver grafts. However, hypothermia can also induce reactive oxygen and nitrogen species (RONS)⁽¹⁾. The initial protection against damage from oxidative stress may require 1) activation of the cellular antioxidant system, and 2) rapid removal of oxidized proteins by the ubiquitin-proteasome system (UPS), among other mechanisms.

Objective To study the cellular mechanisms induced by hypothermic perfusion in isolated and perfused rat liver (IPRL), focusing on the production of oxidative stress, antioxidant activity, and the UPS system.

The procedure was approved by the University of Barcelona's Committee for Animal Care and followed European Community guidelines. Sprague-Dawley male rats (n=6 per group) were anesthetized with I.P. sodium pentobarbital (60 mg/Kg of b.w.) and their livers were removed for perfusion in a non-

recirculating system at a flow rate of 3 mL/ min/g liver with Krebs-Henseleit buffer. Rats were killed by anesthesia overdose. Normothermic livers (NT) were perfused at 37 °C (30 min). Hypothermic livers were perfused at 37° (15 min) and were then switched to cold medium at 26 °C (HT26) or 22 °C (HT22) (15 min). In liver homogenates we determined nitric oxide (NOx), lipid peroxidation (TBARS), modified proteins (4-HNE adducts) and nitrated proteins (nitrotyrosine). As antioxidants, we determined glutathione (GSH) and the activity of the enzymes GSH-peroxidase and GSH-reductase. Expression of the ubiquitin-protein conjugates and the levels of proteasome 26S subunits were also analysed. Data were expressed as means $\pm$ standard error and were analysed by two-way ANOVA.

Oxidative stress is induced in hypothermic perfusion, as demonstrated by increased levels of nitric oxide (from 1.48 ± 0.14 nmol/mg protein in NT to 2.27 ± 0.17 in HT26), TBARS (from 0.36 ± 0.01 nmol/mg prot in NT to 0.48 ± 0.05 in HT 26) and 4-HNE adducts (a 34% increase). Nitrotyrosine levels were not significantly different. In the HT22 group we found increased levels of GSH and higher activity of the GSH-reductase, which may explain the better turnover in the GSH/GSSG system. As regards the UPS system, poly-ubiquitinated proteins were higher in the HT groups; there were no differences in the expression of the 26S proteasome subunits.

Conclusion In the IPRL system, hypothermic perfusion produces oxidative stress, induces the activation of the glutathione antioxidant system and increases ubiquitin-conjugated proteins. A fuller understanding of the cellular mechanisms activated by hypothermic perfusion will help to explore its potential use as a protective strategy for preserving organs.

Alva et al. (2010) Resuscitation 81:609-16

Ferrigno, A et al. Cryobiology 62 (2011) 152–158.

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PC138

Progesterone increases the mobility of non capacitated boar sperm

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The steroid hormone progesterone is released from cumulus cells surrounding the oocyte and is known to influence the physiology of sperm as it moves towards the uterine tubules. Progesterone induces hyperactivation, the acrosome reaction and chemotaxis in capacitated human sperm. Micromolar, but not nanomolar, concentrations of progesterone induce the acrosome reaction in capacitated boar sperm but non capacitated cells are unresponsive (Barboni et al., 1995; Jang & Yi, 2002). However we are unaware of any reports of the effects of progesterone on the mobility of non capacitated and capacitated boar sperm. In this study we sought to address this by using a mobility assay that monitors sperm penetration through an inert cell-separation solution (Vizcarra & Ford, 2006).

Semen samples, diluted in Trixcell extender solution, were obtained commercially. Samples are from PIC 337 champion species of boar. The mobility assay involved using spec-

trophotometry (550nm) to measure the rate at which sperm penetrated through an inert cell-separation solution alone or containing increasing concentrations of progesterone (0.1 – 100nM). The cell-separation solution was placed in the cuvette and 1×10^8 non capacitated sperm cells were carefully loaded onto the surface of the solution and then incubated at 37°C. Absorbance readings were taken over time to monitor the progression of sperm through the solution. Heat inactivated sperm were used in the negative control and did not penetrate the solution. The positive control involved mixing 1×10^8 sperm cells with the cell separation solution before loading into the cuvette.

Statistical significance was determined using general linear mixed effect test (IBM SPSS statistics) and data are presented mean $\pm$ SEM. Curves are fitted using non-linear regression model and Km values (minutes) represent the time at which absorbance was half maximal (Graphpad Prism).

Cells maximally penetrate the solution over 50 minutes. 100nM progesterone significantly ($P < 0.001$) increased the mobility of sperm cells causing a doubling in the rate at which the cells penetrated through the cell-separation solution (control Km = 19 ± 3 ; +100nM progesterone Km = 9 ± 1 , N = 30 assays in each case using cells from at least 3 different boars). Parallel experiments were conducted to assess the acrosome status before and after progesterone treatment. After 20 minutes there was a less than 1% increase in cells demonstrating signs of either acrosome damage or reaction. By 50 minutes this had increased to 10%.

These data demonstrate for the first time that progesterone can enhance mobility of non capacitated boar sperm. Our immediate aim is to examine if capacitation increases the sensitivity of boar sperm cells to progesterone.

Barboni, B., Mattioli, M. & Seren, E. (1995). Influence of progesterone on boar sperm capacitation. J. Endocrinol., 144, 13-18.

Jang & Yi. (2002). RU486 suppresses progesterone-induced acrosome reaction in boar spermatozoa. J. Biochem. Mol. Biol., 35 (6) 604-608.

Vizcarra, J.A. & Ford, J.J. (2006). Validation of the sperm mobility assay in boars and stallions. Theriogenology. 66(5), 1091-97.

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PC139

Effects of the subnormothermic preconditioning against ischemia/reperfusion in isolated perfused rat liver (IPRL): assessment of the perfusate

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The effects of hypothermia depend of the cooling technique, level of hypothermia and tissue status (1). A new alternative approach to preserving isolated organs is hypothermia preconditioning, in which hypothermia perfusion is applied before ischaemia/reperfusion, improving tissue metabolic status and decreasing oxidative stress damage in isolated heart (2). Here, we determined the effects of a model of hypothermic preconditioning in liver when applied before ischemic/reperfusion by means of tissue damage indicators and oxidative stress parameters released in perfusate.

Rats (male, 200-250g) were anesthetized with sodium pentobarbital (60 mg.g-1 i.p) and they underwent laparotomy. Liver

were perfused with Krebs Henseleit buffer equilibrated with 95% O₂/5% CO₂ and placed in an IPRL system. Livers were randomly divided in two experimental groups: preconditioning + ischemia/reperfusion (PC+IR), and ischemia/reperfusion alone (IR), both n=5. The temperature of the medium varied according to group: 10 min of hypothermic perfusion (22 °C) + 10 min of rewarming (37 °C) in PC+ IR and, 20 min of normothermic perfusion (37 °C) in IR. All livers then underwent 40 min of ischemia and 20 min of reperfusion. The levels of oxidants (TBARS, NOx), antioxidants (thiol groups) and tissue-damage indicators (ALT, proteins) were measured at baseline (Basal), just before ischaemia induction (Pre-I), after ischaemia and 10 minutes of reperfusion, (Post-I 10) and after ischaemia and 20 minutes of reperfusion (post-I 20).

The parameters evaluated were mostly similar in the pre-ischemic samples with the exception of thiol groups. At the end of reperfusion tissue damage indicators (ALT and proteins) were significantly higher in IR than in PC+IR livers. TBARS and NOx also increased while antioxidants fell in perfusate (Table 1).

Hypothermic preconditioning improved antioxidant content and attenuated the hepatic oxidant damage induced by ischemia/reperfusion.

Oxidative stress and damage indicators released into the perfusate at different time intervals.

	Group	Basal	pre-Ischemia	post-Ischemia 10	post-Ischemia 20
TBARS (nmol/g.min)	PC+IR	38 ± 5	32 ± 1	44 ± 1	33 ± 2***
	IR	36 ± 4	35 ± 1	45 ± 4	64 ± 5
NOx (nmol/g.min)	PC+IR	2.1 ± 0.1	2.3 ± 0.3	2.3 ± 0.2	1.8 ± 0.2***
	IR	2.4 ± 0.1	1.8 ± 0.1	2.3 ± 0.2	2.5 ± 0.1
Thiol groups (nmol/g.min)	PC+IR	89 ± 7	64 ± 6*	87 ± 8**	88 ± 8*
	IR	93 ± 12	44 ± 5	55 ± 3	66 ± 7
Proteins (μg/ml)	PC+IR	51 ± 6	41 ± 5	45 ± 3***	39 ± 3***
	IR	57 ± 6	42 ± 5	116 ± 3	108 ± 3
ALT (U/L)	PC+IR	7.3 ± 0.2	7.3 ± 0.5	7.3 ± 0.2	6.4 ± 0.3**
	IR	6.3 ± 0.6	5.3 ± 0.7	7.9 ± 0.5	8.7 ± 0.4

Values are mean ± standard error. * P>0.05. ** P>0.01 and ***P>0.001 versus IR

TBARS, Thiobarbituric acid reactive substances; NOx, nitrate plus nitrite content; ALT, Alanine aminotransferase

Carbonell T. et al. (2012) Chapter 2802, in Hypothermia: Prevention, Recognition and Treatment. Nova Publisher, USA.

Khalilulin I. et al. (2007) J Physiol-London; 581: 1147-1161

This work was supported by research grant PI081389

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC140

Migration of the transplanted bone marrow-derived cells into periodontal ligaments due to orthodontic mechanical stress

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Bone marrow-derived cells (BMC) have abilities to migrate and differentiate into teeth and also periodontal ligament fibroblast cells. We examined the effect of orthodontic mechanical stress on the transplanted BMC migration into periodontal tissues using green fluorescence protein (GFP) transgenic

mice. BMC from GFP transgenic mice were transplanted into 8 week-old female C57BL/6 immunocompromised recipient mice (n=10), which had undergone 10 Gy of lethal whole-body irradiation. After successful transplantation, 5 mice under inhalation anesthesia using isoflurane (Isoflu: Dainippon Sumitomo Pharma Co., Osaka, Japan) and gas-air mixture (4.0% concentration) received orthodontic mechanical stress by Wald method 5 times in 5 weeks. Five mice were used as the control without receiving orthodontic mechanical stress. After that, the regional tissues were removed and fixed in formalin fixative. Paraffin-embedded sections were immunohistochemically analyzed using Dako Envision + Kit-K4006 (Dako, Glostrup, Denmark) and a primary anti-GFP-polyclonal rabbit antibody (#598; 1/500; MBL, Nagoya, Japan). For semiquantitative evaluation of immunohistochemical staining, the following procedures were performed. First, immunohistochemical images of the fixed magnification from the periodontal tissues were prepared and pixel density was counted for each image. Then typical immunohistochemically positive staining portion was defined as positive area. The pixel number of the positive area in the periodontal tissue was compared with the previously calculated total pixel number of the periodontal tissue and the ratio of these numbers was obtained. GFP-positive cells were detected in the periodontal tissues, both in the experimental and control specimens. The GFP-positive cells histopathologically differentiated into some cell types, such as osteoclasts and macrophages, and furthermore the positive cells gathered adjacent blood vessels. The data suggest that GFP-positive BMC migrate into periodontal tissues and differentiate into periodontal tissue component-cells. In the experimental group, numerous GFP-positive cells appeared in the periodontal tissues which received intermittent stimulation of orthodontic mechanical stress, but few GFP-positive cells were seen in the control specimens. The pixel ratio number in the experimental group was 5.77 ± 3.24 % (mean ± SD), while in the control group, it was 0.71±0.45 % (mean ± S.D.); thus the ratio in experimental group was significantly greater than that of the control group (Mann-Whitney U test: p<0.001). These results suggest that orthodontic mechanical stress induces migration of transplanted BMC and their differentiation into periodontal tissue cells.

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PC141

Effects of bile acid supplementation and depletion on fatty liver development in obese mice

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Non-alcoholic fatty liver disease (NAFLD) is defined as an abnormal hepatic triglycerides (HTG) accumulation, associated to obesity or insulin resistance (IR). The pathogenesis of NAFLD is not fully understood. Bile acids (BA), as endogenous ligands for the nuclear hormone receptor farnesoid X receptor have been implicated in glucose metabolism and IR. The effects of BA or its derivatives on NAFLD are still unclear as ursodeoxycholic acid (UDCA) has shown discrepant effects in human NAFLD. On the other hand, BA sequestration has been shown to improve glucose tolerance but its effects on NAFLD are unclear. We studied the effects of BA supplementation and sequestration on liver steatosis development in genetically

obese mice. Methods: ob/ob mice (n=4-6), were fed with standard chow (control) or with chow supplemented with UDCA 0.5%, cholic acid (CA) 0.5% or cholestiramine (CTM) 3%, since 4 to 8 weeks-old (prevention experiments) or since 8 to 12 weeks-old (reversion experiments). Anesthesia: ketamine (100 mg/Kg) plus xylazine (10 mg/Kg) i.p. Experiments were approved by the local ethic review committee on animal experiments (2). We assessed serum and biliary parameters, glucose tolerance test (GTT), HTG content, liver histology and hepatic gene expression. Results: 12 weeks-old mice exhibited marked obesity, hyperinsulinemia, fasting hyperglycemia and decreased bile flow and biliary glutathione secretion. Administration of both UDCA and CA, but no CTM, significantly reduced histological NAFLD development in prevention experiments and prevented HTG accumulation (controls: 111 ± 19 mg/g liver, UDCA-fed: 61.8 ± 11.0 mg/g liver, CA-fed: 25.4 ± 13.6 , $p < 0.05$, (mean $\pm$ SD; Student's t-test). A marked steatosis reduction and HTG (UDCA-fed: 53.1 ± 1.4 mg/g liver; CA-fed: 54.5 ± 10.8 mg/g liver) was also seen in established NAFLD (reversion experiments). Although all treated animals exhibited decreased basal glucose levels, GTT improved only after CTM treatment. Gene expression studies showed expected repression of 7- α -hydroxylase (rate limiting enzyme in BA synthesis pathway) in CA and UDCA feeding. Conclusion: BA strongly modulate fatty liver development in mice being able to prevent liver steatosis and reverse established NAFLD. UDCA and CA seem to exert this effect through different pathways. On the other hand, CTM positively affects glucose tolerance but had no effects on hepatic steatosis in this experimental model of NAFLD. More studies are needed to demonstrate the efficacy of novel therapies for NAFLD, linked to the effects of BA on HTG accumulation or the effect of CTM in other pathways related to glucose metabolism (FONDECYT1110455).

Candia R, Riquelme A, Baudrand R, Carvajal CA, Morales M, Solís N, Pizarro M, Escalona A, Carrasco G, Boza C, Pérez G, Padilla O, Cerda J, Fardella CE, Arrese M. Overexpression of 11 β -hydroxysteroid dehydrogenase type 1 in visceral adipose tissue and portal hypercortisolism in non-alcoholic fatty liver disease. Liver Int. 2012 Mar; 32(3):392-9.

Morton, D.B. and P.H. Griffiths, Guidelines on the recognition of pain, distress and discomfort in experimental animals and a hypothesis for assessment. Vet Rec, 1985. 116(16): p. 431-6.

This work was supported by grant from FONDECYT (Fondo Nacional de Ciencia y Tecnología) grant #1110455 to MA

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC142

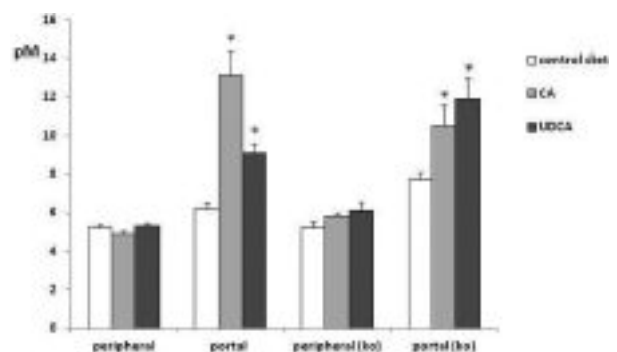
Effect of bile acid feeding on GLP-1 levels in mice

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Bile acids (BA) are synthesized from cholesterol in the peri-central hepatocytes and play an important role in several biological functions. Recent experiments show that BA supplementation modulates energy expenditure in mice; this effect is mediated by the G protein-coupled receptor called TGR5. This receptor is also expressed in the small bowel and is able, upon BA binding, to regulate the intestinal secretion of the incretin Glucagon-like peptide-1 (GLP-1). Thomas et al. (1) and

other authors have shown that TGR5 agonist stimulate GLP-1 release in intestinal cell lines which could enhance glucose tolerance and improve liver and pancreatic function. Aims: to assess the effect of BA administration on portal and systemic GLP-1 levels *in vivo* and to explore the role of TGR5 deficiency in this process. Methods: Wild type (wt) TGR5 and TGR5 knock-out (ko) mice (kindly provided by Dr. J. Auwerx, Strasbourg, France), were fed with standard rodent chow (control group) or supplemented with ursodeoxycholic acid (UDCA) 1% or cholic acid (CA) 0.5%, during 2 weeks. Mice were anesthetized with a mixture of xylazine (10 mg/kg) and ketamine (100 mg/kg) administered i.p. Animal experiments were approved by the local ethic review committee on animals experiments (2). We assessed portal and systemic GLP-1 levels (ELISA Millipore kit, MA, USA), liver histology and serum alanine-aminotransferase levels (ALT). Results were expressed as mean $\pm$ SD and p values < 0.05 were considered statistically significant (t student). Bayesian analysis was carried out in order to test the statistical significance of the observed increment of GLP-1 levels. Results: In wt mice, portal GLP-1 levels were significantly higher than systemic levels (6.2 ± 0.7 vs 5.2 ± 0.5 pM; $p < 0.05$). CA feeding significantly increased portal but not systemic GLP-1 levels. UDCA, a known weak activator of TGR5, modestly increased portal GLP-1 levels. TGR5 ko mice fed with the same diets exhibited an increase in portal GLP1 levels that was significantly less than that observed in wt mice with no differences among both BA treatments (Figure 1). No liver histological differences were observed in control or treated animals. ALT serum levels increased with CA feeding but not with UDCA feeding. Conclusions: These data indicate for first time *in vivo* that CA is able to stimulate intestinal GLP-1 secretion. Our results suggest that this effect is mainly mediated through the action of CA on the TGR5 although additional mechanism may operate. Since CA has a significantly higher affinity for TGR5 compared to UDCA, the latter exerts a weaker stimulation of GLP-1 release. Pharmacological targeting of TGR5 may constitute a promising incretin-based strategy for the treatment of diabetes and associated glucose metabolic disorders (FONDECYT#1110455).



GLP-1 levels. * Significantly different compared to control diet ($p < 0.05$)

Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, Macchiarulo A, Yamamoto H, Matak C, Pruzanski M, Pellicciari R, Auwerx J, Schoonjans K. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab. 2009 Sep;10(3):167-77

Morton, D.B. and P.H. Griffiths, Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. Vet Rec, 1985. 116(16): p. 431-6.

This work was supported by grant from FONDECYT (Fondo Nacional de Ciencia y Tecnología, grant 1110455 to MA)

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PC143

Metabolic effects of GPR120 knock-out: can gene knock-out affect basal metabolism?

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GPR120, an ω -3 fatty acid receptor, is seen as a potential target in diabetes and obesity research enabling indirect control of glucagon-like-peptide-1 (GLP-1) and thus affecting insulin secretion. Previous studies have shown GPR120 mediates insulin sensitization and possesses anti-diabetic properties due to its repression of macrophage-mediated inflammation, a key mechanism for insulin resistance. The study also showed a minor impairment of glucose tolerance and a significant increase in insulin secretion in knock-out (KO) animals (Oh DY *et al.*, 2010). It has also been reported that GPR120 is crucial in energy balance control in rodents and humans and its expression is increased in adipose tissue of obese individuals (Ichimura A *et al.*, 2012).

A metabonomic approach was employed to determine metabolite changes between male, GPR120 wild-type (WT) and KO mice. GPR120 was knocked-out on a C57Bl/6 genetic background (AstraZeneca, Mölndal). This study aimed to determine whether the metabolic pathways affected by GPR120 KO could be identified. Mice were maintained on regular chow diet for 16 weeks before urine samples were collected from metabolically unchallenged animals. The samples were analysed using an integrated liquid chromatography-mass spectrometry (LC-MS) system (Waters, micro Q-TOF) in both positive and negative ionisation mode and the results processed through multivariate statistical analysis and principal components analysis (PCA) (MarkerLynx and Extended Statistics).

Both unsupervised and supervised model plots from PCA showed discrete separation between the WT and KO groups of mice in urine samples. The perturbed metabolites were then statistically validated in accordance with FDA biomarker discovery regulations. From positive ionisation mode, three significantly perturbed ions were identified as causing the metabolite profile differences (ions = 204.1269 m/z, 298.0911 m/z and 339.0597 m/z). In negative ionisation mode, three different ions were identified as causing the main separation of metabolite profiles (ions = 519.9712 m/z, 203.9919 m/z and 269.0130 m/z). Ongoing analysis is being carried out to ascertain the identities of these ions.

Oh DY *et al.* (2010). *Cell* **142**, 687-698.Ichimura A *et al.* (2012). *Nature* **483**, 350-354.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC144

Neuronal nitric oxide synthase mediates angiotensin II impairment of fatty acid-dependent contraction by targeting transglutaminase II in rat left ventricular myocytesY. Zhang¹, C. Jin¹, E. Jeong², J. Jang¹, I. Kim² and S. Kim¹

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Angiotensin II (Ang II) impairs fatty acid (FA) oxidation (Ref 1) which may account for contractile dysfunction in diseased heart. Since nitric oxide is involved in the regulation of FA uptake (Ref 2) and modulates cardiac contractility (Ref 3), we tested whether neuronal nitric oxide synthase (nNOS) contributes to reduced FA-dependent cardiac contractility after Ang II stimulation in rat LV myocytes.

Our results showed that palmitic acid (PA, 1-100 microM) increased the amplitude of sarcomere shortening in LV myocytes from normal rats (at 100 microM, $P < 0.001$, $n = 20$) without affecting the amplitude of Ca^{2+} transient ($P = 0.9$, $n = 11$) or the relationship between Ca^{2+} and myocyte shortening. Similar responses of PA were observed in sham-operated rats ($P < 0.001$, $n = 18$). In contrast, Ang II treatment (via osmotic minipump for 4 weeks) abolished the positive inotropic effect of PA ($P = 0.2$, $n = 18$). 4,5-dihydroxy-1,3-benzenedisulfonic acid (tiron) pretreatment did not affect the response to PA in either group ($P = 0.005$, PA vs. control in the presence of tiron in shams, $n = 7$; $P = 0.2$, PA vs. control in the presence of tiron in Ang II-rats, $n = 6$). Immunoblotting results revealed that nNOS protein expression was greater in LV myocyte homogenates from Ang II-rats (nNOS/GAPDH: $P = 0.03$, $n = 8$); inhibition of nNOS (S-methyl-L-thiocitrulline, SMTc or vinyl-L-N-5-(1-imino-3-butenyl)-L-ornithine, L-VNIO) restored the positive inotropic effect of PA ($P = 0.004$, between PA and control in the presence of SMTc, $n = 14$). Furthermore, nNOS up-regulation was associated with enhanced s-nitrosylation of transglutaminase 2 (TG2, $P < 0.05$, $n = 3$) and reduced activity in LV myocytes from Ang II-rats (63%). Inhibition of TG2 (cystamine or gene deletion, TG2^{-/-}) abolished PA-enhancement of LV myocyte contraction in basal ($P = 0.02$ between PA and PA+cystamine in normal rats, $n = 6$ and $P = 0.91$ between PA and control in TG2^{-/-}, $n = 7$; in TG^{+/+}, $P = 0.007$ between PA and control, $n = 19$) and in Ang II-rats after nNOS inhibition ($P = 0.67$ between cystamine and cystamine+PA in the presence of L-VNIO, $n = 3$). These results indicate that nNOS is up-regulated by Ang II and is responsible for impaired PA-dependent myocyte contraction, at least in part, via modulating TG2 activity. Our findings suggest a novel mechanism through which nNOS modulates myocardial contractility in diseased heart. Rats (of 8 weeks old) were anesthetized with isoflurane (2.5 %). An osmotic minipump (Alzet model 2004) containing Ang II (200 μ l, 6 mM, infusion rate 125 ng/min/kg) was implanted in the midscapular region under sterile condition. Sham-operated animals underwent the same surgical procedure, except for no pump insertion.

Pellieux C, Montessuit C, Papageorgiou I, Lerch R. Angiotensin II down-regulates the fatty acid oxidation pathway in adult rat cardiomyocytes via release of tumour necrosis factor- α . *Cardiovasc Res.* 2009; 82(2):341-50.

Recchia FA, McConnell PI, Loke KE, Xu X, Ochoa M, Hintze TH. Nitric oxide controls cardiac substrate utilization in the conscious dog. *Cardiovasc Res.* 1999; 44(2):325-32.

Zhang YH & Casadei B. Sub-cellular targeting of constitutive NOS in health and disease. *J Mol Cell Cardiol.* 2012; 52(2):341-50.

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PC145

Effect of type 2 diabetes mellitus on intracellular free calcium concentration in human neutrophils and lymphocytes

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Type 2 diabetes mellitus (T2DM) is a major global health problem currently affecting more than 220 million people. T2DM is associated with a number of symptoms and long term complications including neuropathy, cardiomyopathy, nephropathy, retinopathy, delayed wound healing and constant infections. This study investigated the effect of T2DM on intracellular free calcium concentration $[Ca^{2+}]_i$ in neutrophils and lymphocytes from T2DM patients compared to healthy age-matched controls. The study had the relevant ethical clearance from LREC in UK and UNEX in Spain. Both neutrophils and lymphocytes were isolated by density gradient centrifugation using Ficoll histopaque and loaded with fura 2-AM. $[Ca^{2+}]_i$ was measured using established fluorescent method. Changes in $[Ca^{2+}]_i$ were calculated using the fura-2 340/380 ratio.

Mean ($\pm$ SD) basal $[Ca^{2+}]_i$ was 100 ± 11 nM ($n=6$) and 75.0 ± 10 nM, ($n=6$) in healthy age-matched control neutrophils and lymphocytes, respectively. In T2DM patients $[Ca^{2+}]_i$ was 92 ± 10 nM ($n=6$) and 87 ± 10 nM ($n=6$) in neutrophils and lymphocytes, respectively. These results show no significant difference in basal $[Ca^{2+}]_i$ comparing control with T2DM in either neutrophils or lymphocytes. Stimulation of human neutrophils with 10^{-8} M fMLP resulted in transient and marked increases in $[Ca^{2+}]_i$ above basal level reaching maximum within 15-25 sec, followed by a rapid decline in both age-matched control and T2DM cells. Peak $[Ca^{2+}]_i$ was significantly (Student's t-test; $p < 0.05$) decreased in neutrophils from T2DM patients compared to age-matched controls. Typically, $[Ca^{2+}]_i$ was 2.3×10^4 nM ($n=6$) and 0.08×10^4 nM ($n=6$) for control and T2DM neutrophils, respectively. Similarly, stimulation of neutrophils with 10^{-6} M thapsigargin resulted in a gradual increase in $[Ca^{2+}]_i$ reaching maximum within 4-5 min. There was a significant ($p < 0.05$) decrease in $[Ca^{2+}]_i$ in T2DM human neutrophils compared to age-matched controls. $[Ca^{2+}]_i$ was 9.0×10^4 nM ($n=6$) and 2.0×10^4 nM ($n=6$), in control and T2DM neutrophils, respectively. Stimulation of fura-2 loaded lymphocytes with 10^{-6} M thapsigargin resulted in gradual increases in $[Ca^{2+}]_i$ in both age-matched control and T2DM. However, $[Ca^{2+}]_i$ was significantly ($p < 0.05$) less in lymphocytes from T2DM compared to age-matched control. Typically, $[Ca^{2+}]_i$ was 9.0×10^4 nM ($n=6$) and 2.0×10^4 nM ($n=6$) in control and T2DM lymphocytes, respectively. Together, the present results have demonstrated that $[Ca^{2+}]$ homeostasis seemed to be deranged in both neutrophils and lymphocytes of T2DM patients compared to age-matched controls suggesting a relationship

between cellular calcium and frequent infections normally associated with T2DM patients.

I would like to thank all the members of the team at University of Extremadura, Badajoz, Spain for their valuable help and support.

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PC146

Effect of nicotine on sperm parameters and testicular oxidant-antioxidant system in male albino rats

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The use of nicotine through smoking remains a serious health problem. It has been associated with reduced fertility (Oyeyipo et al, 2011), although the mechanism responsible is still unclear. The present study was to investigate the effects of nicotine administration on sperm function and testicular oxidant and antioxidant system in male albino rats. Twenty four male rats weighing between 200-250g (8-10 weeks) were divided into three groups and treated orally for thirty days. Group I, which served as the control received 0.2ml / kg normal saline, Groups II and III received 0.5mg/kg (low dose) and 1.0mg/kg (High dose) body weight of nicotine respectively. At the end of the experimental period, animals were anaesthetized with 1.9% ether and the testis and caudal epididymis were removed. Sperm analysis was done on the sperm collected from the epididymis while the testis was homogenized for lipid peroxidation and anti-oxidant enzyme assays. Values were expressed as mean $\pm$ S.E.M compared by ANOVA. Results showed that nicotine administration significantly ($p < 0.05$) decreased sperm motility (60.2 ± 3.96 ; 40.7 ± 4.63) and count (63.2 ± 5.62 ; 54.7 ± 6.2) when compared with the control group (94.4 ± 3.60 ; 107.5 ± 6.8) respectively. There was a significant decrease ($p < 0.05$) in testicular glutathione peroxidase (1910.4 ± 53.8 IU/ml), testicular glutathione reductase (1071.0 ± 57.1 IU / ml) and superoxide dismutase (1.4 ± 0.1 IU/ml) in the high dose group when compared with the control (2489.8 ± 60.1 ; 1487.6 ± 55.0 and 1.9 ± 0.1 IU / ml) respectively. MDA was significantly ($p < 0.05$) increased in nicotine treated groups (25.4 ± 1.8 μ mol/L) and (30.8 ± 1.7 μ mol/L) when compared with the control (17.9 ± 1.5 μ mol/L). These findings suggest that nicotine administration is associated with decreased sperm function, testicular antioxidant and increase testicular lipid peroxidation which results in oxidative damage thereby causing adverse effects on sperm cells.

I.P. Oyeyipo, Y. Raji, B.O. Emikpe and Adeyombo F. Bolarinwa (2011): Effect of nicotine on sperm characteristics and fertility profile in adult male rats: a possible role of cessation. *J Reprod Infertil.* 12(3):201-207

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PC147

Body composition and resting metabolic rate changes in weight loss programs

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Physical activity in conjunction with moderate dietary energy restriction has been promoted as an important component of a successful weight-loss regimen (1-4). It has been shown in previous studies that the decrease of fat mass (FM) with low-energy diets is accompanied by a reduction of the fat-free mass (FFM) and the resting metabolic rate (RMR), while physical activity contributes to preserve the FFM, and therefore, the RMR (1, 5). Thus, the aim of this study was to investigate the effect of four different weight loss programs on the FM, the FFM and the RMR, comparing men and women. One hundred eighty overweight and obese people (Body Mass Index: 25-34.9 kg/m²), aged from 18 to 50 years, participated in the study (84 males, 96 females) during 6 months. Four types of treatments were randomly assigned: strength training (S, n=43), endurance training (E, n=51), combined S and E training (SE, n=46), and physical recommendations (C, n=40). All participants followed a 25-30% calorie restriction diet. Body composition and RMR were assessed by dual-energy X-ray absorptiometry (DXA) (Lunar ProdigyTM, General Electric, USA) and by indirect calorimetry (Jaeger Oxycon Pro Gas Analyser, Erich Jaeger, Viasys Healthcare, Germany), respectively. A MANOVA test was used to determine differences among types of treatment and sexes in FM, FFM, and RMR changes. Probability level for statistical significance was set at $\alpha=0.05$. FM was significantly reduced in all groups (S: -6.69 ± 3.77 kg; E: -6.38 ± 2.94 kg; SE: -7.81 ± 3.31 kg and C: -6.25 ± 4.17 kg) and in women and men (-6.08 ± 3.38 vs. -7.59 ± 3.62 kg, respectively), being the decrease for men significantly higher than the one reported for women. However, no differences were found among the different treatments performed ($p>0.05$). FFM was maintained in all exercise groups (S, E, and SE), while C group reduced it by -1.15 ± 1.64 kg ($p<0.05$). Both sexes decreased the FFM ($p<0.05$). RMR was decreased in S, SE and C groups (from 1814 ± 322 to 1591 ± 415 kcal/day; from 1727 ± 376 to 1629 ± 329 kcal/day; and from 1755 ± 37 to 1631 ± 321 kcal/day, respectively, $p<0.05$), while in E resulted unaltered (from 1583 ± 351 to 1589 ± 354 kcal/day, $p>0.05$). Moreover, RMR was significantly reduced in women and men (-102.56 ± 292.06 vs. -112.39 ± 367.55 kcal/day, respectively). In a controlled weight loss intervention the FM lost can be achieved throughout all the four types of treatment analyzed, with or without exercise, in both sexes. However, adding supervised exercise to the calorie restriction contributes to preserve the FFM, after a six-month program. On the other hand, the RMR decreased in all the groups, except the E group. Maybe this can be due to the fact that subjects in the E group had a lower RMR at baseline, and they reach the level of the other groups throughout the 6 month treatment.

Stiegler P, Cunliffe A. The Role of Diet and Exercise for the Maintenance of Fat-Free Mass and Resting Metabolic Rate During Weight Loss. *Sports Medicine*. 2006;36(3):239-62.

Volpe SL, Kobusingye H, Bailur S, Stanek E. Effect of diet and exercise on body composition, energy intake and leptin levels in overweight women and men. *J Am Coll Nutr*. 2008 Apr;27(2):195-208.

Catenacci VA, Wyatt HR. The role of physical activity in producing and maintaining weight loss. *Nat Clin Pract Endocrinol Metab*. 2007 Jul;3(7):518-29.

Tsai A, Wadden T. The Evolution of Very-Low-Calorie Diets: An Update and Meta-analysis. *Obesity*. 2006;14(8):1283-93.

Hunter GR, Byrne NM, Sirikul B, Fernandez JR, Zuckerman PA, Darnell BE, et al. Resistance training conserves fat-free mass and resting energy expenditure following weight loss. *Obesity (Silver Spring)*. 2008 May;16(5):1045-51.

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PC148

Relationship between vitamin D deficiency and chronic low back pain in postmenopausal womenH. Rkain^{1,2}, F. Allali² and N. Hajjaj-Hassouni²

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Objective: To examine whether vitamin D deficiency is a determinant risk factor of chronic low back pain (LBP) in Moroccan postmenopausal women.

Methods:

A biochemical assay of serum calcium, phosphate, 25(OH)D, and parathormone (PTH) was performed for 104 patients complaining from a chronic LBP with no obvious causes and compared to those of 45 healthy patients. All participants were postmenopausal. Patients were matched with controls for age and body mass index (BMI). Vitamin D deficiency was defined as a circulating level of 25(OH)D below 20 ng/ml.

Results:

Vitamin D deficiency was significantly more common in patients suffering from chronic LBP than in controls (79 % vs 61.7 %; $P=0.02$). Falls antecedent was also associated with chronic LBP (37.1 % in patients vs 20.5% in controls; $P<0.01$). There was no significant association between chronic LBP and age, BMI, smoking status, nor with number of pregnancies. In multiple logistic regression, after adjusting for potential confounders factors potentially influencing chronic LBP (age, BMI, smoking status, number of pregnancies), the main determinants of chronic LBP were vitamin D deficiency [OR 2.5 (95% IC, 1.1-5.8; $P=0.04$)] and falls antecedent [OR 3 (95% IC, 1.2-7.2; $P=0.01$)].

Conclusion:

Our study shows a significant association between vitamin D deficiency and chronic LBP in Moroccan postmenopausal women. Further studies are clearly warranted to determine the effectiveness and the mechanism(s) of this links between vitamin D deficiency and chronic LBP.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC149

Effects of feeding frequency on the metabolic responses to subsequent feeding in humans

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Post-prandial glucose excursions have been identified as a risk factor for cardiovascular disease (Hanefeld et al., 1999) and all-cause mortality (Balkau et al., 1998). It is therefore important to determine which nutritional therapies can reduce post-prandial glycaemia (Bantle et al., 2008). Diet regimens which are predicated on modifying the frequency of feeding occasions circumvent the limiting factors of other diet regimens, as they can be easily implemented and do not necessitate change in the type of foods consumed (Jenkins, 1997). A second meal effect, or Staub-Traugott effect, describes an improved glycaemic response to a given nutrient load following the ingestion of a clearly defined previous nutrient load and may partially explain why multiple small feeding occasions, compared to fewer larger feeding occasions, may acutely improve glucose control. There is a paucity of literature on the influence of feeding frequency on glycaemia (Betts et al., 2011) and contrasting findings in the extant literature on the occurrence of a second meal effect.

In this study, the influence of feeding frequency on the glycaemic and insulinaemic responses to both an initial nutrient load and a subsequent nutrient load were examined. Five healthy humans (three males and two females) participated in this study (age 26 ± 4 y; body mass index [BMI] 23.4 ± 2.4 kg/m²; waist circumference 80 ± 7 cm; mean $\pm$ SD) and were each exposed to three conditions: one where seven feeding occasions (i.e., a high frequency of feeding) occurred prior to a standardised high-carbohydrate lunch-time meal (HFF trial), another where a single morning meal occurred prior to the lunch-time meal (SMM trial) and another where an extended morning fast occurred prior to the lunch-time meal (EMF trial). Peak venous plasma glucose was significantly lower ($p=0.044$; two-tailed t-test) in the HFF condition (6.6 ± 0.7 mmol/l) compared to the SMM condition (7.8 ± 0.5 mmol/l) in the pre-lunch period (Figure 1). There were no significant differences in incremental area under the curve (iAUC) for plasma glucose between the three conditions for the post-lunch period ($F=0.172$; $p=0.727$; two-way repeated measures ANOVA). There were no significant differences in iAUC for serum insulin between the three conditions for the post-lunch period ($F=1.981$; $p=0.244$; two-way repeated measures ANOVA). These results suggest that spreading a high-carbohydrate nutrient load acutely lowers peak post-prandial glucose concentrations. The inter-individual variation in the overall glycaemic response to each condition suspends the practical application of eating little and often in order to obviate any deleterious effects of the diet regimen. The second meal effect was not observed in the present study, neither following a single morning meal nor following multiple smaller feeding occasions.

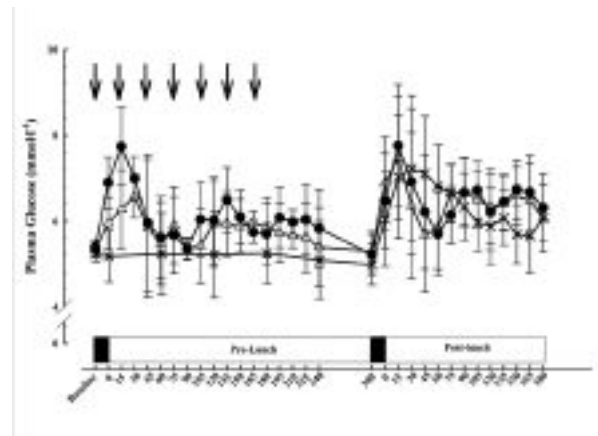


Figure 1. Venous Plasma Glucose Concentrations over Time. Data are mean $\pm$ 95% confidence intervals; $\circ$ denotes High Frequency of Feeding (HFF) condition; $\square$ denotes Single Morning Meal (SMM) condition; $\times$ denotes Extended Morning Fast (EMF) condition. Significant differences for an effect of Time are denoted by * ($p \leq 0.05$) for a given time point, after the Ryan-Holm-Bonferroni correction was applied. The seven arrows denote the temporal pattern of each of the seven feeding occasions in the pre-lunch period in the HFF condition. The black blocks denote the single morning feeding occasion in the SMM condition and the standardised lunch-time meal, respectively.

Balkau, B., Shipley, M., Jarrett, R.J., Pyorala, K., Pyorala, M., Forhan, A., & Eschwege, E. (1998). High blood glucose concentration is a risk factor for mortality in middle-aged nondiabetic men – 20-year follow-up in the Whitehall Study, the Paris Prospective Study, and the Helsinki Policemen Study. *Diabetes Care*, 21, 360-367.

Bantle, J.P., Wylie-Rosett, J., Albright, A.L., Apovian, C.M., Clark, N.G., Franz, M.J., Hoogwerf, B.J., Lichtenstein, A.H., Mayer-Davis, E., Mooradian, A.D., & Wheeler, M.L. (2008). Nutrition recommendations and interventions for diabetes: a position statement of the American Diabetes Association. *Diabetes Care*, 31, 61-78.

Betts, J.A., Thompson, D., Richardson, J.D., Chowdhury, E.A., Jeans, M., Holman, G.D., & Tsintzas, K. (2011). Bath Breakfast Project (BBP) – Examining the role of extended daily fasting in human energy balance and associated health outcomes: Study protocol for a randomised controlled trial [ISRCTN31521726]. *Trials*, 12 (172).

Hanefeld, M., Koehler, C., Schaper, F., Fuecker, K., Henkel, E., & Temelkova-Kurktschiev, T. (1999). Postprandial plasma glucose is an independent risk factor for increased carotid intima-media thickness in non-diabetic individuals. *Atherosclerosis*, 144, 229-235.

Jenkins, D.J.A. (1997). Carbohydrate tolerance and food frequency. *British Journal of Nutrition*, 77 (1), 71-81.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC150

Quality of life in children and adolescents with type 1 diabetes in Kuwait

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Aim: To evaluate the health related quality of life (HRQoL) of children and adolescents with type 1 diabetes (T1DM) in Kuwait using the PedsQL 4.0 Generic Core Scale (GCS) and PedsQL 3.0 Diabetes Module (DM), identify the risk factors associated with unsatisfactory QoL and their effect of metabolic control. **Methods:** A total of 377 children and adolescents (2-

18 years) with T1DM (> 6months) and 289 without diabetes, with their parents, completed the Arabic GCS. Those with T1DM also completed the Arabic DM. Data on disease history and patient biography were collected. Results: Mean total score reported by children was 70.2 and that reported by the parents 59.3 (higher scores indicating better QoL). Young age and long duration of diabetes were associated with poor QoL ($p < 0.001$). Males had better total scores ($p < 0.001$), however, females did better in treatment barriers and adherence items ($p < 0.005$). Higher HbA1c values were associated with lower QoL scores. Conclusion: This is the first large study in the Middle East on children and adolescents evaluating HRQoL. Efforts to achieve optimal metabolic control appear justified on QoL together with clinical grounds. QoL assessment should be an integral part of management, at least on yearly basis.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC151

Gonadotropin inhibitory hormone attenuates kisspeptin-activated intracellular calcium increase in immortalized rHypo8 cells

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During the last decade our understanding of the hypothalamic-pituitary-gonadal axis (HPG) has dramatically expanded. Kisspeptins are reported to be the most potent activators of HPG to date. *In vivo* and *in vitro* studies suggest that the kisspeptin/GPR54 system is very important to fertility control, and kisspeptin is the main triggering factor for puberty onset. A rise in intracellular free calcium concentration ($[Ca^{2+}]_i$) is the common trigger for exocytosis in most cell types. Several neuropeptides are known to regulate GnRH secretion from the hypothalamus by changing $[Ca^{2+}]_i$. Kisspeptins have been recently suggested to be the potent activator of GnRH neurons. Kisspeptin-10 causes a triphasic change in $[Ca^{2+}]_i$ in GT1-7 cells (1). But, very little is known about regulation of this newly identified regulator of reproduction, kisspeptin. The novel hypothalamic RFamide peptides, avian gonadotropin-inhibitory hormone (GnIH) and its mammalian orthologous peptides, discovered in mammals belonging to the RF-amide peptide superfamily (RFRP) seem to be important regulators of the reproductive axis. Kisspeptin and its receptor (GPR54) are expressed in rHypo8 cells, which are immortalized kisspeptin neurons newly developed from rat hypothalamus (2). Whether GnIH/RFRP has modulatory effects on kisspeptin-activated rHypo8 cell line remains to be determined. Thus, in this study for the first time the effects of GnIH/RFRP-1 on calcium signaling in rHypo8 cells were investigated. rHypo8 cells were cultured on poly-L-lysine-coated coverslips and maintained in DMEM supplemented with heat-inactivated fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin at 37 °C in 5% CO₂. After loading the cells with 1 µmol Fura-2 AM, $[Ca^{2+}]_i$ responses were quantified by the changes in 340/380 ratio for individual rHypo8 cells. Kisspeptin-10 at 100 nM caused a significant

increase ($p < 0.001$) in $[Ca^{2+}]_i$ compared to basal levels in rHypo8 cells ($n=58$). GnIH at 1 µM ($n=47$) inhibited ($p < 0.001$) kisspeptin-induced $[Ca^{2+}]_i$ increase. RFRP-1 at 1 µM ($n=72$) did not have any significant effect on $[Ca^{2+}]_i$ in rHypo8 cells. These results suggest that GnIH may exert its effects on HPG axis by modulating kisspeptin neurons.

Ozcan M. et al. (2011) *Neurosci Lett* **492** 55-58.

Gingerich E. et al. (2009) *Neuroscience* **162** 1134-1140.

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PC152

Protective effect of exenatide treatment in STZ-induced diabetic rats *in vivo* and *in vitro*

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Diabetes mellitus (DM) is a major disorder currently affecting 250 million worldwide, including 3 million in the UK. DM can lead to a number of long term complications including nephropathy, retinopathy, neuropathy and cardiomyopathy. These diabetic-induced complications are mainly related to either insulin deficiency or resistance resulting in prolonged hyperglycaemia, hyperlipidaemia and exaggerated levels of reactive oxygen species with impaired antioxidant defense system. Methods which can improve insulin secretion can be of help in the treatment of DM. GLP-1 and its analogue, exenatide are known insulin secretagogues. Adult male Wistar rats ($n=20$) were rendered diabetic using 60 mg/kg body weight (i.p.) of streptozotocin (STZ) according to Home Office regulations. One week after STZ injection and after DM was confirmed, the rats were divided into two groups of 10 each. One group of diabetic (type 1) rats was treated with exenatide (1 µg/kg body weight, daily intravenously) for 10 weeks compared to age-matched diabetic controls (treated with saline). During, and at the end of the experimental period, blood samples were taken from control and diabetic rats for the measurement of a number of biochemical parameters. Pancreatic tissues were also taken for analysis after the rats were humanely killed according to Home Office regulation.

After 10 weeks of exenatide administration, levels of serum insulin increased significantly (3.5 ± 0.2 µIU/ml vs 4.7 ± 0.1 µIU/ml; Student's t-test; $p < 0.01$) accompanied with a decrease in serum cholesterol (49 ± 2.4 mg/dl vs 44 ± 5.0 mg/dl) and a significant reduction of serum triglyceride (79 ± 6.7 mg/dl vs 48 ± 5.9 mg/dl; $p < 0.01$), for treated rats compared to untreated control. Simultaneously, the percentage (%) of both catalase (66.3 ± 2.9 vs 91.6 ± 0.8 ; $p < 0.001$) and glutathione reductase (66.2 ± 3.7 vs 86.1 ± 2.1 ; $p < 0.01$) positive cells was significantly elevated in exenatide treated diabetic rats compared to untreated diabetic rats. At molecular level, the study revealed a significant increase in the gene expression of both glutathione peroxidase (0.97 ± 0.06 to 1.44 ± 0.09 $p < 0.01$) and GLP-1 receptor (1.00 ± 0.07 vs 12.46 ± 0.44 $P < 0.001$) with significant reduction in glucagon gene (1.03 ± 0.06 vs 0.75 ± 0.06 $P < 0.05$) expression in diabetic rats compared to untreated diabetic control. These findings confirmed the protective role of exenatide

in enhancing insulin secretion with reduction in blood lipid levels and improving the antioxidant protective system in pancreatic β -cells. These actions may be mediated through enhancement of both glutathione peroxidase and GLP-1, all of which may contribute to the beneficial effect of exenatide on diabetes mellitus.

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PC153

BACE1 inhibitors as a novel treatment for obesity and diabetes

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Aims: A key enzyme involved in Alzheimer's disease (AD) progression is the β -secretase enzyme (β -site APP Cleaving Enzyme 1 – BACE1). Individuals with type 2 diabetes have an increased risk of AD, and AD is closely associated with insulin resistance and central glucose impairment. We previously reported that BACE1^{-/-} mice are resistant to diet induced obesity and have improved glucose homeostasis. Therefore the aim of the study was to determine the effect of inhibition of β -secretase (BACE1) on the diabetic phenotype of mice on a high fat diet.

Methods: Age matched C57BL/6 mice were fed a high fat diet (HFD, 45% fat) for 20 weeks to engender a diet-induced obesity (DIO) phenotype. Mice were then anesthetised by inhalation of isoflurane and a mid-scapular incision was made to enable subcutaneous implantation of an osmotic minipump. A minipump containing BACE1 inhibitor (BACEi; 10 mg/kg) or vehicle (DMSO/PBS) was implanted and remained for 4 weeks. Body weight and food intake were monitored over the course of the study, while glucose homeostasis was measured at the end of the study. Intraperitoneal glucose (2 mg/kg) and insulin (0.75 U/kg) tolerance tests were performed following an overnight or 4 hour fast respectively. Blood glucose measurements were monitored, from blood taken from the tail vein (~5 μ l), at regular intervals for 2 hours post administration. All data are expressed as mean $\pm$ standard error of mean, and statistical significance determined by two-way ANOVA or Student's t-test. * p <0.05, ** p <0.01, *** p <0.001 versus control. **Results:** Infusion of BACE1 inhibitor to obese mice caused a significant reduction in body weight ($-8.79\% \pm 1.45$; $P < 0.001$) relative to their starting weight, while vehicle treatment had no effect ($1.35\% \pm 0.96$). Food intake was not significantly different between vehicle and BACEi treated mice. Glucose disposal was significantly improved in the BACEi treated mice (AUC: 2004 ± 119 , 1561 ± 90.0 ; $P < 0.01$), whereas insulin sensitivity, as determined by insulin tolerance test, was unaltered. **Conclusions:** Peripheral administration of an inhibitor of the protease, BACE1 reduced body weight and improved glucose disposal in DIO mice. As BACE1 has been validated as a therapeutic target for Alzheimer's disease these data suggest that BACE1 inhibitors, currently in clinical trials, may also be useful therapeutic agents for the treatment of obesity associated with type 2 diabetes.

Meakin PJ, et al. (2012) Biochem. J. 441(285-296).

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PC154

Losartan mitigates type 1 diabetes-induced oxidative DNA damage in corpus cavernosum

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Losartan, an angiotensin II receptor type 1 (AT₁) antagonist, is widely used in the treatment of hypertension. Losartan alleviates oxidative stress and inhibits nitric oxide metabolism by blocking AT₁ receptors. The current study investigated putative modulatory effects of Losartan on type 1 diabetes mellitus (T1DM)-induced oxidative DNA damage in corpus cavernosum (CC). Adult male Wistar rats (250 g; 15-16-week-old) were treated (i.p.) as follows (n=6/group [G]): G1-water (vehicle control), G2-Losartan (300mg L⁻¹ drinking water), G3-single injection of Streptozotocin (i.p., 55 mg kg⁻¹; induces pancreatic β cell destruction leading to T1DM within 72 h) and G4-G3 + Losartan. The drug was administered during weeks 4-6 after the induction of DM. The animals were anesthetized with a mixture of ketamine and rompun (2 mL kg⁻¹, i.m.) and sacrificed at the end of sixth week. The CC was dissected out from the penis and then either fixed in 10% formaldehyde or homogenized in lysis buffer. Paraffin sections were stained with Masson's trichrome and structural changes were observed under a light microscope. Total antioxidant status (TAS) and total oxidant status (TOS) were quantified in a plate reader by using Trolox and H₂O₂ as standards, respectively. Oxidative DNA damage was evaluated by quantifying the activities of an oxidized base, 8-hydroxy-2'-deoxyguanosine (8-oxo-dG) by competitive ELISA (Narayana & Raghupathy, 2012) and immunohistochemistry (Narayana, 2010). Data are values $\pm$ S.D., and compared by one way ANOVA and LSD test. The CC in G3 (T1DM) showed degeneration of smooth muscle cells and collagen fibers compared to G1 and G2. In G4, Losartan mitigated the induced structural changes although the CC still showed degenerative changes. The TAS showed an increase in G2 (0.009 ± 0.002 ; $p < 0.05$), a decrease in G3 (0.002 ± 0.0004 ; $p < 0.05$) and a significant recovery in G4 (0.007 ± 0.002 ; $p < 0.05$) compared to G1 (0.006 ± 0.001). Conversely, TOS was significantly increased in G3 (0.095 ± 0.008 ; $p < 0.05$) compared to G1 (0.029 ± 0.004), G2 (0.043 ± 0.014) and G4 (0.031 ± 0.01). A decrease in TAS/TOS ratio in G3 (0.016 ± 0.003) compared to G1 (0.20 ± 0.04) indicated the induction of an oxidative stress status in the CC of T1DM rats. The ratio showed a recovery in G4 (0.26 ± 0.27) compared to G3 ($p < 0.05$). Enhanced intensity of 8-oxo-dG labeling in G3, in both nuclei and cytoplasm of the CC cells, indicated nuclear and mitochondrial oxidative DNA damage. Activity of 8-oxo-dG significantly increased in G3 (0.19 ± 0.005 ; $p < 0.05$) compared to G1 (0.06 ± 0.001), but recovered in G4 (0.07 ± 0.004 ; $p < 0.05$) compared to G3. In conclusion, Losartan significantly ameliorates T1DM-induced oxidative stress, oxidative DNA damage and structural changes in the CC.

Narayana K & Raghupathy R (2012). Drug Chem Toxicol 35, 208-217

Narayana K (2010). Eur J Pharmacol 641, 238-245

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PC155

Skeletal muscle acylcarnitines and lipid induced insulin resistance in healthy humans

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Intramyocellular lipid (IMCL) accumulation results in skeletal muscle insulin resistance (IR; 1). Intravenous lipid emulsion infusion in healthy humans during euglycaemic hyperinsulinaemia leads to impaired insulin stimulated whole body glucose uptake (2, 3), likely due to substrate competition (2) and/or accumulation of IMCL metabolites (1, 3). Skeletal muscle long-chain acylcarnitine (AC) accumulation from excess mitochondrial β -oxidation flux is associated with IR (4), but a direct link *in vivo* needs definitive evidence. Fish oil omega (n) 3 fatty acids can reduce lipid induced skeletal muscle IR in animal models (5). We hypothesised that i.v. infusion of n3 lipid emulsion (Omegaven, Fresenius Kabi) in healthy humans will lessen the degree of IR normally observed with an n6 emulsion (Intralipid, Fresenius Kabi) due to reduced muscle accumulation of long-chain ACs. Six healthy men (age 26 ± 2 y, BMI 27 ± 2 kg/m²) participated in the present study, which was approved by the local ethics committee. On three randomised occasions at least one week apart subjects underwent a 6 h euglycaemic hyperinsulinaemic clamp (50 mU/m²/min) accompanied by infusion of saline (Con), 10% Intralipid (n6), or 10% Intralipid + 10% Omegaven (2:1; n3) at 100 ml/h. Arterialised-venous blood samples were taken hourly, and *vastus lateralis* muscle biopsy samples were taken before and after the clamp. Statistical analysis was performed using repeated-measures two-way ANOVA and data are expressed as means $\pm$ SEM. Steady state glucose disposal during the 6 h clamp was 28% lower in n6 compared to Con (57.3 ± 3.0 vs. 41.5 ± 2.6 nmol/kg/min; $P < 0.01$). However, glucose disposal in n3 (51.4 ± 2.4 nmol/kg/min) was no different to Con, such that it was 24% greater than n6 ($P < 0.05$). Skeletal muscle pyruvate dehydrogenase complex activation status (PDCa) increased during clamp in Con and n3 from 0.58 ± 0.08 to 1.15 ± 0.30 ($P < 0.01$) and 0.73 ± 0.10 ($P < 0.05$) mmol/kg/min wet mass, respectively, but remained unchanged in n6 (0.43 ± 0.09 mmol/kg/min wet mass). From a mean baseline content of 52.0 ± 0.1 μ mol/kg dry mass, total AC (carbon lengths 3-20) content was suppressed after the clamp in Con (21.3 ± 4.7 μ mol/kg dm; $P < 0.001$), but not in n6 or n3 (52.0 ± 14.3 and 49.6 ± 6.8 μ mol/kg dm). However, whereas long-chain AC (12-20 carbons) was suppressed by 54% ($P < 0.001$), and there was a trend for muscle acetyl-CoA content to be 3-fold greater ($P = 0.09$) in n6, they were unchanged in n3. In conclusion, i.v. n3 lipid infusion results in a reduced degree of lipid induced IR compared to equimolar n6 lipid infusion in healthy males. This effect was not due to a reduction in long-chain AC accumulation, but appears to be due to reduced shorter-chain mitochondrial β -oxidation flux resulting in a reduced degree of acetyl-CoA mediated inhibition of PDCa.

Savage et al (2007). *Physiol Rev* 87: 507–520

Boden et al (1991). *J Clin Invest* 88: 960–966

Tsintzas et al (2007) *J Clin Endocrinol Metab* 92: 3967–3972

Bosma et al (2012) *Prog Lipid Res* 51: 36–49

Fedor & Kelley (2009). *Curr Opin Clin Nutr Metab Care* 12: 138–46

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PC156

Sympathetic neurovascular transmission in the mouse tail artery

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In order to analyse the postjunctional mechanisms involved in sympathetic neurovascular transmission, we are developing transgenic mice, particularly α 1-AR knockouts. The first step, however, is to define neurovascular transmission in the tail artery of normal mice using pharmacological antagonists, as has been done for the rat. Perivascular stimulation of rat tail artery evokes depolarizations mediated by ATP and noradrenaline (1) but contraction is mediated largely by α 1- and α 2-adrenoceptors (ARs), probably acting synergistically (2). *In vivo*, α 2- but not α 1-AR blockers cause profound vasodilation, increasing tail skin temperature (3). The α 2-AR-mediated effects are more readily shown distally (4). Further, activation of capsaicin-sensitive peptidergic afferent nerves, present in small numbers around the rat tail artery (5), can elicit relaxation.

Male C57Bl mice (4–6 months) were killed with CO₂. Ring segments of the tail artery 2mm long were prepared from proximal (2cm) and distal (5cm) sites. Vessels were mounted on a wire myograph in oxygenated physiological saline at 37°C. Responses to 62.5mM KCl were recorded. Vessels were incubated for 30 minutes in 1 μ M capsaicin to block release of calcitonin gene-related peptide from afferent nerves. Frequency response curves (FRC) to maximal stimuli were constructed before and after capsaicin (0.5Hz–8Hz; 20 pulses; 0.3ms pulse width; 20V) and in the presence of various combinations of 100nM prazosin (α 1-AR antagonist), 100nM rauwolscine (α 2-AR antagonist), and 1mM suramin (P2X receptor antagonist). Data were analysed using unpaired and paired t-tests as well as one way ANOVA with Bonferroni's post-hoc test.

Capsaicin induced relaxation if vessel tone was raised, but caused no change in responses to nerve stimulation at either location ($P > 0.05$, $n = 23$, proximal; $n = 6$, distal). However, responses in proximal segments at high frequencies were significantly greater than for distal segments after, but not before, capsaicin. In proximal segments, prazosin reduced responses at all frequencies by $\sim 40\%$ ($P < 0.05$, $n = 6$, one way ANOVA). Rauwolscine also reduced responses, exerting more effect at 0.5Hz ($\sim 80\%$ block) than at 8Hz ($\sim 30\%$ block). Suramin alone reduced responses at each frequency by $\sim 40\%$. In distal segments, neither prazosin nor suramin significantly reduced the responses, which were virtually abolished by rauwolscine. While both α 1- and α 2-ARs are involved in nerve evoked contraction of the proximal mouse tail artery, contractile responses mediated by α 2-ARs dominate at low stimulation frequencies, especially in the distal part. Unlike in the rat tail artery, the contribution of P2X receptors to nerve evoked contraction is substantial and will be clarified in mice that lack alpha-adrenoceptors.

Sneddon P, Burnstock G. (1984) ATP as a co-transmitter in rat tail artery. *Eur. J. Pharmacol.* 106, 149–152.

Yeoh M, McLachlan EM, Brock JA. (2004) Chronic decentralization potentiates neurovascular transmission in the isolated rat tail artery, mimicking the effects of spinal transection. *J Physiol.* 561, 583-596.

Redfern WS, MacLean MR, Clague RU, McGrath JC. (1995) The role of alpha 2-adrenoceptors in the vasculature of the rat tail. *Br J Pharmacol* 114, 1724-1730.

Medgett, I.C. (1985). α 2-Adrenoceptors mediate sympathetic vasoconstriction in distal segments of rat tail artery. *Eur. J. Pharmacol.* 108, 281-287.

Li Z, Duckles SP. (1993) Acute effects of nicotine on rat mesenteric vasculature and tail artery. *J Pharmacol Exp Ther.* 264, 1305-1310.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC157

Immediate effects of hyperbaric oxygenation on blood pressure, acid-base and blood gas status and parameters of oxidative stress in healthy male rats

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INTRODUCTION: Hyperbaric oxygenation (HBO) is the medical use of pure oxygen at a level higher than atmospheric pressure. There are different protocols of HBO being used for research purposes; however, data on the changes in blood pressure, oxidative stress and acid-base and gas status induced by some of the possible oxygenation patterns/protocols are scarce and conflicting.

AIM: In this work we aimed to investigate the immediate influence of oxygenation at a pressure of 2 bar during 2 hours on blood pressure, acid-base and blood gas status and parameters of oxidative stress in healthy rats.

METHODS: Healthy male 12-15 week old Sprague-Dawley rats were anesthetized with ketamine (75 mg/kg) and midazolam (2.5 mg/kg) i.p. Femoral artery was cannulated for blood pressure measurements (AP) and blood sampling prior and immediately after single time hyperbaric oxygenation (HBO) in a hyperbaric oxygen chamber (100% oxygen at a pressure of 2 bar for 2 hours, with additional 15 minutes for compression and decompression). Arterial blood samples were collected to analyze acid-base and blood gas status and to determine Ferric reducing ability of plasma (FRAP) and Thiobarbituric Acid Reactive Substances (TBARS; based on reaction of malonaldehyde (MDA) with thiobarbituric acid). Paired t-test for AP, blood gases and acid-base status (N of rats = 12) and t-test for FRAP and TBARS (N of rats = 12) were used; $p < 0.05$ was considered significant. Data are expressed as mean $\pm$ SD. The study was approved by Ethical Committee of Faculty of Medicine University of Osijek

RESULTS: After HBO there was significant decrease in systolic and diastolic AP (control 138 ± 14 / 103 ± 13 vs. 113 ± 12 / 72 ± 16 kPa after HBO), although in normal AP range. pH significantly decreased after HBO (control 7.34 ± 0.05 vs. 7.28 ± 0.05 after HBO). After HBO, pCO₂ was significantly decreased (7.07 ± 0.89 vs. 5.76 ± 0.50 kPa) and pO₂ significantly increased (12.48 ± 0.88 vs. 13.68 ± 2.4 kPa). Plasma bicarbonate significantly decreased (control 27.04 ± 3.25 , vs. 20.52 ± 3.02 after HBO). Exposure to

HBO significantly increased MDA concentration (from 0.173 ± 0.092 to 21.79 ± 1.05 microM/MDA), while FRAP was significantly decreased compared to control condition (0.152 ± 0.035 to 0.117 ± 0.013 mM/L TE).

CONCLUSION: Control values are in agreement with previously published data on anesthetized rats (1). Acute exposure to HBO had significant effects on all observed parameters. Acidosis may be contributed to prolonged anesthesia (2). However, there is immediate improvement in pCO₂ and pO₂, although with increase in oxidative stress products and acute decrease in plasma antioxidative capacity, after single time HBO exposure (3).

1. Fish RE, Brown MJ, Danneman PJ, Karas AZ. Pharmacology of Injectable Anesthetics, Sedatives, and Tranquilizers in "Anesthesia and analgesia in laboratory animals". American College of Laboratory Animal Medicine Series, Second edition, USA (2008). Pp : 65-66

2. Wixson SK, White WJ, Hughes HC Jr, Lang CM, Marshall WK. The effects of pentobarbital, fentanyl-droperidol, ketamine-xylazine and ketamine-diazepam on arterial blood pH, blood gases, mean arterial blood pressure and heart rate in adult male rats. *Laboratory animal science* 1987; 37:736-42

3. Pakulla MA, Obal D, Loer SA. Continuous intra-arterial blood gas monitoring in rats. *Laboratory animals* 2004; 38:133-137

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PC158

Level of Fetuin-A, Endothelin-1 and sex hormones in the serum of isolated systolic hypertensive patients

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Isolated systolic hypertension (ISH) is characterized by increased arterial stiffness (Wallace, et al, 2007). The mechanisms underlying arterial stiffness and subsequently ISH in human are not fully understood. Several factors have been demonstrated to be involved in arterial stiffness such as the liver-produced anti-classificatory hormone, fetuin-A, endothelial-derived vasoconstrictor, endothelin-1 (ET-1), and the sex hormones, testosterone and progesterone (Mori, et al, 2007; McEniery, et al, 2007; Dockery, et al, 2002; Natoli, et al, 2005). Changes of blood level of these parameters in ISH and hypertensive patients (both SBP and DBP are high) might reflect the level of their expression and production, therefore contributing to the pathophysiology of these disease statuses, which still not clear. ELISA was used to measure serum level of these parameters in three groups: (I) ISH patients; (II) hypertensive patients and (III) normal subjects. Samples were collected from several hospital in Makkah, Saudi Arabia during the period of 2009 and 2010. The serum level of the parameters is expressed as mean $\pm$ standard error of the mean. Comparisons between groups was made by using t-test. In ISH, there is decrease in fetuin-A (0.17 ± 0.03 ; $n=39$) as compared to normal subjects ($P < 0.05$) and no changes were observed in serum level of testosterone (19.2 ± 1.9 ; $n=15$), progesterone (0.72 ± 0.2 ; $n=19$) and ET-1 (0.09 ± 0.02 ; $n=30$) as compared to normal subjects ($P > 0.05$). In hypertension, there is an increase in ET-1 (0.2 ± 0.03 ; $n=14$) and decrease in testosterone (8.125 ± 1.8 ; $n=9$) as compared to normal subjects ($P < 0.05$) and no changes were

observed in fetuin-A (0.29 ± 0.06 ; $n=18$) and progesterone (0.67 ± 0.3 ; $n=7$) as compared to normal subjects ($P>0.05$). Low level of the anti-calcification hormones, fetuin-A, in ISH suggest its possible contribution in the arterial stiffness and therefore increase in the SBP in this group of patients. Increase in the potent vasoconstrictor, endothelin-1, and decrease in testosterone in hypertensive patients suggest their possible involvement in the pathophysiology of this vascular disease. These findings open a new understanding of the pathophysiology of ISH and hypertension and bring hope for better treatment of these diseases status.

Dockery F, Bulpitt CJ, Agarwal S, Rajkumar C. Testosterone suppression in men with prostate cancer is associated with increased arterial stiffness. *Aging Male*. 2002 Dec;5(4):216-22.

McEniery CM, Qasem A, Schmitt M, Avolio AP, Cockcroft JR, Wilkinson IB. Endothelin-1 regulates arterial pulse wave velocity in vivo. *J Am Coll Cardiol*. 2003 Dec 3;42(11):1975-81.

Mori K, Emoto M, Araki T, Yokoyama H, Teramura M, Lee E, Motoyama K, Koyama H, Shoji T, Inaba M, Nishizawa Y. Association of serum fetuin-A with carotid arterial stiffness. *Clin Endocrinol (Oxf)*. 2007 Feb;66(2):246-50.

Natoli AK, Medley TL, Ahimastos AA, Drew BG, Thearle DJ, Dilley RJ, Kingwell BA. Sex steroids modulate human aortic smooth muscle cell matrix protein deposition and matrix metalloproteinase expression. *Hypertension*. 2005 Nov;46(5):1129-34.

Wallace SM, Yasmin, McEniery CM, Mäki-Petäjä KM, Booth AD, Cockcroft JR, Wilkinson IB. Isolated systolic hypertension is characterized by increased aortic stiffness and endothelial dysfunction. *Hypertension*. 2007 Jul;50(1):228-33.

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PC159

Effects of testosterone on vascular reactivity in male Sprague – Dawley rats fed a high salt diet

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Sex hormone-dependents vascular reactivity is an underlying factor contributing to gender differences in cardiovascular diseases (Sader and Celermajer, 2002). This study evaluated the role of androgens on vascular reactivity in the perfused hind limb preparation in salt - induced hypertension. Thirty six weanling male Sprague - Dawley rats (8 weeks old; weight, 180 – 200g) were either bilaterally orchidectomised ($n = 24$) or sham-operated ($n = 12$) (under ketamine and xylazine anaesthesia (90mg and 10mg/kg/body weight i.m) respectively, with or without testosterone replacement (10mg/kg sustanon 250® i.m once in 3 weeks), and were placed on normal (0.3%) or high (4%) NaCl diet for 6 weeks. Arterial blood pressure (BP) was determined via carotid artery cannulation under 25% urethane and 1% α -chloralose anaesthesia (5ml/kg body weight i.p) at the end of the feeding period. Thereafter renal cortical blood flow was measured and renovascular resistance (RVR) was also determined. Vascular responses to PE, ACh and SNP were studied in the perfused hind limb. Fluid balance (FB) and

plasma concentration of nitrite (PCN) were also determined. Data were analyzed using one way analysis of variance (ANOVA) and Student-Newman-keuls post Hoc test. Confidence interval was placed at 95%. Rats fed a high salt diet (HSD) showed increases in BP (100 ± 2.0 vs. 124 ± 3.0), RVR (0.29 ± 0.02 vs. 0.34 ± 0.01), FB (5.0 ± 1.34 vs. 9.6 ± 1.83), that was accompanied by decreased plasma nitric oxide (NO) production (0.64 ± 0.09 vs. 0.22 ± 0.05). Orchidectomy reversed but testosterone replacement restored the increased BP (117 ± 2.0 vs. 127 ± 3.0), RVR (0.33 ± 0.01 vs. 0.37 ± 0.01), FB (6.8 ± 1.39 vs. 11.2 ± 1.24) and PCN (0.69 ± 0.15 vs. 0.50 ± 0.04) observed in the rats fed a HSD. High salt diet increased response to PE (60.4 ± 7.61 vs. 84.0 ± 8.63) but this was reversed and restored by orchidectomy and testosterone replacement (70.4 ± 7.13 vs. 79.0 ± 5.34) respectively. HSD also impaired vasorelaxation to ACh (68.8 ± 4.54 vs. 48.4 ± 6.32) and SNP (79.6 ± 5.40 vs. 44.4 ± 8.39). Orchidectomy attenuated the impaired relaxation response to ACh (56.6 ± 4.47 vs. 48.4 ± 3.47) and SNP (64.8 ± 3.60 vs. 58.6 ± 4.14) observed in the HSD fed rats. Testosterone promotes the impairment of vascular function observed in HSD and this may involve the NO pathway.

Sader, M. and Celermajer, D.S. (2002) Endothelial functions, vascular reactivity and gender differences in the cardiovascular system. *Cardiovasc Res* 53: 597 – 607.

Federal Republic of Nigeria Education Trust Fund (ETF) Academic Staff Training & Development [AST&D]

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PC160

Mechanisms of the sarcoplasmic reticulum Ca^{2+} release induced by P2X receptor activation in small mesenteric artery myocytes

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Sympathetic nervous system controls total peripheral vascular resistance and blood flow via regulation of the contraction of small arteries. One of the principal sympathetic neurotransmitters, ATP, acts on arterial smooth muscle cells (SMCs) via activation of P2X receptors (P2XRs) leading to an increase of $[\text{Ca}^{2+}]_i$ and SMC contraction. We have recently demonstrated that phasic contractions of the guinea-pig small mesenteric arteries induced by P2XR stimulation are sensitive to inhibitors of voltage-gated Ca^{2+} channels (VGCCs), ryanodine receptors (RyRs) and inositol 1,4,5-trisphosphate receptors (IP_3Rs) [1]. Here we analysed the respective role of RyRs and IP_3Rs in elevation of $[\text{Ca}^{2+}]_i$ induced by selective stimulation P2XRs in smooth muscle cells (SMCs) from the guinea-pig small mesenteric arteries. Data are presented as mean $\pm$ S.E.M. and compared using Student's t-test. Freshly isolated fluo-3-loaded SMCs were stimulated with $10 \mu\text{M}$ α, β -methyleneadenosine 5-triphosphate (α, β -meATP). Fast x-y confocal imaging revealed that activation of P2XRs evoked an abrupt sub-plasmalemmal (sub-PM) $[\text{Ca}^{2+}]_i$ upstroke (SPCU) followed by Ca^{2+} wave rapidly propagating towards SMC center. The effect of IP_3R inhibition ($30 \mu\text{M}$ 2-APB) was significantly ($p<0.00001$) stronger than that of RyR inhibition ($100 \mu\text{M}$ tetracaine): reduction of the SPCU amplitude by $67 \pm 7\%$ ($n=16$) and by $40 \pm 5\%$ ($n=26$),

respectively. The effect of simultaneous block of IP₃Rs and RyRs was similar ($p < 0.001$) to the effects of calcium store depletion with 10 μ M CPA: attenuation of the response by $93 \pm 2\%$ ($n=7$) and by $81 \pm 3\%$ ($n=24$), respectively. The effect IP₃R inhibition on SPCU was significantly ($p < 0.0001$) attenuated by initial block of VGCCs (5 μ M nicardipine): under these conditions 2-APB reduced SCPU amplitude only by $37 \pm 3\%$ ($n=9$), thus, suggesting that IP₃R-mediated Ca²⁺ release is facilitated by Ca²⁺ entry via VGCCs. Immunostaining of RyRs and IP₃Rs in the SMCs with identified sarcoplasmic reticulum (SR) and nucleus revealed that sub-PM SR elements are enriched with type 1 IP₃Rs while RyRs are located mainly in deeper SR. This structural peculiarity makes IP₃Rs more accessible to Ca²⁺ entering the cell via VGCCs. As a result, IP₃Rs may serve as a functional link ("intermediate amplifier") between voltage-gated Ca²⁺ entry and RyR-mediated Ca²⁺ release. We conclude that depolarization of mesenteric artery SMCs following P2X receptor activation induces IP₃R-mediated Ca²⁺ release from sub-PM SR, which is facilitated Ca²⁺ entry via VGCCs. This mechanism seems to be fundamental (as we have recently demonstrated this in renal microvascular SMCs [2]) and provides convergence of signalling pathways engaged in electro-mechanical and pharmacomechanical coupling [3] in vascular smooth muscle.

Sukhanova KYu et al. (2010) *Int J Physiol Pathophysiol* 1: 67-77.

Povstyan OV et al. (2011) *Br J Pharmacol* 162: 1618-1638.

Somlyo AV, Somlyo AP (1968) *J Pharm Exp Ther* 159: 129-145.

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PC161

C-C Chemokine Signalling in Human Monocytes Involves a Concomitant Release of ATP and Activation of Purinergic Receptors

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Monocytes are pivotal in the innate immune response and progression of chronic inflammatory and degenerative disease. The recruitment of monocytes driven by chemical mediators is essential for host defence but also contributes to monocyte involvement in disease pathogenesis. C-C chemokine (C-C motif) CCL2 is a chemoattractant that acts through G-protein coupled receptors (GPCRs) to produce changes in cytosolic calcium levels that drive monocyte function. Similarly, Adenosine-5'-Triphosphate (ATP) is a signalling molecule that acts through GPCRs and is released by dying cells as a "come find me" signal for leukocytes. Exploring the signalling pathways of these molecules and how they interrelate may therefore aid in identifying the chemical imbalances involved in disease.

Studies were performed with human THP-1 cells (acute monocyte leukaemia) or peripheral blood mononuclear cells (PBMCs). All calcium (Ca²⁺) mobilisation studies were performed with FLUO-4-AM with or without (w/o) Ca²⁺ (1.5mM). Initial studies explored the effect of apyrase, an ATP/ADP scavenger. THP-1s and PBMCs were pre-incubated for 10min with or w/o apyrase (2U/mL) and were stimulated with CCL2 (1-500ng/mL). The effect of apyrase on THP-1 chemotaxis was

also studied using a transwell migration assay. To further elucidate an association between purinergic and chemokine signalling, the ecto-ATPase inhibitor ARL-67156 (100 μ M), P1 antagonist CGS-15943 (2.5 μ M), and the P2Y/P2X antagonist Suramin (100 μ M) were explored. ATP secretion was tested using the luciferase-luciferin assay. Hypothesis testing performed by means of paired Student t-test.

Chemokine-evoked Ca²⁺ responses in THP-1 cells were inhibited by apyrase. The inhibitory effect of apyrase was also observed in PBMCs. In THP-1s, apyrase inhibited CCL2 (50ng/mL:E_{max}) responses by 60% ($n=6$, $p < 0.05$) and also produced a 5-fold shift in the CCL2 EC₅₀ response ($p < 0.05$). THP-1 chemotaxis toward CCL2 was significantly attenuated in the presence of apyrase ($n=3$, $p < 0.05$). ARL-67156 potentiated Ca²⁺ responses at 10 (EC₂₀) and 20ng/mL (EC₅₀) CCL2 ($n=4-9$, $p < 0.05$). An involvement of P1 receptors was explored through CGS-15943 and showed a potentiation of CCL2 EC₂₀ Ca²⁺ responses ($n=4$, $p < 0.05$). P2 receptor involvement was tested with Suramin and gave a >90% inhibition of CCL2 E_{max} responses ($n=3$, $p < 0.05$). Our data also demonstrates that Ca²⁺ responses to CCL2 are associated with a secretion of ATP ($n=3$, $p < 0.05$).

In conclusion, CCL2 evoked calcium signalling in human leukocytes is facilitated by a concomitant release of ATP acting through purinergic receptors. Ecto-ATPases and adenosine P1 receptors also act to modulate this process. Our data suggests that purinergic receptors offer a new route for therapeutic intervention of C-C chemokine mediated diseases.

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PC162

EPAC mediates the antiproliferative effects of estrogen on human arteriolar smooth muscle cells

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Hypertension is a major contributor to cardiovascular mortality, which remains the leading cause of death. Reduced arteriolar lumen due to increased proliferation of smooth muscle cells (SMCs) could precipitate hypertension. Although SMCs from large vessels have been extensively studied, very little is known about mechanisms regulating proliferation of arteriolar SMCs, particularly in response to 17 β estradiol (estrogen). We hypothesized that estrogen inhibits proliferation of arteriolar SMCs by activating exchange protein activated by cAMP (EPAC). Cells were isolated from dermal arterioles of healthy subjects using 2% lidocaine as local anesthetic, after written informed consent. Statistical analysis was done using student's t-test for comparing two means. Data are reported as means $\pm$ SEM. We show that estrogen significantly inhibits proliferation of arteriolar SMCs in a concentration- (10^{-11} - 10^{-7} M) and time- (0-96 hours) dependent fashion. A $37 \pm 3.9\%$ inhibition in proliferation was observed after 48 hours of treatment with 10^{-9} M of estrogen ($n=3$; $p < 0.01$). Pretreatment with the estrogen receptor (ER) antagonist ICI-182,780 (ICI; 10^{-8} M) significantly ($n=3$; $p < 0.05$) reduced estrogen's inhibition ($39 \pm 4\%$ inhibition with estrogen versus $21 \pm 3\%$ inhibition with ICI + estrogen). Moreover, transfection with ER alpha or beta potentiated estrogen's effect (61 ± 7 or $47 \pm 4\%$ inhibition with ER alpha or beta respectively; $n=3$ and $p < 0.01$ for either). Treatment of cells with the cell-impermeable form of estrogen, estro-

gen:BSA (10^{-8} M) also caused a significant $27.3 \pm 4.1\%$ inhibition in proliferation, suggesting that a membrane estrogen receptor mediates the estrogen effect ($p < 0.05$). Moreover, pretreatment with the adenylate cyclase inhibitor SQ-22536 ($400 \mu\text{M}$) abolished the estrogen effect, indicating that cAMP mediates the noted estrogen effect. Additionally, we show that the cAMP analogue, 8-(4-chloro-phenylthio)-2'-O-methyladenosine-3',5'-cyclic monophosphate ($0-150 \mu\text{M}$) which selectively activates EPAC, mimics the antiproliferative effects of estrogen. PKA does not appear to be involved since pretreatment with the PKA inhibitor (H89; $5 \mu\text{M}$) did not significantly affect the estrogen-induced effect (100% (no H89) versus $111 \pm 4.7\%$ (+H89); $p > 0.05$). Transfection with a dominant negative mutant of EPAC (EPAC-R279E) reduced estrogen's effect (39 ± 2.1 versus $22 \pm 3.2\%$; $p < 0.05$), suggesting that EPAC mediates the observed estrogen effect. Taken together, our data show that estrogen elicits its antiproliferative effect via a Gs-coupled membrane ER, which elevates cAMP levels leading to EPAC activation. This novel effect of estrogen could partially account for its protective role in premenopausal women compared to age-matched men, particularly in regard to hypertension incidence.

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PC163

Microelectrode analysis of gut pacemaker activity in mice lacking IL-10

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Interstitial cells of Cajal (ICC) play multiple roles in the gut. they act as pacemaker cells to generate basic electric rhythms for gut motility, such as peristalsis and segmentation. ICC also serve as a interface between the autonomic and enteric nervous systems and smooth muscle. Furthermore, their long processes prompt us to speculate that these cells connect electric excitation in the gut, acting as a syncytium. Interleukin-10 (IL-10) is known to suppress gut immunity by antagonizing interferon- γ . IL-10-deficient mice (IL-10^{-/-}) therefore suffer from chronic inflammatory bowel disease (IBD). These mice are also implicated in prominent anal prolapse, although the underlying mechanisms are unclear. We hypothesize that basal electric activity, including in ICC, may be accelerated in IL-10^{-/-} mice, thus spatiotemporal electrical activity was examined and compared with wild-type (WT) mice (C57BL/6J). WT and IL-10^{-/-} mice were sacrificed by cervical dislocation after deeply anaesthetising with diethyl ether. The ileum was quickly excised, and the whole-muscle layer (5 mm $\times$ 20 mm) containing the myenteric plexus was isolated. A microelectrode array (MEA; MED 64 System, Alpha Med Science, Osaka, Japan) was employed to measure spontaneous electric activity in ileal musculatures of both WT and IL-10^{-/-} mice. A set of 8×8 field potentials over a $\sim 1 \text{ mm}^2$ area was simultaneously recorded with the high-pass filtering of 0.1 Hz. In some experiments, nifedipine ($1 \mu\text{M}$) and tetrodotoxin (TTX, 250 nM) were applied to predominantly measure ICC electric activity. Power spectrum, auto-correlation and cross-correlation analyses were performed using commercial add-in software (Kyowa Electronic Instruments, Tokyo, Japan). Statistical differences were evaluated

by an independent *t*-test. Spontaneous electric activity was synchronized throughout the recording area in WT mice, while unsynchronized electric activity was frequently observed in IL-10^{-/-} mice. A cross-correlation function was derived in 63 channels other than the centre channel used as the reference. The peak value of cross-correlation was significantly lower in IL-10^{-/-} mice than in WT mice (mean $\pm$ SD: 0.63 ± 0.14 , $n=9$ vs 0.77 ± 0.06 , $n=9$; $P < 0.01$) in the presence of nifedipine and TTX. The spectral power in the frequency range of 9.4 to 27.0 cpm ($P_{w_{9.4-27.0}}$) was estimated in all 64 channels. $P_{w_{9.4-27.0}}$ was larger in IL-10^{-/-} mice than in WT mice (0.014 ± 0.024 vs $0.007 \pm 0.015 \text{ mV}^2$), but there was no statistical significant difference ($P=0.48$). The frequency of oscillation estimated from auto-correlation was significantly higher in IL-10^{-/-} mice than in WT mice (20.47 ± 3.50 vs $14.96 \pm 1.46 \text{ cpm}$; $P < 0.01$). These results suggest that unorganised hyper gut activity may contribute to the ethiology of IBD, for instance, by altering anti-bacterial factors from epithelial cells.

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PC164

Pharmacological differences of endothelin receptors-mediated modulation in cultured interstitial Cells of Cajal from the murine small and large intestine

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Interstitial cells of Cajal (ICCs) are pacemaker cells that activate the periodic spontaneous depolarization (pacemaker potentials) responsible for the production of slow waves in gastrointestinal smooth muscle. Under current clamping, ICCs had a mean resting membrane potential of $-58 \pm 3 \text{ mV}$ and externally applied ET produced membrane depolarization in a dose-dependent manner. These effects were reduced by intracellular GDP beta S. A comparison of the concentration-dependent membrane depolarizations on pacemaker potentials to ET-1, ET-2 and ET-3 showed a rank order of potency $\text{ET-1} \geq \text{ET-2} \geq \text{ET-3}$ in cultured murine small intestinal ICCs. The pretreatment with Ca^{2+} -free solution and thapsigargin, a Ca^{2+} -ATPase inhibitor in endoplasmic reticulum, abolished the generation of pacemaker potentials and suppressed the ET-1 induced membrane depolarizations. Chelerythrine and calphostin C, protein kinase C inhibitors or naproxen, an inhibitor of cyclooxygenase, did not block the ET-1 induced effects on pacemaker potentials. Pretreatment with BQ-123 (ETA receptor antagonist) or BQ-788 (ETB receptor antagonist) blocked the ET-1 induced effects on pacemaker potentials in cultured murine small intestinal ICCs. However, pretreatment with BQ-788 selectively did not block the ET-1 induced effects on pacemaker potentials in cultured murine large intestinal ICCs. Also, only externally applied selective ETB receptor agonist, IRL 1620 did not show any influence on pacemaker potentials in cultured murine large intestine ICCs. RT-PCR results indicated the presence of the ETA and ETB receptor in ICCs. These results suggested that ET-1 modulates pacemaker potentials through ETA and ETB receptor activation in murine small intestinal ICCs and ETA receptor activation in murine large intestinal ICCs by

external Ca²⁺ influx and internal Ca²⁺ release via protein kinase C or cyclooxygenase-independent mechanism. Therefore, the ICCs are targets for ET and their interaction can affect intestinal motility.

Huisinga JD et al. (1995). *Nature* 373, 347-352.

Jun JY et al. (2004). *Br J Pharmacol* 141, 670-677.

Choi S et al. (2006). *Cell Physiol Biochem* 18, 187-198.

Sakurai T et al. (1990). *Nature* 348, 732-735.

Lin WW et al. (1990). *Eur J Pharmacol* 190;176:57-62.

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PC165

Influence of temperature on erythrocyte ATP release in humans

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Heating and cooling alter local limb tissue blood flow and its surrogate oxygen and blood supply in humans and animals, but the temperature sensitive mechanisms remain unknown. In the clinical and athletic settings heating [1] and cooling [2] are routinely used to improve recovery or alleviate symptoms. We have recently demonstrated that passive heating increases limb tissue blood flow in humans in association with the increases in muscle temperature and arterial plasma adenosine 5'-triphosphate (ATP) [3, 4] and that the erythrocytes are the sole blood source of ATP (3). However, the effect of cooling on erythrocyte ATP release has never been investigated. Here we tested the hypothesis that the release of the vasodilator and sympatholytic mediator ATP from human erythrocytes is sensitive to both increases and reductions in blood temperatures. To accomplish this aim, erythrocytes, plasma and serum were isolated from blood taken from 12 volunteers. To investigate the effects of heating and cooling, samples were exposed to physiological temperatures in water baths set at 39°C (hot), 33°C (control), 27°C (cool) and 20°C (coldest) for 20 mins. ATP was measured after the addition of a "stop solution" which prevented any further release or degradation and analysed by using a luciferin-luciferase technique while free haemoglobin was assessed as an indicator of haemolysis. Values reported are means $\pm$ SEM for n=12. Erythrocyte ATP release was increased from 0.61 ± 0.13 μ mol/L at 33°C to 1.14 ± 0.19 μ mol/L at 39°C ($P < 0.05$). ATP release from erythrocytes exposed to 27°C was 0.49 ± 0.08 μ mol/L whilst at the coldest temperature of 20°C ATP release was significantly reduced to 0.39 ± 0.05 μ mol/L ($P < 0.05$) compared to control. Consequently, the release of ATP from erythrocytes was tightly associated with changes in temperature from 20°C to 39°C ($r^2 = 0.97$; $P < 0.05$). Free haemoglobin ranged from 0.079 ± 0.007 g/L at 33°C, 0.073 ± 0.012 g/L at 39°C, 0.074 ± 0.014 g/L at 27°C and 0.060 ± 0.007 g/L at 20°C indicating that temperature did not increase haemolysis. In contrast, no changes in ATP levels were observed in either isolated plasma or serum samples at these temperatures. In conclusion, these results demonstrate that erythrocyte ATP release is sensitive to phys-

iological changes in temperature produced by local heating and cooling. Furthermore, they imply that heat and cooling can be used as a non-pharmacological means of manipulating the supply of oxygen and blood to human tissues for therapeutic benefit.

Green DJ, Carter HH, Fitzsimons MG, Cable NT, Thijssen DH, Naylor LH. Obligatory role of hyperaemia and shear stress in microvascular adaptation to repeated heating in humans. *J Physiol.* 2010;588(Pt 9):1571-1577.

Swenson C, Sward L, Karlsson J. Cryotherapy in sports medicine. *Scand J Med Sci Sports.* 1996;6(4):193-200.

Kalsi KK, González-Alonso J. Temperature-dependent release of ATP from human erythrocytes: mechanism for the control of local tissue perfusion. *Exp Physiol.* 2012;97(3):419-432.

Pearson J, Low DA, Stohr E, Kalsi K, Ali L, Barker H, González-Alonso J. Hemodynamic responses to heat stress in the resting and exercising human leg: insight into the effect of temperature on skeletal muscle blood flow. *Am J Physiol Regul Integr Comp Physiol.* 2011;300(3):R663-673.

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PC166

Structure activity relationships of novel BK channel modulators: The GoSlo-SR family

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We have synthesized novel anthraquinone derivatives called the GoSlo-SR family, which open large conductance Ca²⁺ activated K⁺ (BK) channels.

All experiments were approved by DKIT Animal Care and Use Committee. Rabbits were humanely killed with pentobarbital (125 mg/kg, i.v.), their bladder removed and smooth muscle strips cut into 1 mm³ pieces. Single bladder smooth muscle cells (UBSMC) were isolated as previously described (Hollywood et al., 1998) and plated in 35mm Petri dishes prior to study. RBSMC were isolated as previously described (Hollywood et al., 1998) and plated in 35mm Petri dishes. Experiments were performed at 37°C using excised inside/out patch configuration with symmetrical K⁺ solutions (pH 7.2) containing 140 mM KCl, 10 mM Glucose, 10 mM HEPES and either 1 μ M EGTA (for [Ca²⁺] 100 nM) or 1 μ M HEDTA (for [Ca²⁺] > 300 nM). Free [Ca²⁺] was determined using Chelator software (Schoenmaker et al., 1992).

Patches were held at -60 mV and currents evoked by voltage ramps (100 mV sec⁻¹). Single channel conductance was 335 ± 5 pS, (mean $\pm$ SEM, n=9). Currents reversed at K⁺ equilibrium potential, were abolished by the BK channel blocker penitrem A (100 nM, n=5) and a 10 fold increase in [Ca²⁺]i to 1 μ M shifted the activation V_{1/2} from 120 ± 6 mV to 5 ± 9 mV ($p < 0.01$, paired t test; $\Delta V_{1/2} = -115 \pm 5$ mV, n=9).

Compounds (structure shown Fig 1) were synthesized by microwave irradiation using the copper-catalyzed Ullmann coupling reaction (Baqi & Müller, 2007), their structures assigned by ¹H NMR and high resolution mass spectra and purity determined by HPLC. All compounds were screened at 10 μ M.

When aliphatic D ring size was changed from cyclopropane to cycloheptane, $\Delta V_{1/2}$ changed from 8 ± 6 mV (n=5) to -61 ± 6 mV (n=6). Substitutions of the cyclohexane ring with a ben-

zene ring did not alter $\Delta V_{1/2}$ further (-51 ± 10 mV, $n=4$) but replacement with bromine slightly inhibited the channels ($\Delta V_{1/2} +14 \pm 9$ mV, $n=6$). In subsequent syntheses, aniline derivatives were used to produce a library of compounds in order to study the structure activity relationships (SAR) further. Addition of electron withdrawing or hydrophilic groups in the meta position of ring D significantly reduced efficacy ($\Delta V_{1/2}$ by <25 mV), whereas hydrophobic meta-substituents, such as ethyl, isopropyl, and tert-butyl increased efficacy and shifted $V_{1/2}$ by >90 mV. Enhancing phenyl ring lipophilicity with meta-CF₃ and para-methyl groups produced the most efficacious member of this family, GoSlo-SR-5-44, which shifted $V_{1/2}$ by -142 ± 8 mV ($n=12$). This was more effective than the meta-CF₃ substituent, GoSlo-SR-5-6 (-107 ± 7 mV, $n=12$, $p<0.05$). However, these compounds were equipotent (EC₅₀ of 2.3 μ M and 2.4 μ M respectively). The effects of GoSlo-SR-5-6 were abolished in the presence of penitrem A (100 nM, $n=6$). The GoSlo-SR family are novel BK channel openers and increasing hydrophobicity of ring D enhances efficacy.

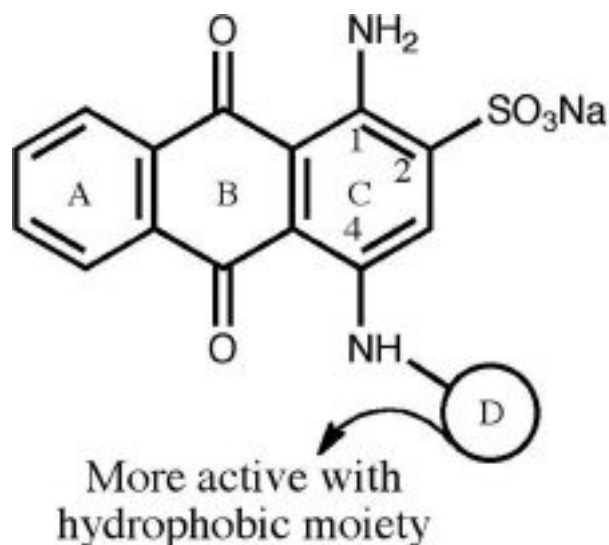


Fig 1. General structure of GoSlo-SR family of BK channel openers.

Baqi Y & Müller CE. (2007). *Org Lett.* 9:1271-4.

Hollywood, M.A., Cotton, K.D., McHale, N.G. & Thornbury, K.D. (1998). *Pflugers Archives* 435, 631-636

Schoenmakers T.J., Visser, G.J., Flik, G. and Theuvsen AP. (1992). CHELATOR: An Improved Method for Computing Metal Ion Concentrations in Physiological Solution. *BioTechniques*. 12(6) 870-879.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC167

The regulation of Ca²⁺-activated chloride channels by cholesterol and phosphatidylinositol 4,5-bisphosphate in rat pulmonary artery

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Introduction. Ca²⁺-activated chloride channels (ClCa) are important excitatory mechanism in vascular myocytes because activation of ClCa leads to Cl⁻ ion efflux and membrane depolarisation sufficient to enhance voltage gated Ca²⁺ channels. Our lab recently showed that the amplitude and pharmacology of ClCa currents (I_{ClCa}) in portal vein myocytes was influenced by lipid raft integrity (1). The present study aimed to ascertain whether I_{ClCa} in rat pulmonary arteries (PA) myocytes were also regulated by cholesterol as well as phosphatidylinositol 4, 5-bisphosphate (PIP₂) levels.

Methods. Whole-cell configuration of the patch clamp technique was used to measure I_{ClCa} currents in myocytes dispersed from first and secondary-order branches of PA, from male Wistar rats (200-225g). The pipette solution contained a known amount of free Ca²⁺ (25-500 nM) which immediately induced an I_{ClCa} upon rupture of the cell membrane. Upon stabilisation of the I_{ClCa} , current voltage (IV) relationships were constructed by stepping from a holding potential (-50mV) to test potentials between -100 mV and 100 mV for 1s. All experiments were recorded after a control IV with 500nM [Ca²⁺]_i, apart from wortmannin which was pre-incubated with the cells for 1 hour, here untreated myocytes I_{ClCa} was recorded on the same day. I_{ClCa} was normalised to cell capacitance.

Results. I_{ClCa} in rat PA were activated in a Ca²⁺-dependent manner and displayed the distinctive voltage-dependent kinetics and anionic permeability profile of previous studies. Application of niflumic acid (100 μ M) produced a characteristic complex effect whereas the novel TMEM16A inhibitor T16Ainh-A01 (5 μ M) produced simple inhibition. I_{ClCa} was augmented rapidly by the cholesterol depleting agent methyl- β -cyclodextrin (3mg/ml) and after 1 h incubation with the phosphatidylinositol 4-kinase inhibitor wortmannin (20 μ M) (~73.5% and 80.8% respectively, $n=5$). Conversely, the α 1-adrenergic receptor agonist methoxamine (1 μ M) attenuated the I_{ClCa} by ~32% ($n=3$).

Conclusion. This study demonstrates that I_{ClCa} are augmented by cholesterol depletion from the cell membrane and reduction of PIP₂. An augmented I_{ClCa} in a response to a reduction in levels of PIP₂ suggests that PIP₂ has an inhibitor function on the channel. However, activation of Gq linked α 1-adrenergic receptors produced a marked reduction in I_{ClCa} suggesting the regulation of ClCa by PIP₂ is complicated.

Sones et al., 2010 Cardiovascular Research 87 (3): 476-484

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PC168

Reactive oxygen species inhibit Slo1 BK_{Ca} channels in undifferentiated human mesenchymal stem cellsA. Soloviev¹, S. Tishkin¹, I. Ivanova¹, I. Prudnikov² and V. Tsyvkin²¹*Institute of Pharmacology and Toxicology, National Academy of Medical Sciences, Kiev, Ukraine and* ²*Bogomoletz Institute of Physiology, National Academy of Sciences, Kiev, Ukraine*

Human mesenchymal stem cells (hMSCs) are non-hemopoietic stem cells possessing multidifferential potential. One possible application of the hMSCs is the repair of high conductance Ca²⁺-sensitive voltage-dependent K⁺ channels (Slo1 BK_{Ca} or Maxi-K⁺ channels) function in vascular smooth muscle cells (SMC) damaged by oxidative stress following ionizing irradiation (Soloviev et al., 2010). Ionic channels are important for hMSCs differentiation but there is a little information on electrophysiological characteristics (Li, Deng, 2011) and function of hMSCs under oxidative stress. Experimental design of the study comprised patch-clamp technique and RT-PCR analysis. Bone marrow was aspirated in heparin from the sternum of 4 human male healthy volunteers of ages 38 - 44 years after informed consent and Institute Hematology and Transfusion Ethics Committee approval. To reduce the burning effect of lidocaine solution, local anesthesia was achieved using 1% lidocaine (up to 4 microgram/kg) in 1% sodium bicarbonate solution injected subcutaneously and into periosteum. hMSCs derived from bone marrow were separated using negative selection procedure with monoclonal antibodies and cultured as monolayer 20 - 32 days. At the end of cultivation hMSC were detached from the bottom of the flasks using trypsin-EDTA and the suspension was prepared for electrophysiological studies. Phenotypic RT-PCR analysis showed that hMSCs were negative for hematopoietic cell markers (CD14, CD34, CD45) and positive for hMSCs markers (CD29, CD44, CD71, CD73, CD90, CD105, CD166). Membrane currents were measured in the whole cell configuration of the patch clamp technique at 210 C. hMSCs were stimulated with increasing depolarizing 10 mV voltage steps 300 ms duration from a holding potential of -60 mV. All hMSCs demonstrated a rapidly activating at potentials positive to +10 mV non-inactivated outward currents with noisy oscillation typical for Ca²⁺-activated K⁺ currents. Mean current density in hMSCs was 41 ± 3 pA/pF at +70 mV vs. 71 ± 6 pA/pF in rat thoracic aorta SMC, and membrane potential was -72 ± 5 mV vs. -51 ± 3 mV in SMC. The currents were identified as carrying through BK_{Ca} channels preferentially since severely inhibited by externally applied BK_{Ca} inhibitor, paxilline (500 nM), from 41 ± 3 pA/pF to 10 ± 2 pA/pF (P<0.05) vs. 71 ± 6 and 23 ± 2 pA/pF (P<0.05) in SMC. hMSCs capacitance as a measure of cell size was 44 ± 5 pF vs. 4 ± 0.3 pF in SMC. Xanthine (X, 0.1 mM) plus xanthine oxidase (XO, 0.015 U/ml) were used to generate reactive oxygen species (ROS). 15 s of radicals exposure results in decrease of outward current amplitude from 41 ± 3 pA/pF to 13 ± 1 pA/pF (P<0.05). Whole body ionized irradiation (6 Gy) - induced oxidative stress decreased outward currents in SMC from 71 ± 6 to 24 pA/pF (P<0.05). Paxilline application was without effect in both hMSC and SMC treated with X - XO combination or irradiation, respectively, suggesting the absence or inability of BK_{Ca} channels in plasma membranes. In conclusion, ROS possess the ability to inhibit Slo1 BK_{Ca} channels embedded into undifferentiated hMSCs plasma membrane in a manner similar to damaging effect of ionized irradiation on BK_{Ca} in SMC.

Soloviev A. Prudnikov I., Tsyvkin V. et al. Electrophysiological and contractile evidence the ability of human mesenchymal stromal stem cells to correct vascular malfunction in rats after ionizing irradiation. The J. of Physiol. Sci. (Japan), 2010, 60, 2, 161-172.

Li GR, Deng XL. Functional ion channels in stem cells. World J. Stem Cells, 2011, 26; 3(3): 19-24.

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PC169

Functional Ca²⁺ sensors do not contribute to the effect of the novel BK channel agonist GoSlo-SR5-6R. Large², T.I. Webb², A. Akande¹, S. Roy², G.P. Sergeant^{1,2}, K.D. Thornbury^{1,2}, N.G. McHale^{1,2} and M.A. Hollywood^{1,2}¹*Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Ireland and* ²*Ion Channel Biotechnology Centre, Dundalk Institute of Technology, Dundalk, Ireland*

We have synthesised a novel BK channel opener GoSlo-SR5-6, that mimics the effect of Ca²⁺ by shifting the voltage required for half maximal activation in 100nM Ca²⁺ by -107±7 mV, (n=12, 10 µM) in native rabbit bladder smooth muscle cells. We hypothesised that this molecule interacts with the Ca²⁺ sensing apparatus of the BK channel.

All experiments were carried out on BK channel α subunits cloned from the rabbit urethra and expressed in human embryonic kidney cells (HEK293). Site-directed mutagenesis on the resulting cDNA was carried out using the method of Sawano & Miyawaki (2000) and confirmed by sequencing. HEK cells were grown in DMEM medium supplemented with 10% FCS, penicillin and streptomycin. HEK cells were dissociated with trypsin (1%), plated onto 35 mm Petri dishes and maintained in culture at 37°C in 5% CO₂ prior to use. All experiments were performed at 37°C using the excised inside/out patch configuration with symmetrical K⁺ solutions containing 140 mM KCl, 10 mM Glucose, 10 mM HEPES and either 1 mM EGTA (for free [Ca²⁺] 100 nM) or 1 mM HEDTA (for free [Ca²⁺] > 300 nM). All solutions had a pH of 7.2 and the pipette solution contained 100 nM free Ca²⁺.

The BK α subunit has a large cytoplasmic tail that contains two high affinity Ca²⁺ binding sites in the RCK1 domain and Ca²⁺ bowl respectively. When we examined the Ca²⁺ sensitivity of BK α subunits re-expressed in HEK 293 cells, a 10-fold increase in Ca²⁺ from 100 nM to 1 µM shifted the activation V_{1/2} from 177±4 mV to 92±6 mV (Δ V_{1/2}= -85±5, n=6, p<0.05 paired t test). When GoSlo-SR5-6 (10 µM) was applied in the presence of 100 nM Ca²⁺ the Δ V_{1/2} was -90 mV (n=6, p<0.05). To examine if abolition of Ca²⁺ sensing in the RCK1 domain reduced these effects, we mutated D367A (Xia et al., 2002) and found that although the Ca²⁺ sensitivity of the channels was decreased compared to the normal BK α , the effect of GoSlo-SR5-6 was not diminished, suggested that a functional Ca²⁺ sensor in the RCK1 domain was not required for these molecules to exert their effects.

We carried out a double mutation on M513I in the RCK1 domain and D898A in the Ca²⁺ bowl (Bao et al., 2004). This double (RCK & BOWL) mutant was practically insensitive to Ca²⁺ below 100 µM and a hundred-fold increase in Ca²⁺ from 100 nM to 10 µM only shifted the activation V_{1/2} from 144±8 mV to 115±10 mV (n=4). Despite this reduction in the chan-

nel's Ca^{2+} sensitivity, application of 10 μM GoSlo-SR5-6 still shifted the activation $V_{1/2}$ of the channel to $47 \pm 8 \text{ mV}$ ($\Delta V_{1/2} = -97 \pm 10 \text{ mV}$, $n=4$).

These data suggest that GoSlo-SR-5-6 can still activate $\text{BK}\alpha$ subunits in the absence of functional high affinity Ca^{2+} sensors.



Fig 1. Structure of GoSlo-SR-5-6

Bao L, Kaldany C, Holmstrand EC, Cox DH. (2004) Mapping the BKCa channel's " Ca^{2+} bowl": side-chains essential for Ca^{2+} sensing. *J Gen Physiol.* 23(5):475-89.

Bian S., Favre I, and E. Moczydlowski F. (2001). Ca^{2+} -binding activity of a COOH-terminal fragment of the *Drosophila* BK channel involved in Ca^{2+} -dependent activation. *Proc. Natl. Acad. Sci.* 98:4776-4781

Sawano A & Miyawaki A. (2000) Directed evolution of green fluorescent protein by a new versatile PCR strategy for site-directed and semi-random mutagenesis. *Nucleic Acids Res.* 28(16):E78.

Xia XM, Zeng X, Lingle CJ. (2002) Multiple regulatory sites in large-conductance calcium-activated potassium channels. *Nature.* 22:418(6900):880-4.

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PC170

Aldosterone regulates the TRPM7 chanzyme

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Recently, it has been shown that transient receptor potential melastatin 7 (TRPM7) expression is modified by vasoactive agents (aldosterone and angiotensin II), suggesting a potential role for TRPM7 in hypertension. TRPM7 is a ubiquitously expressed chanzyme (channel + enzyme) that contains a cation permeable pore, as well as a kinase domain at the COOH-terminus. It is also essential for life, as genetic deletion of TRPM7 in mice is embryonic lethal. At pH 7.4 and with Mg^{2+} in solution, TRPM7 exhibits non-rectifying inward kinetics, rectifying outward kinetics, and a reversal potential of 0 mV. Under these conditions, TRPM7 is permeant to divalent cations at negative potentials and monovalent cations at positive potentials. Presently, it is not known if the vasoactive agents mentioned above alter TRPM7's functional characteristics. Thus, the pur-

pose of this study was to determine the effects of aldosterone on TRPM7's biophysical properties in tetracycline-induced, stably-expressed HEK293 cells. Using whole-cell voltage-clamp, currents were characterized using a ramp protocol from -100 mV to +100 mV (50 ms), at a frequency of 0.5 Hz. Current-voltage (IV) relationships were statistically compared between aldosterone treated and control cells using repeated measures two-way ANOVAs with post bonferroni tests. Currents (pA) are expressed as mean $\pm$ S.E.M. The results indicate that TRPM7 is functionally modulated by 100 nM aldosterone, only after 18 hour (h) induction (control, $n=7$; aldosterone, $n=8$; $P = 0.0099$). Direct application of aldosterone using superfusion (0 h; $n=6$), or after a 4 h induction period ($n=5$), generated superimposed IV relationships compared to controls ($n=8$). As well, the aldosterone effect at 18 h is only seen in the rectifying component of the IV relationship, becoming significant at 80 mV. At this voltage, current values for control, time 0 h, time 4 h, and time 18 h aldosterone were $2204.3 \pm 226.8 \text{ pA}$ ($n=8$), $2247.6 \pm 182.0 \text{ pA}$ ($n=6$), $2304.2 \pm 249.9 \text{ pA}$ ($n=5$), and $3172.2 \pm 104.3 \text{ pA}$ ($P < 0.0001$, $n=6$), respectively. Also, both control (+80mV, 1680.1 ± 109.8 ; $n=7$) and 18 h aldosterone stimulated currents (+80mV, 2827.0 ± 341.4 ; $n=8$) were blocked by application of 50 μM 2-aminoethoxydiphenyl bromate (control: $601.6 \pm 139.6 \text{ pA}$, $n=6$; 18 h aldosterone: 669.0 ± 204.9 , $n=4$), a known TRPM7 blocker. Finally, when comparing western blot analysis of control and 18 h induced aldosterone cell lysates ($n=1$), there was no effect on the amount of TRPM7 or mineralocorticoid receptor protein. Thus, aldosterone has a very specific biophysical effect on TRPM7 ion channels, requires over 4 hours of induction time to do so and is not dependent on cellular TRPM7 protein levels. These results suggest that aldosterone is transcriptionally activating a regulatory factor that modulates TRPM7. The connection between this modulation and hypertension remains to be determined.

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Multiple actions of TRPM8 agonists in rat tail artery vascular smooth muscle

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The non-selective cation channel TRPM8 is present and functional on vascular smooth muscle (Yang *et al.*, 2006; Johnson *et al.*, 2009). The TRPM8 agonist menthol causes vasoconstriction in relaxed vessels or vasodilatation in precontracted vessels (Johnson *et al.*, 2009). These diverse actions suggest multiple actions of this and other TRPM8 agonists. Previous studies have revealed that menthol can potentially inhibit voltage-gated Ca^{2+} currents (I_{Ca}) in DRG and cardiac muscle cells (Swandulla *et al.*, 1987; Baylie *et al.*, 2010). In the present study we investigated whether menthol and other TRPM8 agonists may have actions in vascular smooth muscle mediated by such an additional mechanism.

Tail arteries were obtained from humanely-dispatched male Sprague-Dawley rats (12 weeks). Single vascular smooth muscle cells (VSMC) were isolated by enzymatic digestion for patch clamp analysis. Vascular rings were endothelium denuded for isometric contraction studies.

In voltage-clamped tail artery VSMCs, TRPM8 agonists icilin (50 μ M), menthol (300 μ M) and WS-12 (50 μ M) were found to significantly reduce peak I_{Ca} compared to their respective vehicle-only controls (one-way ANOVA, $P < 0.001$; $n = 8$; $N = 3$, where N = number of rats, n = number of cells). Isometric contraction studies demonstrated that menthol (300 μ M) caused vessels precontracted with phenylephrine (PE: 2 μ M; 0.71 ± 0.06 g (mean $\pm$ SEM), $N = 15$, $n = 19$, where N = number of rats, n = number of preparations) to initially contract (108.4 ± 7.3 % of PE control, $P < 0.01$, one-way ANOVA, $n = 26$, $N = 19$) and then relax (73.5 ± 2.4 % of PE control, $P < 0.001$). However, when vessels were pre-incubated with L-type calcium antagonist, nifedipine (10 μ M), PE-induced contractions were decreased by 46.5 ± 3.0 % ($n = 41$, $N = 15$) compared to control conditions and the major response to menthol application was a strong contraction (140 ± 8 % of control, $P < 0.001$, $N = 7$, $n = 10$). When vessels were incubated with the TRPM8 antagonist, AMTB (10 μ M), this menthol-induced contraction was strongly inhibited (TRPM8: 80 ± 9 % of control, $P < 0.001$, $N = 9$, $n = 9$). We conclude that the vasodilatory actions of TRPM8 agonist, menthol, on precontracted vascular smooth muscle is likely to reflect effective L-type calcium channel blockade. Without this additional effect of menthol on calcium channels (e.g. in the presence of nifedipine), a TRPM8-specific contraction is revealed.

Yang *et al.* (2006) *Am J Physiol* 290, L1267-L1276

Johnson *et al.* (2009) *Am J Physiol* 296, H1868-H1877

Baylie *et al.* (2010) *J Physiol Pharmacol* 61, 543-550.

Swandulla *et al.* (1987) *Pflügers Archiv* 409, 52-59

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PC172

The role of endothelium in VIP-induced relaxation of the human submandibular artery

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It was established in animal models that vasoactive intestinal peptide (VIP) significantly increases blood flow in the salivary glands, potentiates vasodilatory response to acetylcholine, and stimulates salivation. We hypothesized that endothelium can modulate vascular response of the human submandibular artery to VIP, therefore the aim of the study was to evaluate the contributions of endothelial diffusible vasodilatory factors on VIP-induced relaxation. This was achieved using following substances: the nitric oxide synthase inhibitor N(G)-nitro-L-arginine (L-NOARG), the potassium channel blocker 4-aminopyridine (4-AP), and the cyclo-oxygenase inhibitor indomethacin. Seven patients (ages 30-45 yrs; four females and three males), undergoing surgery for salivary duct calculus excision, were enrolled in this study. During surgery, submandibular arteries were ligated and dissected into segments (2 segments from each patient). Segments were immediately

immersed in ice-cold Krebs-Ringer bicarbonate solution and transported to in vitro experimental set-up within 20 minutes. All experimental procedures were conducted after obtaining written informed patient consent and approval of the University of Nis Faculty of Medicine Ethical Committee and were performed in accordance with the Declaration of Helsinki. VIP (3×10^{-9} - 3×10^{-7} mol/L) induced a concentration-dependent relaxation in endothelium-containing arterial segments ($pEC_{50} = 7.62 \pm 0.01$; maximal relaxation = 80.4 ± 4.2 %, $n = 7$), but not in endothelium-denuded segments of the human submandibular artery. This VIP-induced relaxant response was significantly reduced after pretreatment with L-NOARG (10-5 mol/L) ($pEC_{50} = 7.50 \pm 0.10$ vs. 7.30 ± 0.09 ; maximal relaxation = 79.3 ± 6.1 vs. 39.3 ± 3.8 %, $n = 5$, $p < 0.05$) and almost completely inhibited after pretreatment with 4-AP (10-5 mol/L) ($pEC_{50} = 7.67 \pm 0.03$ vs. 7.58 ± 0.13 ; maximal relaxation = 84.5 ± 1.5 vs. 18.0 ± 2.7 %, $n = 5$, $p < 0.01$). On the other hand, pretreatment of the arterial segments with indomethacin (10-5 mol/L) failed to induce any effect ($pEC_{50} = 7.56 \pm 0.14$ vs. 7.66 ± 0.05 ; maximal relaxation = 78.0 ± 3.1 vs. 85.5 ± 2.6 %, $n = 4$, $p > 0.05$). Since vascular effects of VIP are usually mediated through cAMP signaling pathway, they were tested in the presence of an adenylate cyclase inhibitor, forskolin (10-5 mol/L). Our results shown that relaxant effect of VIP was not significantly changed. We concluded that VIP has direct action on the human submandibular artery inducing endothelium-dependent relaxation presumably by releasing the endothelium-derived hyperpolarizing factor and NO.

Roganovic J, Radenkovic M, Stojic D. ACh- and VIP-induced vasorelaxation in rabbit facial artery after carotid artery occlusion. *Arch Oral Biol*. 2010;55(5):333-42.

Stojic D, Pesic S, Radenkovic M, Popovic-Roganovic J, Pesic Z, Grbovic L. Responses of the human submandibular artery to ACh and VIP. *J Dent Res*. 2007;86(6):565-70.

Pesic S, Grbovic L, Radenkovic M, Stojic D, Nikolic V, Cvetkovic Z. The relaxant effect of vasoactive intestinal polypeptide in the isolated canine uterine artery: the role of endothelium. *J Vet Med A Physiol Pathol Clin Med*. 2004;51(9-10):394-9.

Henning RJ, Sawmiller DR. Vasoactive intestinal peptide: cardiovascular effects. *Cardiovasc Res*. 2001;49(1):27-37.

McIntyre CA, Buckley CH, Jones GC, Sandeep TC, Andrews RC, Elliott AI, Gray GA, Williams BC, McKnight JA, Walker BR, Hadoke PW. Endothelium-derived hyperpolarizing factor and potassium use different mechanisms to induce relaxation of human subcutaneous resistance arteries. *Br J Pharmacol*. 2001;133(6):902-8.

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PC173

The AGE-breaker ALT-711 restores high-blood-flow-dependent remodeling in mesenteric resistance arteries in type 2 diabetic rats

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Objective: Advanced glycation end products (AGEs) are generated by glucose in diabetes. Flow-mediated remodeling of resistance arteries (RA) is essential for revascularization in ischemic diseases, but this is impaired in diabetes. We hypoth-

esized that breaking AGE crosslinks could improve flow-dependent remodeling in mesenteric RA in Zucker Diabetic Fatty (ZDF) rats.

Research Design and Methods: The animals were anesthetized (Isoflurane, 2.5%) and mesenteric resistance arteries were exposed to high (HF) or normal blood flow (NF) after alternate arterial ligation *in vivo* and were compared to age-matched lean Zucker (LZ) rats. All animals were treated with buprenorphine (Temgesic®; 0.1 mg/kg, s.c.) before and after surgery. Half of the rats were simultaneously treated by i.p. injection with ALT-711 (3 mg/kg per day).

Results: In LZ rats, HF artery diameter was larger than for NF vessels, but this was not the case in ZDF rats. Furthermore, endothelium-mediated dilation in ZDF rats, which was lower than in LZ rats, was further

decreased in HF arteries. Treatment of rats with the AGE-breaker ALT-711 reversed the diabetes-induced impairment of HF-dependent remodeling, as shown by an increased arterial diameter. Breaking AGE crosslinks also improved cross-sectional compliance and endothelium-dependent relaxation in mesenteric RA. Additionally, contribution of the major endothelium-derived relaxing factor, nitric oxide, increased in acetylcholine-induced relaxation in NF and HF arteries in ZDF rats treated with ALT-711.

Accumulation of AGEs and the receptor for AGE expression was reduced by ALT-711 in arteries in ZDF rats. Finally, ALT-711 increased eNOS protein expression in both HF and NF arteries. **Conclusions:** AGE crosslinks oppose outward remodeling of RA in response to chronic increases in blood flow. Breaking AGE crosslinks provides a therapeutic potential for overcoming the microvascular complications of ischemic diseases in long-term diabetes.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC174

Effects of hyperbaric oxygen treatment on response of middle cerebral arteries to reduced pO₂ in diabetic rats

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INTRODUCTION: Diabetes mellitus leads to impaired endothelial function. Clinical studies established beneficial role of hyperbaric oxygen therapy in diabetic wound healing, however, the mechanisms are not clearly defined. The aim of this study was to determine the effect of intermittent hyperbaric oxygenation (HBOT) on vascular responses to reduced pO₂ in diabetic rats.

METHODS: Prior to experiments, rats were anesthetized with ketamin (75 mg/kg) and midazolam (2.5 mg/kg) i.p. Isolated middle cerebral arteries (MCA) of male control, diabetic DM (streptozocin 60mg/kg i.p.) and HBOT treated diabetic rats (DM+HBOT) Sprague-Dawley rats (N=10 per group) were mounted on glass pipettes of DMT 110P pressure myograph system for internal diameter measurements in normoxia (21% O₂ in superfusate and perfusate, and reduced pO₂ (0%) condition. HBOT rats were treated in a hyperbaric chamber with 100% O₂ (2 bar) 2 hours/day for 4 consecutive days. To assess the role of CYP450-epoxygenases' vasodilating metabolites (EETs), the CYP450 inhibitor - clotrimazole was used. The role of KATP channels in hypoxic response was assessed using glibenclamide (KATP channels blocker). The study was

approved by the Ethical Committee of the Faculty of Medicine University J.J. Strossmayer Osijek.

RESULTS: Results are shown as mean ± SEM (microns). For statistical analysis One way ANOVA was used. Control rats exhibit significant vasodilation in response to reduced pO₂ (20±4) compared to DM rats (0±3) while vasodilation was restored in DM+HBOT rats (14±2). In the presence of clotrimazole, vasodilation to reduced pO₂ was preserved in control group (18±2), and attenuated in DM+HBOT (2±1), similarly to impaired response in DM group (7±3). Glibenclamide eliminated dilation in all groups.

CONCLUSION: This study demonstrated that HBOT restored vasodilation in response to reduced pO₂ in diabetic rats which is mediated via KATP channels, possibly due to effect of EETs on KATP channels.

Unfirer, S., Kibel, A., Drenjancevic-Peric, I. 2008. The effect of hyperbaric oxygen therapy on blood

vessel function in diabetes mellitus. *Med Hypotheses* 71, 776-780.

Harder DR, Narayanan J, Birks EK, et al. Identification of a putative microvascular oxygen sensor. *Circ Res* 1996; 79, 54-61.

Ye D, Zhou W, Lu T, Jagadeesh SG, Falck JR, Lee HC. Mechanism of rat mesenteric arterial KATP channel activation by 14,15-epoxyeicosatrienoic acid. *Am J Physiol Heart Circ Physiol* 2006; 290(4):H1326-36.

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PC175

Sex differences in vascular endothelial cell function reveal a gender specific role for nitroxyl

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Nitric oxide (NO; which plays a critical role in vascular health) is produced by eNOS in endothelial cells (EC) and activates soluble guanylate cyclase (sGC) to induce vasorelaxation. NO can also exist as the vasorelaxant nitroxyl (HNO) although the precise role for this form is not fully understood. Given that gender-specific variations exist in vascular activity we investigated if HNO generation underlies any differences between the sexes. Using isolated rings of aorta from female (n=16) and male (n=20) Sprague Dawley rats, and following sub-maximal contraction with phenylephrine (PE), responses to ACh (1nM - 3µM) were measured isometrically in EC-containing or -denuded rings with and without a range of pharmacological inhibitors. In separate experiments, the depression of PE (1nM - 3µM)-induced contraction was used as a measure of the tonic depressor influence of the EC in the absence of ACh. Data are given as means ± SEM, compared by ANOVA, n≥6 for all observations.

In aortic rings from both females and males, ACh-induced EC-dependent relaxation was completely abolished following incubation with either L-NAME (300µM; P<0.001), hydroxocobalamin (100µM; P<0.001) or ODQ (30nM; P<0.001) suggesting that the relaxation was dependent entirely on eNOS activity, generation of NO and sGC activation, respectively. As determined by the depression of PE-induced contraction, the tonic vasodepressor influence of the EC in aortic rings from females was shown to be completely reliant on eNOS, the NO radical and sGC activation (i.e. treatment with L-NAME, hydroxocobalamin and ODQ, respectively, altered PE-induced con-

traction so that it was indistinguishable from EC-denuded vessels, $P>0.05$). In aorta from males, EC-dependent depression of PE (max contraction in EC-containing rings was 1.0 ± 0.3 g vs. 2.0 ± 0.2 g in EC-denuded preparations; $P<0.001$) was also shown to be eNOS and sGC dependent (contraction in L-NAME and ODQ-treated tissue was 1.9 ± 0.1 g and 2.1 ± 0.2 g, respectively; neither statistically different from EC-denuded rings, $P>0.05$). However, in powerful contrast to females, inactivation of NO with hydroxocobalamin only partly impaired the influence of EC (1.6 ± 0.2 g; $P<0.01$ compared to EC-denuded rings) suggesting the presence of a non-NO mediator. Use of the established HNO scavenger L-cysteine (3mM) produced a partial inhibition of the vasodepressor influence of EC (1.5 ± 0.1 g; $P<0.05$ compared to EC-containing rings) but in combination with hydroxocobalamin produced an effect greater than when either was used independently (1.9 ± 0.2 g; indistinguishable from EC-denuded rings, $P>0.05$).

In conclusion, tonically active eNOS in aortic EC from male, but not female, rats generates both NO and HNO but the HNO contribution is lost when the EC are stimulated by ACh. These data identify a gender-specific role for HNO.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC176

Withdrawal of statin treatment leads to increased constriction in the mesenteric arteries of adult rats

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HMG-CoA reductase inhibitors (statins) are widely used for secondary prevention of cardiovascular disease and increasing evidence indicates benefits from primary prevention in risk groups. Chronic statin treatment from weaning has been shown to attenuate endothelial dysfunction in adult rats in a model of cardiovascular disease (Torrens et al. 2009) and evidence suggests that early targeted interventions can have long term benefits in this same model (Sherman and Langley-Evans 2000; Vickers et al. 2005). It was hypothesised that early age acute statin treatment would also improve the cardiovascular function in later life in a rat model of developmentally induced cardiovascular disease.

Pregnant Wistar rat dams were fed either 18% protein, control (C) or 9% protein restricted (PR) diet throughout pregnancy and were returned to standard chow on delivery. The pups were divided into controls and those receiving atorvastatin, 10mg/kg, (S) for two weeks from weaning at 3 weeks. This gave four experimental groups: control (C), control + statin (CS), protein restricted (PR) and protein restricted + statin (PRS). At 16 weeks blood pressure was assessed with non-invasive tail-cuff plethysmography and vascular reactivity of the mesenteric arteries (MA) was measured *ex vivo* by wire myography. The differences were assessed with one-way ANOVA and results are expressed as mean $\pm$ SEM. Significance was assumed at $p\leq0.05$.

Increased constriction to PE was observed in PRS groups in both males (C, 4.12 ± 0.16 mN/mm, $n=6$; CS, 4.57 ± 0.16 mN/mm, $n=6$; PR, 4.66 ± 0.29 mN/mm, $n=5$; PRS, 5.27 ± 0.21 mN/mm, $n=5$; $p<0.01$) and females (C, 3.22 ± 0.26 mN/mm, $n=5$; CS, 4.17 ± 0.23 mN/mm, $n=6$; PR, 3.82 ± 0.40 mN/mm, $n=5$;

PRS, 4.71 ± 0.24 mN/mm, $n=6$; $p<0.01$). In female MA increased constriction to endothelin was also shown in the PRS group (C, 3.27 ± 0.56 mN/mm, $n=4$; CS, 4.43 ± 0.36 mN/mm, $n=5$; PR, 3.44 ± 0.62 mN/mm, $n=4$; PRS, 6.20 ± 0.89 mN/mm, $n=5$; $p<0.01$). No differences were seen in systolic or diastolic blood pressure or heart rate between the groups.

These data show increased constriction in the statin treated PR offspring in adulthood. As data during statin treatment at 5 weeks indicated reduced constriction, the detrimental effects are attributed to statin withdrawal and subsequent rebound. The PR offspring appear to be more susceptible to this as no increase in constriction was observed in the control MA. Investigation into the mechanisms is ongoing.

Sherman, R.C. & Langley-Evans, S.C. 2000. Antihypertensive treatment in early postnatal life modulates prenatal dietary influences upon blood pressure in the rat. Clin.Sci.(Lond), 98, (3) 269-275 available from: PM:10677384

Torrens, C., Kelsall, C.J., Hopkins, L.A., Anthony, F.W., Curzen, N.P., & Hanson, M.A. 2009. Atorvastatin restores endothelial function in offspring of protein-restricted rats in a cholesterol-independent manner. Hypertension, 53, (4) 661-667

Vickers, M.H., Gluckman, P.D., Coveny, A.H., Hofman, P.L., Cutfield, W.S., Gertler, A., Breier, B.H., & Harris, M. 2005. Neonatal leptin treatment reverses developmental programming. Endocrinology, 146, (10) 4211-4216 available from: PM:16020474

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PC177

Modulation of aortic reactivity to angiotensin-(1-7) with intermittent hyperbaric oxygenation in diabetic rats

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INTRODUCTION: We previously reported a facilitating effect of hyperbaric oxygen therapy (HBOT) on responses of rat aortic rings to angiotensin-(1-7) (ANG-(1-7)) in healthy rats ('Physiology 2011')[1]. It was suggested that this influence was mediated by changes in production or sensitivity to epoxyeicosatrienoic acids (EETs). The aim was to assess whether HBOT exerts similar effects in diabetic rats and to further explore the role of cytochrome P450 (CYP) enzymes in changes induced by HBOT.

METHODS: Diabetes was induced by streptozocin in 6 weeks old male Sprague-Dawley rats. After 8 weeks, they were divided into control and HBOT group (exposed to 100% O₂(2 bars) 2 hours/day for 4 consecutive days). Before decapitation, rats were anesthetized with 75 mg/kg ketamine with 2.5 mg/kg midazolam. Thoracic aortic rings were used to test responses to 1 μ M ANG-(1-7) after precontraction with noradrenaline for 5 minutes (tension was read 3 minutes after ANG-(1-7) addition and the response expressed as percentage of precontraction decrease). Intactness of endothelium was previously tested. A separate series of HBOT rats was used to test ANG-(1-7) responses after the epoxygenases inhibitor MS-PPOH (10 μ M) – inhibitor of the CYP4A2 AND CYP4A3 epoxygenation reactions – was added for 15 minutes to the tissue bath. CYP 4A1, 4A2, 4A3 and 2J3 mRNA expression was determined with Quantitative real time PCR and was normalized to the expres-

sion of two housekeeping genes -HPRT and 18S. The study was approved by Ethical Committee of Faculty of Medicine University of Osijek.

The route of administration of both drugs is intraperitoneal (i.p.).

RESULTS: Mean percentage of precontraction decrease after ANG-(1-7) addition was significantly higher in the HBOT group ($19.2\% \pm 7.3$ [STDEV]) ($n=18$) than in the control group ($12.1\% \pm 6.4$) ($n=14$), $P=0.007$ (t-test). The ANG-(1-7) ring response of HBOT rats after MS-PPOH addition was $2.3\% \pm 3.3$ ($n=8$), significantly less pronounced compared to HBOT rings when no MS-PPOH was used, $P<0.001$ (Mann Whitney U). Median CYP2J3/18s expression was 12.2 (control) ($n=7$) and 94.8 (HBOT) ($n=6$), significantly different ($P=0.008$, Mann Whitney U). Median CYP2J3/HPRT expression was 7.5 (control) ($n=9$) and 49.7 (HBOT) ($n=9$), with significant difference ($P=0.042$). CYP4A1, CYP4A2 and CYP4A3 expression was similar between groups.

CONCLUSION: HBOT increases the reactivity of thoracic aortic rings to ANG-(1-7) in diabetic rats. The epoxygenase inhibitor MS-PPOH reverses these changes. HBOT increases mRNA expression of the epoxygenase CYP2J3 in diabetic rats, further suggesting an important role of EETs in HBOT-induced modulation of vascular function.

Kibel A, Cavka A, Drenjanecvic I. Effects of hyperbaric oxygen therapy on reactivity of rat aortic rings to angiotensin II and angiotensin-(1-7) [abstract]. Physiology 2011, Oxford, UK.

This study was partially supported by grant of Ministry of Science, Education and Sports, Croatia, #219-2160133-2034 and USPHS NIH GM31278 (to JRF).

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

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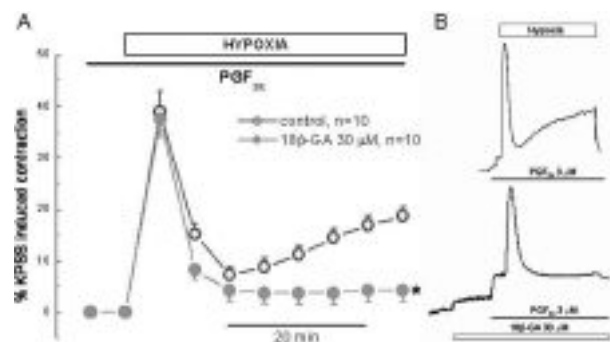
Possible role of gap junctions in the mechanisms of hypoxic pulmonary vasoconstriction in rats

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It is known that hypoxia causes pulmonary artery constriction normally maintaining optimal ventilation-perfusion matching in the lung but leading to pulmonary hypertension development. Although it is known that sustained hypoxic pulmonary vasoconstriction (HPV) is critically dependent on both the endothelium and glycolysis, the signaling pathways remain unclear [1]. The aim of this study was to determine the role of gap junctions in HPV development. The vascular tone was measured on isolated small intrapulmonary arteries (IPA) from male Wistar rats (200-250g) using a wire myograph technique. Animals were killed by pentobarbital injection (80 mg/kg). Values are means $\pm$ S.E.M., compared by Student's *t*-test for paired data. Hypoxia (PO_2 2–3 mm Hg) elicited a biphasic response in tension in IPA. The transient HPV phase I consisted $38.89 \pm 3.97\%$ of the contraction elicited with 80 mM K^+ (T_K) ($n=10$) and the sustained phase II reached a level of $18.62 \pm 1.78\% T_K$ ($n=10$) after 40 min of hypoxia in the vessels precontracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 3 μ M). Application of gap junctions inhibitor 18 β -glycyrrhetic acid (18 β -GA, 30 μ M) abolished HPV phase II ($4.2 \pm 2.36\% T_K$, $n=10$,

$P<0.001$) but had no effect on the phase I ($37.48 \pm 3.49\% T_K$, $n=10$, $P>0.05$) in $PGF_{2\alpha}$ -precontracted IPA. In IPA precontracted with 25 mM K^+ 18 β -GA (30 μ M) also inhibited HPV phase II ($1.86 \pm 2.49\%$, $n=7$ vs. $12.67 \pm 2.32\% T_K$ in control, $n=7$, $P<0.05$) and had no effect on the phase I ($22.06 \pm 3.16\% T_K$, $n=7$ vs. $25.39 \pm 2.59\% T_K$ in control, $n=7$, $P>0.05$). In nonprecontracted IPA 18 β -GA (30 μ M) led to reduction of the sustained HPV phase ($2.92 \pm 0.29\% T_K$, $n=8$ vs. $4.66 \pm 0.61\% T_K$ in control, $n=8$, $P<0.05$) without effect on the transient HPV phase I ($3.75 \pm 1.15\% T_K$, $n=8$ vs. $2.24 \pm 0.69\% T_K$ in control, $n=8$, $P>0.05$). Endothelium removing in IPA resulted in reduction in HPV transient phase amplitude ($10.71 \pm 1.79\% T_K$, $n=8$ vs. $38.48 \pm 7.01\% T_K$ in intact control, $n=8$, $P<0.01$) and abolished the sustained HPV ($-1.03 \pm 2.21\% T_K$, $n=8$ vs. $15.26 \pm 2.22\% T_K$ in intact control, $n=8$, $P<0.001$), whereas 30 μ M 18 β -GA enhanced this effect ($-17.27 \pm 4.04\% T_K$, $n=8$ vs. $-1.03 \pm 2.21\% T_K$ in endothelium-denuded control, $n=8$, $P<0.01$). 18 β -GA had no effect on the phase I ($11.28 \pm 2.1\% T_K$, $n=8$, vs. $10.71 \pm 1.79\% T_K$ in deendothelised control, $n=8$, $P>0.05$). Taken together, these data indicates that myoendothelial gap junctions can be involved to HPV development reflecting possibility of a novel pathway existing for signaling during hypoxia in pulmonary artery that supports the sustained phase of HPV.



The effect of gap junctions inhibitor 18 β -glycyrrhetic acid (18 β -GA) on HPV in isolated rat IPA precontracted with 3 μ M $PGF_{2\alpha}$. A: summarised data showing the effect of 30 μ M 18 β -GA ($n=10$) on HPV development in IPA vs. control condition ($n=10$). Each symbol represents the mean $\pm$ SE. * - $P<0.05$. B: example traces of HPV in isolated rat IPA precontracted with 3 μ M $PGF_{2\alpha}$ in control (left upper trace) and in the presence of 18 β -GA at concentrations of 30 μ M.

Sylvester J.T., Shimoda L.A., Aaronson P.I., and Ward J.P.T. Hypoxic Pulmonary Vasoconstriction.

Physiol. Rev. 92: 367–520, 2012.

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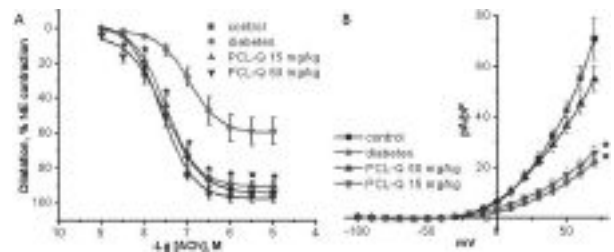
PC179

Quercetin-filled phosphatidylcholine liposomes possess restore vasodilator potential in vascular smooth muscles of diabetic rats

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Macrovascular and microvascular diseases are currently the principal causes of morbidity and mortality in patients with diabetes mellitus. Abnormalities in vascular endothelial and smooth muscle cells (SMCs) function contribute to the vascular complications in diabetes. The aim of this study was to evaluate the effect of antioxidant quercetin enclosed in phosphatidylcholine liposomes (PCL-Q) on diabetic endothelial dysfunction and changes in total outward K^+ current in SMCs at diabetes. Experimental design of the study comprised SM contractile recordings and whole-cell patch-clamp technique. Data are presented as mean $\pm$ SEM with n indicating the number of vascular preparations tested. Dilator responses to acetylcholine (ACh, 10^{-9} - 10^{-5} M) were expressed as a percentage of the maximum response produced by norepinephrine (10^{-6} M). The endothelial function was assessed in aortic rings isolated from streptozotocin-treated male Wistar rats (STZ, 65 mg/kg, intraperitoneal injection). Diabetes was verified by the presence of hyperglycaemia measured in blood. Glucose level was 30.7 ± 0.9 mM, $n=11$, $P<0.05$ in month after STZ injection. Diabetic rats were treated with PCL-Q (15 mg/kg or 50 mg/kg) intravenously twice with 1 day interval. Animals were killed in 2 weeks by cervical dislocation following ketamine (45 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) anesthesia. The concentration-response curve to ACh in the aortic rings precontracted with norepinephrine (10^{-6} M) was significantly shifted to the right (EC_{50} : 6.97 ± 0.1 , $n=11$, $P<0.05$ in diabetic animals vs. 7.59 ± 0.08 , $n=7$ in control) and maximum relaxation in control and diabetic rats were $96.5 \pm 3.05\%$, $n=7$ and $58.98 \pm 7.75\%$ ($n=11$; $P<0.05$), respectively. Treatment with PCL-Q significantly improved endothelium-dependent ACh-induced relaxation in diabetic rats and the maximum relaxation increased to $90.16 \pm 2.48\%$, $n=16$; $P>0.05$ (EC_{50} : 7.55 ± 0.07 ($n=16$; $P>0.05$) for animals treated with 15 mg/kg PCL-Q and $94.1 \pm 1.89\%$, $n=16$, $P>0.05$ (EC_{50} : 7.55 ± 0.13 , $n=16$, $P>0.05$) for treated with 50 mg/kg PCL-Q. Whole-cell patch-clamp technique showed a reduction of total K^+ outward current in SMCs of diabetic rats versus controls (peak density was 22.1 ± 1.6 pA/pF, $n=8$, $P<0.05$ for diabetes vs. 70.8 ± 8.3 pA/pF, $n=6$ for control). SMCs from animals treated with PCL-Q (50 mg/kg) showed significant increase in total K^+ currents with peak density 54.8 ± 4.9 pA/pF ($n=8$, $P>0.05$) while the treatment with 15 mg/kg PCL-Q showed no change in total K^+ current (peak density was 26 ± 2.8 pA/pF, $n=7$, $P<0.05$). In conclusion, quercetin-filled phosphatidylcholine liposomes significantly improve endothelium-dependent relaxation and restore total K^+ outward current in rats with diabetes mellitus.



A: Dose-response curve for ACh in precontracted with norepinephrine (NE, 10^{-6} M) isolated rat aorta in control ($n=7$), from diabetic animals ($n=11$), diabetic animals treated with 15 mg/kg ($n=16$), and 50 mg/kg PCL-Q ($n=16$). B: Steady-state current-voltage relationships for whole-cell outward K^+ current density isolated rat aorta SMC in control ($n=6$), in SMC from diabetic animals ($n=8$), diabetic animals treated with 15 mg/kg ($n=7$), and 50 mg/kg PCL-Q ($n=8$). Data are shown as means $\pm$ SEM. * $P < 0.05$ versus control.

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PC180

Vascular relaxation to acetylcholine in female diabetic rats that underwent hyperbaric oxygenation; the role of EETs and 20-HETED. Manojlovic¹, A. Cavka² and I. Drenjancevic²¹*Surgery, General Hospital Vukovar, Vukovar, Croatia and*²*Physiology and Immunology, Faculty of Medicine University of Osijek, Osijek, Croatia*

Introduction: Impaired vascular relaxation has been described previously in diabetes mellitus (DM), mostly in male. A few functional studies were made in female animal models of DM or in diabetic women. 20-HETE (20-hydroxyeicosatetraenoic acid) and epoxyeicosatrienoic acids (EETs) play an important role in the regulation of vascular tone, production of which increases with increase in pO_2 (1). Hyperbaric oxygenation (HBO) is used successfully in therapy of chronic vascular complications of DM; however, the mechanisms of its action are not clarified yet. **Aims:** This study aimed to elucidate relaxation mechanisms in response to acetylcholine (ACh) in aortic rings of DM female rats, exposed to HBO and to determine the possible role of 20-HETE and EETs in that response. **Methods:** Female Sprague-Dawley rats (12 weeks-old at the time of experiment, 6 weeks DM duration) were divided into 4 groups: control group, DM group, and control-HBO rats and DM-HBO rats that underwent HBO (120 minutes in duration, at 2.0 atm for 4 consecutive days). Prior to decapitation, rats were anesthetized with Ketanest (ketamine, generic name 75 mg/kg) and midazolam (2.5 mg/kg) intraperitoneally. $N=9-18$ rings/group/treatment. Vascular responses to cumulative concentration of ACh (10^{-10} to 10^{-5} M) in norepinephrine-precontracted aortic rings were measured with/without L-NAME, clotrimazol (CYP450 epoxygenase inhibitor) or HET0016 (CYP450-omega hydroxylase inhibitor) and to endothelium-independent NO donor, sodium-nitropruside (SNP). The data are presented as mean $\pm$ SD and analyzed with Two-way ANOVA Repeated Measures and Bonferroni posttest; $p<0.05$ was considered to be statistically significant. The study was approved by the Ethical Committee of Faculty of Medicine University of Osijek. **Results:** Basal ACh response was preserved in all groups of rats, possibly due to short duration of DM. However, administration of HET0016, selective inhibitor of 20-HETE production, increased vasorelaxation to ACh in DM-HBO compared to DM rats, while clotrimazol significantly reduced relaxation in

response to ACh in control-HBO rat and DM-HBO rats compared to their respective controls. L-NAME significantly decreased ACh-induced dilation in all groups of rats. Relaxation in response to SNP was slightly reduced in DM group compared to DM-HBO group. Conclusions: These results suggest that HBO could affect the mechanisms of relaxation response to ACh in both, control-HBO and DM-HBO treated rats. ACh-induced relaxation could be mediated not just with NO, but also with partial contribution of EETs, in both control-HBO and DM-HBO rats. Enhanced relaxation after blockade of 20-HETE production in DM-HBO rats suggest its' contribution to vascular tone under the HBO treatment in female diabetic rats.

1. Harder DR, Narayanan J, Birks EK, et al. Identification of a putative microvascular oxygen sensor. *Circ Res* 1996; 79, 54–61.

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PC181

High D-glucose increases the NADPH oxidase 2 and 4 mRNA levels and synthesis of reactive oxygen species involving the activity of PKC and p38^{MAPK} in HUVEC

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Cardiovascular problems associated with diabetes mellitus involves endothelial dysfunction in a mechanism that could be triggering by hyperglycemia (Ding *et al.*, 2000). There is evidence that the endothelial dysfunction would be caused by an increase of reactive oxygen species (ROS) induced by high extracellular concentrations of D-glucose (Srinivasan *et al.*, 2004). Protein kinases C (PKC) and p38^{MAPK} could be involved as modulators of the synthesis of superoxide by NADPH oxidase (NOX), the main source of superoxide in human endothelium (Li *et al.*, 2009). Our aim was to determine if the signaling pathway involves in regulation of NOX expression is dependent of PKC and/or p38^{MAPK} activity.

Biological samples were obtained from normal pregnancies (ethics committee approval and informed patient consent were obtained). Human umbilical vein endothelial cells (HUVEC) were isolated by collagenase digestion (37 Celsius degree) and cultured in medium 199 (M199) supplemented with 20% newborn and fetal calf sera. HUVEC were incubated (24 h) with M199 containing 5 mM (control) or 25 mM of D-glucose (high D-glucose) in absence or presence of calphostin C (CC, PKC inhibitor), PD169316 (p38^{MAPK} inhibitor) and/or apocynin (NOX inhibitor). mRNA levels were determined by RT-PCR from total RNA using primers for NOX2, NOX4, p47phox, myeloperoxidase (MPO) and 28S. The ROS abundance was determined by fluorescence derivated from oxidation of dichlorofluorescein (DCF).

High D-glucose increased (ANOVA unpaired Student's t test, P<0.05, n=5-10) the mRNA levels of p47phox (0.5-fold), NOX2 (0.5-fold) and NOX4 (0.5-fold) in HUVEC. Increases of NOX2 and NOX4 mRNA levels were blocked by co-incubation with CC, PD169316 and apocynin. Co-incubation of CC and PD169316 do not show an additive response in presence of high D-glucose. Moreover, MPO was detected in HUVEC, which is a key protein in the inhibition mechanism of apocynin. As

well as the case of mRNAs, high D-glucose increased the synthesis of ROS (2-fold), an effect blocked by co-incubation with CC, PD169316 and apocynin.

In conclusion, high D-glucose increases the ROS synthesis in a mechanism mediated by an increase of the mRNA expression of NOX2 and NOX4. The mechanism underlying the transcriptional activation of NOX genes is related with a signaling pathway that involve PKC and p38^{MAPK}. Also, high D-glucose increased the MPO expression, validating the use of apocynin like NOX inhibitor in HUVEC and, most importantly, showing a potential role of MPO in cellular responses to stress induced by high D-glucose.

Ding Y *et al.* (2000). *Am J physiol Endocrinol Metab* **279**, E11-E12

Srinivasan S *et al.* (2004). *Diabetologia* **47**, 1727-1734.

Li JM *et al.* (2004). *Am J Physiol Regul Integr Comp Physiol* **287**, 1014-1030.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC182

In vivo imaging provides a novel insight into autoimmune disease and cardiovascular health

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Background:

Disruption to regulation of nuclear factor-kappa beta (NF-κB) has been associated with a number of inflammatory conditions including atherosclerosis. The protein A20-binding inhibitor (ABIN) has a polyubiquitin binding domain homologous to that present in NF-κB essential modulator (NEMO), a key component of the inhibitor NF-κB (IκB) kinase (IKK) complex. Mice bearing a mutation in ABIN-1 [D485N] produce numerous physiological changes typical of autoimmunity. Remodelling of the vascular wall and invasion of mononuclear cells has previously been reported, although the functional consequences of this are yet to be established.

Aim:

To better understand the relationship between ABIN-1 protein mutation and vascular function in an *in vivo* mouse model.

Method:

Skin microcirculation was assessed on the flanks of anaesthetised (1.5-2% isoflurane with oxygen) C57BL/3 ABIN-1 knock-in (KI) (n=17) and wild-type (WT) (n=17) mice aged 12-18 weeks. Laser Doppler Imaging (LDI) with drug iontophoresis of phenylephrine (PE), acetylcholine (ACh), sodium nitroprusside (SNP), and localised skin heating (45°C) were used to measure vascular function. Results are expressed as mean percentage change in perfusion (maximum response-baseline) ± standard error of mean (SEM). Additionally, skin fluorescence measurements in arbitrary units (AU) ± SEM were made to quantify levels of NADH, flavins, pyridoxine and carotene. One-way ANOVA and T-test analysis were used for statistical significance (p<0.05).

Results:

Significant differences between ACh induced vasodilation in KI (change in skin perfusion $23\pm3\%$) and WT (change in skin perfusion $34\pm3\%$) mice were observed ($p=0.007$). No significant differences were noted for SNP (KI change in skin perfusion $35\pm4\%$, WT change in perfusion $31\pm3\%$) or for localised skin heating (KI change in skin perfusion $40\pm3\%$, WT change in perfusion $40\pm4\%$). Significant differences for pyridoxine (KI 197 ± 15 AU, WT 243 ± 10 AU) were evident ($p=0.037$). No significant changes were observed for NADH (KI 314 ± 47 AU, WT 396 ± 4 AU), flavins (KI 320 ± 64 AU, WT 400 ± 17 AU), or carotene (KI 169 ± 6 AU, WT 188 ± 7 AU).

Conclusion:

ACh induced vasodilation was significantly reduced in KI mice. However maximal dilatory capacity as tested by SNP and localised skin heating were not significantly different between groups, suggesting localised damage to the endothelium. A reduction in pyridoxine, an existing independent risk factor for vascular disease, might be one factor responsible for this dysfunction. The exact cellular consequences and resulting physiology of this interaction requires further scrutiny.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

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Human skin microcirculation assessment by wavelet transforms and detrended fluctuation analysis

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Wavelet analysis from human skin's microcirculation signals obtained by Laser Doppler Flowmetry (LDF) shows components at characteristic frequency ranges related to the heart (0.6-2Hz), to respiration (0.15-0.6Hz), to the vessel wall myogenic activity (0.052-0.15Hz), to sympathetic activity (0.021-0.052Hz) and to endothelial metabolic activity (0.0095-0.021Hz). These wavelets components additionally show amplitude modulation in time and frequency, and may also hold important information regarding blood flow physiology. Detrended Fluctuation Analysis (DFA) is an interesting method to study blood flow fractal properties. Analyses of wavelet component amplitudes and DFA-derived alpha exponents have typically been used separately. Here we applied a combined approach of these two methods in order to get a cleared picture of microvascular blood-flow regulation.

The study involved 9 female healthy young subjects (age=20.3±4.0 years) giving previous informed written consent. All procedures fully respected Helsinki principles and respective amendments. LDF measurements were recorded for 30 minutes - 10 min baseline; 10 min after a perfusion restriction of the ankle with a cuff, and 10 min recovery. Data sampling was 32Hz and, after data segmentation in the 3 time segments, analysis involved a home-built MATLAB script based on MATLAB's wavelet toolbox and DFA algorithm. Frequency components from wavelet decomposition were analyzed regarding amplitude ratios (mean amplitude of each component over the total signal amplitude) and alpha exponents. Comparison between data segments (baseline, perfusion restriction, and recovery) was done regarding amplitude ratios and alpha exponents for each frequency component by using paired t-test or Wilcoxon test, accordingly.

Data suggest that each frequency component has a distinct amplitude ratio, with the metabolic and sympathetic components showing the highest values. Additionally, these components show the highest DFA alpha exponents, which translate a non-stationary correlated signal of the fractional Brownian motion type. During perfusion restriction, a significant increase of the amplitude ratios and alpha exponents of the heart, respiratory and myogenic components, which are expected to follow vasodilation, happens. Additionally, a decrease in metabolic activity is observed (NO release from the endothelium?). After cuff release, recovery of heart and respiratory components is observed, particularly in the alpha exponents. No significant changes were observed for the sympathetic component, probably due to the reduced sample size. The combined approach of wavelet analysis and DFA may provide a powerful way to analyze vasomotion in LDF signals. In particular, the method could differentiate both myogenic and endothelium responses in microcirculation territories.

Landsverk SV et al. (2007). *Anesthesia & Analgesia* 105, 1012-1019.

Sosnovtseva OV et al. (2004). *Physical Review E* 70, 031915.

Esen F and Esen H (2006). *Physiological Measurement* 27: 1241-1253

Liao F et al. (2010). *Microvascular Research* 80, 44-53.

Esen F et al. (2011). *Microvascular Research* 82, 291-295.

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PC184

Activation of Epac and PKA antagonise thrombin-induced endothelial hyperpermeability via different mechanisms

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Aims: Inflammatory mediators-induced endothelial barrier failure leads to severe oedema that impedes functional recovery of the organ. We and others have shown that activation of cAMP signalling protects endothelial barrier against imminent failure via inactivation of the contractile machinery and strengthening of adherens junctions, two major determinants of endothelial barrier function. cAMP mediates its effects via its effectors i.e. PKA and Epac (exchange protein directly activated by cAMP), however, precise role of these effectors in the control of endothelial barrier is not well described. Here the relative contribution of PKA and Epac in the regulation of endothelial barrier function was studied. **Methods:** Endothelial contractile machinery and barrier function were analyzed in cultured human umbilical vein endothelial cells (HUVEC). 8-CPT-cAMP, 6-Bnz-cAMP, and forskolin were used to activate Epac, PKA or adenylyl cyclase, respectively. Thrombin was used as an inflammatory mediator.

Results: Activation of either PKA or Epac partially antagonised thrombin-induced hyperpermeability. Simultaneous activation of PKA and Epac had additive effect, which was comparable to forskolin. Similarly, activation of either PKA or Epac lead to increased translocation of VE-cadherin to cell-cell junctions and simultaneous activation of both PKA and Epac had additive effects. However, activation of PKA but not Epac inhibited thrombin-induced phosphorylation of MLC and regulatory sub-

unit of MLCP, MYPT1. Moreover, activation of PKA but not Epac induced an increased MLCP holoenzyme complex formation, dephosphorylation of inhibitory protein CPI-17 of the catalytic subunit, PP1 and its dissociation from PP1 leading to MLCP activation. Down-regulation of CPI-17 attenuated thrombin-induced hyperpermeability and abolished the antagonistic effect of the PKA activator, whereas the Epac activator retained its antagonistic effect. Similarly, activation of both PKA and Epac enhanced Rac1 activity, however, activation of PKA but not Epac caused an inactivation of RhoA.

Conclusion: cAMP/PKA regulates the endothelial barrier via inhibition of endothelial contractile machinery mainly by activation of MLCP. This is mediated mainly via increased MLCP holoenzyme complex formation and inhibition of CPI-17. The permeability lowering effect of the cAMP/Epac pathway is via strengthening of adherens junctions and is independent of contractile machinery.

Aslam M, Härtel FV, Arshad M, Gündüz D, Abdallah Y, Sauer H, Piper HM, & Noll T (2010). cAMP/PKA antagonizes thrombin-induced inactivation of endothelial myosin light chain phosphatase: Role of CPI-17. *Cardiovasc Res* 87, 375-384.

Aslam M, Pfeil U, Gunduz D, Rafiq A, Kummer W, Piper HM et al. Intermedin/adrenomedullin2 stabilises endothelial barrier and antagonises thrombin-induced barrier failure. *Br J Pharmacol* 2011.

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PC185

Nonlinear parameters of heart rate variability during mental stress test

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Introduction. This study investigates the variations of nonlinear parameters of heart rate variability (HRV) due to an arithmetic stress test.

Material and methods. We tested 83 healthy students with a mean age of 21.12 ± 0.21 years. The students were examining in the following sequence: recording of HRV at rest (10 minutes), during mental arithmetic (10 minutes) and during the recovery period (10 minutes). HRV was assessed by applying Poincare plot analysis (SD1, SD2, SD2/SD1, SS) to the R-R interval. The traditional (GI) and redefined Guzik's index (GIp) (Karmakar, 2009) was calculated for each subjects in each phase of the experiments.

Results and discussion. SD1 during mental arithmetic was significantly smaller (24.81 ± 1.80) than that at rest (34.90 ± 2.08 ; $p < 0.0001$). During recovery from mental arithmetic tasks SD1 return to baseline level (39.01 ± 3.65). SD2 tended to decrease during the mental arithmetic task, reaching its highest levels during the period of recovery (from 70.74 ± 2.94 during tasks to 81.51 ± 4.23 during post stress; $p < 0.004$).

SD1/SD2 ratio during mental stress (0.333 ± 0.013) was significantly lower than that at rest (0.474 ± 0.017 ; $p < 0.00001$) and during the period of recovery (0.441 ± 0.017 ; $p < 0.00001$). SS was significantly lower during the test (2802.32 ± 320.04) than that at rest (3860.98 ± 377.56 ; $p < 0.001$) and recovery phase (5812.81 ± 971.32 ; $p < 0.001$). There was no significant differ-

ence in the GI during mental stress (0.491 ± 0.004) than that at rest (0.496 ± 0.004 ; $p > 0.05$) and recovery period (0.486 ± 0.004 ; $p > 0.05$). After redefinition traditional asymmetry indices, we have found pronounced changes in GIp parameter with a maximum at rest and minimum during arithmetic test (0.436 ± 0.012 vs 0.478 ± 0.013 ; $p < 0.05$).

This findings suggest that nonlinear HRV analysis could be effective in automatically detecting functional status during mental stress.

Karmakar C. K., Khandoker A. H., Gubbi J., Palaniswami M. Defining asymmetry in heart rate variability signals using a Poincare plot // *Physiol. Meas.* 30 (2009) 1227-1240.

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PC186

Heart rate variability in type 2 diabetes mellitus

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Diabetes mellitus is a metabolic disorder affecting various organs of the body. In many patients with diabetic mellitus, typical symptoms associated with the disease manifest only after sufficient cumulative effect of diabetes mellitus. As a result, diabetes related complications and associated clinical conditions may be present by the time it is clinically diagnosed. One of such complications which remain subclinical in diabetes is autonomic neuropathy. Because of its association with a variety of adverse outcomes including cardiovascular deaths, cardiovascular autonomic neuropathy is the clinically most important and well-studied form of diabetic autonomic neuropathy. Heart rate variability of 60 patients with type 2 diabetes mellitus (T2DM) and 30 controls were assessed. Resting electrocardiogram (ECG) at spontaneous respiration was recorded for 5 minutes in supine position. The data are expressed as median (interquartile range). The p value < 0.05 was considered statistically significant. All the time domain measures, standard deviations of normal to normal R-R intervals (SDNN) [26 (15.5-35) vs 36 (30-40.25) ms, $p = 0.002$], square root of the mean of the squared R-R intervals (RMSSD) [25.9 (11.95-40.45) vs 36.65 (27.05-44.13) ms, $p = 0.002$], number of R-R intervals that differ by more than 50 ms (NN50) [13.5 (1-70) vs 58 (15.5-86.5) count, $p = 0.002$] and percentage of NN50 (pNN50) [3.5 (0.23-21.83) vs 16.4 (4.45-27.63) %, $p = 0.002$] were significantly lower in T2DM. In frequency domain, low frequency (LF) power [81 (32-148.75) vs 126 (85.25-237.75) ms², $p = 0.007$], LF power [28.8 (20.78-36.13) vs 36.05 (24.6-43.5) %, $p = 0.008$], high frequency (HF) power [81 (16.75-187.75) vs 182.5 (121.5-281.75) ms², $p = 0.001$] and HF power [42.15 (27.73-50.65) vs 51.6 (43.58- 62.05) %, $p = 0.001$] were significantly lower in T2DM whereas LF nu [45.25 (35.28-63.93) vs 44.65 (33.7-65.35)] and LF/HF ratio [0.776 (0.543-1.13) vs 0.7005 (0.397-0.999)] were comparable. In Poincare plot, standard deviation (SD)1 [18.5 (8.73-28.98) vs 26.2 (19.33-31.65) ms, $p = 0.003$] and SD2 [37 (26.13-51.18) vs 48.6 (39.63-56.15) ms, $p = 0.002$] were lower in T2DM. These data suggest that patients with

T2DM have reduced cardiovascular autonomic control especially parasympathetic control.

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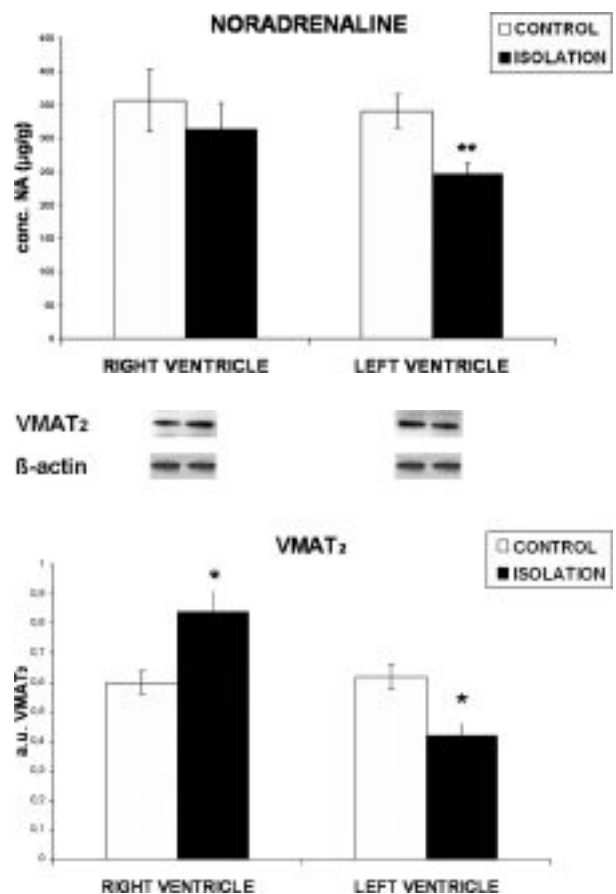
PC187

Alternation in noradrenaline synthesis, reuptake and stores in ventricles of chronic stressed rats

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Stress exposure activates sympathoneural system resulting in noradrenaline release. The sympathoneural system plays an important role in regulating cardiac function in both health and disease. The activity of sympathoneural system is dependent on the synthesis of noradrenaline, as determined by the rate-limiting enzymes tyrosine hydroxylase (TH), its reuptake through the noradrenaline transporter (NET), synaptic release, degradation and vesicular transport mediated by the vesicular monoamine transporter 2 (VMAT2). Functional noradrenergic transmission consists of a balance between noradrenaline synthesis, release and reuptake. Depletion of cardiac noradrenaline stores contribute to the progressive deterioration of cardiac function and decrease myocardial contractility (Liang C, 2007). Therefore, we examined changes in noradrenaline stores and protein levels of TH, NET and VMAT 2 in the right and left ventricle of chronic individually housed Wistar male rats. Animals remained for 12 weeks in individual housing, during this period visual isolation was guaranteed by white paper walls surrounding the cage. Chronic social isolation reduced noradrenaline content in the left ventricle. The protein levels of TH and NET in both ventricles were unchanged. However, in the right ventricle protein levels of VMAT2 were increased ($p < 0.05$), whereas in the left ventricle VMAT2 protein levels were significantly decreased ($p < 0.05$). Guillot and Miller (2009) described protective actions of the VMAT 2 in monoaminergic neurons, so packaging of catecholamine into vesicle serves two purposes: neurotransmission and neuroprotection. Since VMAT2 provides neuroprotection from toxicant, it could be hypothesized that decreased VMAT2 protein in the left ventricle may enhance oxidative stress in sympathetic nerves which innervate heart. Kristen and coworkers (2006) reported that in volume-overloaded hypertrophic hearts depletion of cardiac noradrenaline stores is caused by reduction of the sympathetic nerve density. Adult sympathetic neurons whose axons have been damaged decrease expression of noradrenergic genes and production of noradrenaline (Pellegrino et al., 2011). Our data indicate differential changes in the status of sympathetic nerves in the right and left ventricles. Thus, the finding of increased expression of VMAT2 in the right ventricle would be consistent with increased capacity of noradrenaline storage, as an important adaptation to chronic stress, whereas depletion of noradrenaline stores in the left ventricle may be due to reduced VMAT2.



Guillot TS and Miller GW (2009). Protective actions of the vesicular monoamine transporter 2 (VMAT2) in monoaminergic neurons. *Mol Neurobiol* 39, 149-170.

Kristen AV, Kreusser MM, Lehmann L, Kinscherf R, Katus HA, Haass M, Backs J (2006). Preserved noradrenaline reuptake but reduced sympathetic nerve endings in hypertrophic volume-overloaded rat hearts. *J Card Fail* 12, 577-583.

Liang C (2007). Cardiac sympathetic nerve terminal function in congestive heart failure. *Acta Pharmacol Sin* 28, 921-927.

Pellegrino MJ, Parrish DC, Zigmond RE, Habecker BA (2011). Cytokines inhibit noradrenaline transporter expression by decreasing Hand2. *Mol Cell Neurosci* 46, 671-680.

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PC188

The influence of transcutaneous vagus nerve stimulation on cardiac autonomic control in healthy human subjects

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Heart failure is a debilitating and fatal condition characterised by sympathetic activation and parasympathetic withdrawal. Current therapies target sympathetic activation (e.g. beta-blockers) and have improved the clinical outcome of heart failure. However, the prevalence of heart failure is increasing, demanding the development of new therapeutic approaches. Tackling the underlying autonomic imbalance through parasympathetic stimulation can improve cardiac function in heart failure patients, however, the current method of cervical vagus nerve stimulation (VNS) is invasive and associated

with side effects¹. This study investigated the autonomic effects of a non-invasive method of VNS - transcutaneous stimulation of the auricular branch of the vagus nerve (tVNS) - in healthy humans (n=89; 49 female, 40 male; aged 20-66 years). The study was approved by the University of Leeds Ethics Committee. Heart rate (ECG), blood pressure and respiration were recorded continuously. Heart rate variability (HRV) was calculated using spectral analysis of beat-to-beat (R-R) intervals derived from ECG data. Low frequency (LF; 0.04-0.15Hz) and high frequency (HF; 0.15-0.40Hz) power were calculated. HF power represents parasympathetic/vagal modulation of heart rate and the ratio of low to high frequency power (LF/HF) can be used as an indicator of sympathovagal balance, such that a decrease in LF/HF indicates a shift in autonomic balance towards parasympathetic dominance. tVNS was applied at either low pulse width and pulse frequency (L-tVNS) or high pulse width and pulse frequency (H-tVNS). Data was analysed at baseline, during tVNS and during recovery. Repeated measures ANOVAs were used to analyse results (data presented as mean $\pm$ S.E.M). There was a significant difference in HRV response between L-tVNS and H-tVNS groups ($p=0.045$). L-tVNS had no significant effect on HRV ($n=63$) whereas HRV improved significantly in the H-tVNS group with a decrease in LF/HF ratio from 1.45 ± 0.22 at baseline to 1.21 ± 0.15 during H-tVNS ($n=26$; $p=0.048$). HRV improved in 49% of the L-tVNS group compared to 63% of the H-tVNS group. Baseline LF/HF was significantly higher in subjects whose HRV improved during L-tVNS or H-tVNS compared to those who did not ($p=0.038$ and $p=0.012$ respectively). Based on the results of this study, tVNS can improve HRV in healthy subjects although the effects may be underestimated due to the limitation of using subjects with healthy autonomic balance. This is supported by the fact that baseline LF/HF was significantly higher in responders to tVNS indicating that these subjects had higher sympathetic and/or lower parasympathetic activity at baseline. tVNS could be a practical, non-invasive and economical therapy for heart failure patients and warrants further investigation.

De Ferrari GM *et al.* (2011) *European Heart Journal* **32**, 847-855.

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PC189

Molecular characterisation of atrial volume receptors

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Hypertension is a major risk factor for stroke and heart failure, and is also a leading cause of chronic kidney failure. To maintain an appropriate cardiac output the central nervous system (CNS) requires information from the periphery regarding blood pressure and volume. Sensors detect changes in these parameters and signal to the CNS where the information is integrated so that the brain can regulate efferent sympathetic activity to maintain cardiovascular homeostasis. Atrial volume receptors (AVRs) are specialised nerve endings found in the atrial wall and the entrances of the major blood vessels [1]. They detect the change in volume as blood returns to the heart. The literature provides classic descriptions of the electrical output from

these receptors [2,3], however knowledge of their morphology is limited and they still await molecular characterisation. We have shown previously that members of the epithelial sodium channel (ENaC)/Degenerin /acid sensing ion channel (ASIC) family are major contributors to mechanotransduction in rat muscle spindles [4]. Transient receptor potential (TRP) proteins have also been implicated in mechanosensation in heart as well as other tissues [5].

Three male Hooded Lister rats were killed according to Schedule 1 of the Animals (Scientific Procedures) Act 1986. The atria were removed and fixed in 4% formaldehyde at 4°C. Following cryoprotection in sucrose/phosphate buffered saline (PBS), venoatrial regions were dissected, frozen and 16 μ m cryosections collected. Labelling was carried out with a range of anti-channel antibodies (ASIC2, ASIC3, ENaC α , ENaC β , ENaC γ , TRPC1, TRPC4/5, TRPC6, TRPV4) together with either anti-synaptophysin (SYN) as a vesicle marker or anti-neurofilament (NF) antibodies to identify nerves. For controls anti-channel antibodies were omitted. Following washing in PBS, secondary antibodies (AF 594 + AF 488) were applied for one hour. The slides were washed in PBS and mounted in PBS/glycerol. Slides were viewed under a Zeiss Fluorescent microscope.

TRPC1-like immunoreactivity (IR) was observed in ganglion cells and nerves of the epicardium; and also in sensory endings of the endocardium, where it was coincident with both SYN-IR and NF-IR. Strong TRPV4-like IR was likewise seen in nerves and ganglion cells. It was coincident with SYN-IR in all three layers of the heart wall. Immunoreactivity was not detected with any of the other anti-channel antibodies.

TRPC1 and TRPV4 channels merit further investigation as potential contributors to mechanosensation in AVRs. Elucidation of the process whereby changes in returning blood volume are detected and signalled to the CNS is necessary to inform our understanding of how normal cardiovascular homeostasis malfunctions in disease. This will require a comprehensive knowledge of the proteins involved.

Hainsworth R (1991) "Reflexes from the heart." *Physiol. Revs.* **71**: 617-658.

Paintal, A. S. (1953). "A study of right and left atrial receptors." *Journal of Physiology-London* **120**(4): 596-610.

Paintal, A. S. (1953). "The conduction velocities of respiratory and cardiovascular afferent fibres in the vagus nerve." *Journal of Physiology-London* **121**(2): 341-359.

Simon, A., F. Shenton, *et al.* (2010). "Amiloride-sensitive channels are a major contributor to mechanotransduction in mammalian muscle spindles." *Journal of Physiology-London* **588**(1): 171-185.

Inoue, R., Z. Jian, *et al.* (2009). "Mechanosensitive TRP channels in cardiovascular pathophysiology." *Pharmacology & Therapeutics* **123**(3): 371-385

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PC190

Role of NO and angiotensin AT2 receptors in the cardiopulmonary baroreflex control of renal sympathetic nerve activity in anaesthetised ratsM.H. Abdulla¹ and E.J. Johns^{1,2}¹Physiology, University College Cork, Cork, Co Cork, Ireland and²Medical Sciences, Alliance University College, Kapala Batas, Penang, Malaysia

The low-pressure baroreceptors are sensitive to circulatory volume and initiate a reflex renal sympatho-excitation or inhibition. This study investigated the contribution of NO to low pressure baroreceptor regulation of RSNA and whether there was a role for AT2 receptors. Groups of Wistar rats (275-350g) were anaesthetised with 1ml chloralose/urethane mixture (16.5/250mg/ml) IP. Cannulae were inserted into the right femoral artery, for mean arterial pressure (MAP) and heart rate (HR) measurement, and vein for saline (NaCl, 9g/L) infusion and supplementary anaesthetic. A cannula was placed in the right intracerebroventricle (ICV) for infusion of drugs. The left kidney was exposed, a renal sympathetic nerve dissected and sealed onto recording electrodes. Saline was infused ICV at 30µl/h for 10min followed by a maintenance infusion of 7.5µl/h for 30min after which the first volume expansion was performed by infusing saline at 0.25% bwt/min IV for 30min. Thereafter, the ICV infusion was switched to either PD123319 (PD, 50µg/kg/min), an AT2 receptor antagonist (n=6) or L-NAME (LNM, 150µg/kg/min), a NO synthase inhibitor (n=8) after which a second volume expansion was performed. Another group of rats received ICV PD or LNM initially followed by PD+LNM as combination (n=6 and 7, respectively). Baseline MAP, HR and RSNA were recorded for 5 min before starting the first and second volume expansion. Data, mean±SEM were analysed using the Student's t-test with significance at P<0.05. Animals were killed with an anaesthetic overdose. MAP, HR and RSNA following either PD (75±6mmHg, 319±20bpm, 0.69±0.17µV.s) or LNM (82±7mmHg, 304±14bpm, 0.95±0.33µV.s) were not different from those following saline infusion (67±4mmHg, 307±15bpm, 0.82±0.17µV.s; 77±6mmHg, 312±16bpm, 1.00±0.1µV.s respectively). MAP was increased after ICV PD+LNM compared to either PD or LNM (PD+LNM vs. PD, 77±5 vs. 64±7mmHg and PD+LNM vs. LNM 89±5 vs. 80±4mmHg; both P<0.05). Volume expansion decreased RSNA in all groups by some 64±10% (P<0.05) at the end of the 30min. Infusion of PD ICV had no effect on the magnitude of the renal sympatho-inhibition. Following ICV LNM, the reduction in RSNA was less compared to that obtained during saline infusion (46±11 vs. 64±10%, P<0.05, respectively). Similarly, in the PD+LNM group where PD preceded LNM, the fall in RSNA was smaller than when PD was infused alone (37±8 vs. 63±7%, P<0.05, respectively) but not if PD was given after the LNM. These findings suggest that NO is important in allowing the normal renal sympatho-inhibition to occur in response to a volume load but that AT2 receptors do not contribute to this reflex mechanism.

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Maternal haemodynamic changes with advancing gestation in normal healthy pregnancyR. Davies¹, L. D'Silva¹, M. Lewis¹, S. Emery² and O. Uzun³¹College of Engineering, Swansea University, Swansea, UK,²Singleton Hospital, Swansea, UK and ³University Hospital of Wales, Cardiff, UK

Aim: To characterise maternal haemodynamic changes using non-invasive methods throughout normal healthy pregnancy. **Method:** The Task Force haemodynamic monitor (CNSystems, Austria) was used to assess 54 healthy pregnant women aged 20-41 years at 15.1±1.8 weeks (T1, n=26), 26.0±1.0 weeks (T2, n=18) and 35.8±1.1 weeks (T3, n=10). Cardiac interval (RR), stroke volume (SV), cardiac output (Q), left ventricular ejection time (LVET), end diastolic volume index (EDI), acceleration index (ACI), systolic and diastolic blood pressure (BPs, BPD) and vascular compliance (C) were quantified. Participants performed postural manoeuvres (supine, standing), stepping exercise, seated recovery (all 6 minute blocks), mental arithmetic, controlled and spontaneous breathing (all 3 minute blocks). Mixed between-within repeated measures ANOVA assessed the influence of main factors 'Protocol Stage' and 'Trimester' on each haemodynamic variable. Post-hoc analysis with Bonferroni correction identified the locations of significant differences. One way ANOVA additionally assessed the stage-specific influence of trimester on haemodynamic variables.

Results: When subjects moved from supine to standing we observed the following responses (Δ indicates change between stage, + or - indicates a positive or negative between-state change, = indicates no change): During the supine-to-standing change (1) Δ RR- in T1 was diminished during T3 (p=0.002), (2) Δ SV- in T1 increased to Δ SV= in T3 (p<0.002), (3) Δ Q- in T1 increased to Δ Q+ in T3 (p<0.05), (4) Δ LVET- was diminished between T1, T2 and T3 (p<0.01), (5) Δ EDI- increased to Δ EDI+ in T2 and T3 (p<0.05). During the metronomic-to-spontaneous breathing change we observed that: (7) Δ C- in T1 increased to Δ C+ in T3 (p<0.01), (8) Δ ACI- became more pronounced from T1 to T2 and T3 (p<0.0001).

Conclusion: Despite these being early results, we observed marked gestation-related changes in cardiovascular and haemodynamic responses to postural change and breathing rate. The altered physiology during the change from supine to standing might be related to autonomic function changes as pregnancy progresses and also due to increased systemic blood flow with gestation. At the early stage of pregnancy (T1) metronomic breathing is associated with greater compliance of the blood vessels whereas in T3 the opposite applies. This might be associated with haemodynamic restrictive changes caused by the baby. We are planning to develop this protocol to be used as a screening tool in the future to help assess pathophysiological complications during pregnancy.

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Extracellular Cathepsin-L alters spontaneous and triggered sarcoplasmic reticulum-mediated calcium release in adult rat cardiomyocytes

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Cathepsin-L (CatL) is a lysosomal cysteine protease which can be released from a variety of cell types into the extracellular space. CatL is increased in the serum of patients with heart disease¹ and has been linked to post-infarct extracellular matrix (ECM) remodelling². However, whether extracellular CatL can directly alter cardiac function remains unknown. Sarcoplasmic reticulum (SR)-mediated Ca^{2+} release in cardiomyocytes is a major determinant of cardiac function and is modified during cardiac disease. The aim of this study was to determine whether extracellular CatL can directly alter SR-mediated Ca^{2+} release in cardiomyocytes. Adult male Wistar rats (~250g) were killed in accordance with Schedule 1 of the Animal (Scientific Procedures) Act 1986 and ventricular cardiomyocytes isolated. Recombinant mammalian CatL activity at physiological pH was confirmed using a Z-LR-AMC fluorometric assay. Spontaneous Ca^{2+} wave characteristics in Fluo-3-loaded cardiomyocytes incubated for 30min with 5.4nM CatL/vehicle were measured by confocal microscopy. In separate experiments, Ca^{2+} transient parameters in field stimulated (0.5Hz) Fura-2AM loaded cardiomyocytes incubated for 30min and subsequently perfused with a modified Krebs-Henseleit solution containing 5.4nM CatL/vehicle were measured using epifluorescence microscopy. SR Ca^{2+} content in these cells was determined by the amplitude of caffeine-induced Ca^{2+} release after 6min of field stimulation. Data are presented as mean $\pm$ SEM, CatL vs. control; statistical comparisons were made by Student's paired t-test. Confocal microscopy revealed that Ca^{2+} wave frequency was increased by CatL (0.16 ± 0.02 vs. 0.12 ± 0.02 , CatL:n=73, control:n=70) and time constant (τ) of Ca^{2+} wave decay significantly shortened (69 ± 3 vs. 79 ± 4 ms; $P<0.05$). Field stimulated Ca^{2+} transient amplitude was decreased by CatL (0.22 ± 0.03 vs. $0.51\pm 0.06\mu\text{M}$, CatL:n=18, control:n=16; $P<0.05$) via both a reduced transient peak [Ca^{2+}]_i (0.31 ± 0.029 vs. $0.58\pm 0.068\mu\text{M}$, $P<0.05$) and minimum [Ca^{2+}]_i (73.4 ± 4.89 vs. $93.5\pm 6.40\text{nM}$; $P<0.05$). The maximum rate of rise ($d\text{Ca}^{2+}/dt_{\text{max}}$), was slower (3.8 ± 0.4 vs. $9.6\pm 1.2\mu\text{M}\cdot\text{s}^{-1}$; $P<0.05$) as was the maximum rate of fall, ($d\text{Ca}^{2+}/dt_{\text{min}}$), (0.1 ± 0.02 vs. $0.6\pm 0.1\mu\text{M}\cdot\text{s}^{-1}$; $P<0.05$). The τ of Ca^{2+} transient decay was prolonged (2.4 ± 0.2 vs. 0.9 ± 0.1 s; $P<0.05$). The amplitude of the caffeine-induced Ca^{2+} transient was reduced by CatL (0.5 ± 0.1 vs. $0.8\pm 0.1\mu\text{M}$; $P<0.05$) and the τ prolonged (1.1 ± 0.07 vs. 0.9 ± 0.04 s; $P<0.05$) suggesting reduced SR Ca^{2+} content and slowed cellular extrusion of Ca^{2+} via sarcolemmal fluxes. This study demonstrates for the first time that extracellular CatL reduces Ca^{2+} transient amplitude and increases Ca^{2+} wave frequency. Therefore CatL may not only play a role in ECM remodelling but also has the potential to directly alter cardiac function during heart disease.

Y. Liu *et al.* American Journal of Cardiology 2009; 103: 476-481

M. Sun *et al.* Cardiovascular Research 2011; 89: 374-383

BBSRC

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC193

Age dependent effects on Heart Rate Variability and QT Variability Index in a sheep model of heart failure

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Background

Heart failure is associated with an increased risk of ventricular arrhythmias and sudden death. It is important to identify high risk individuals in order to target therapies effectively. Previous studies have demonstrated that heart rate variability (HRV) is reduced in heart failure, and that this evidence of autonomic dysfunction has prognostic significance. QT variability index (QTVI) measures the dynamic relationship between heart rate and QT interval; an increased QTVI represents increased lability of repolarisation and correlates with sudden death. The relationship between the two measurements is not clear, and we therefore undertook preliminary studies to examine these measures in a sheep model of heart failure with particular reference to the effect of age.

Methods

All experiments were performed, under license, in accordance with current UK legislation. Heart failure was induced in 4 old and 4 young Welsh Mountain Sheep, by tachycardic pacing at 210 – 240 bpm via the right ventricular apex for 4–6 weeks. Pacemaker implantation was performed under general anaesthesia (isoflurane 1–4% v/v in oxygen) with postoperative analgesia (Meloxicam 0.5mg/kg IM) and antibiotic prophylaxis (Enrofloxacin 2.5mg/kg SC). 256 second ECG recordings were made in conscious restrained animals at baseline and at experiment end. Heart rate variability was assessed using standard time domain measures and power spectrum analysis. QTVI was calculated using a modification of Berger's algorithm as a logarithmic ratio of QT to RR variance. Results are expressed as mean change $\pm$ SD, with significant differences detected by 2 way ANOVA at $p<0.05$.

Results

Heart failure was associated with a reduction in all time domain measures (SDNN: relative change -50.3% $\pm$ 25.6, Triangular Index: relative change -44.7% $\pm$ 23.3, RMSSD: relative change -60.7% $\pm$ 40.5). Power spectrum analysis demonstrated changes in high and low frequency bands. No significant effect of age was seen for HRV measures. QTVI at baseline was lower in young animals (young: -1.68 $\pm$ 0.60, old: -0.69 $\pm$ 0.33); in these animals QTVI increased significantly with heart failure in the young group resulting in no difference between groups at end-stage (young: -0.77 $\pm$ 0.27, old: -0.95 $\pm$ 0.20).

Conclusion

In this model, there is heart failure induces a broad spectrum reduction in heart rate variability. This indicates the presence of autonomic dysfunction, which is common to young and old animals. On the other hand, QTVI increases only in young animals; it appears that old animals already have abnormal repolarisation lability prior to the induction of heart failure. Mechanisms for this age-dependent interaction between autonomic function and cardiac repolarisation warrant further investigation.

Berger RD *et al.* (1997). *Circulation* **96**,1557–65.

Atiga WL *et al.* (1998). *J Cardiovasc Electrophysiol* **9**,899–908.

Piccirillo G *et al.* (2007). *Eur Heart J* **28**,1344–50.

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PC194

Does transverse tubule formation and maintenance depend on the presence of amphiphysinII?

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Transverse tubules (t-tubules) are regular invaginations of the surface sarcolemma membrane of ventricular cardiac myocytes. T-tubules enable a rapid and synchronous release of calcium (Ca²⁺) throughout the cell, thus facilitating contraction. Whilst evidence shows that t-tubules are remodelled in the ventricles of the failing heart, it still remains unclear how t-tubule structure and function changes during cardiac disease. Amphiphysin II (AMPII), a member of the Bin/Amphiphysin/Rvs (BAR) domain family, is known to be involved in membrane invaginations and t-tubule biogenesis (Muller et al, 2003). The aims of this study are to investigate 1) if AMPII is reduced in a tachypacing induced sheep model of heart failure; 2) the role of AMPII in the formation and maintenance of t-tubules in rat ventricular myocytes.

Under isoflurane anaesthesia (2–4% v/v in oxygen) sheep were instrumented with a pacemaker and pacing lead. Post-operative analgesia (meloxicam 0.5 mg/kg) and antibiotics (enrofloxacin 2.5 mg/kg) were provided for 24 hours. Heart failure was induced by rapid ventricular pacing (Briston et al. 2011) for 4–5 weeks. Following pentobarbitone euthanasia (200 mg/kg iv) samples of left ventricular and left atrial myocardium were snap frozen. Age-matched non-instrumented animals served as controls. T-tubule density was assessed using the potential sensitive dye di-4-ANEPS and confocal microscopy (Dibb et al. 2009). Data are presented as mean $\pm$ S.E.M and statistical significance determined using a students t-test and considered significant when $p < 0.05$. Western blotting performed on protein samples obtained from the left ventricular wall and left auricle showed a significant reduction in AMPII protein in heart failure (ventricle, decreased by $38 \pm 12.1\%$, atria, decreased by $43 \pm 12.1\%$, $n = 7$, $p < 0.05$). Transfecting ventricular myocytes, enzymatically isolated from rat hearts, with small interfering RNA (siRNA) against AMPII causes t-tubule loss. The distance at which 50% of pixels in target cells are from a membrane (sarcolemma or t-tubule) compared with control cells is increased by $41 \pm 22.3\%$, $p < 0.05$. Western blotting performed on transfected cells shows that AMPII protein is significantly reduced in target cells when compared with control (decreased by $44 \pm 4.6\%$, $p < 0.05$).

AMPII protein expression is significantly reduced in the sheep model of tachypacing induced heart failure in both the atria and the ventricle. There is also a loss of t-tubules in cells transfected with siRNA against AMPII, suggesting that AMPII plays a key role in the maintenance of t-tubules. This reduction is likely to cause dysynchronous Ca²⁺ release, leading to reduced contractility and heart failure.

Muller AJ, Baker JF, DuHadaway JB, Ge K, Farmer G, Donover PS, Meade R, Reid C, Grzanna R, Roach AH, Shah N, Soler AP, Prendergast GC (2003). Targeted disruption of the murine Bin1/Amphiphysin II gene does not disable endocytosis but results in embryonic cardiomyopathy with aberrant myofibril formation. *J Mol Cell Biol* 23, 4295-306

Briston SJ, Caldwell JL, Horn MA, Clarke JD, Richards MA, Greensmith DJ, Graham HK, Hall MC, Eisner DA, Dibb KM & Trafford AW (2011). Impaired [beta]-adrenergic responsiveness accentuates dysfunctional excitation contraction coupling in an ovine model of tachypacing induced heart failure. *J Physiol* 589, 1367-1382.

Dibb KM, Clarke JD, Horn MA, Richards MA, Graham HK, Eisner DA, Trafford AW (2009). Characterization of an extensive transverse tubular network in sheep atrial myocytes and its depletion in heart failure. *Circ Heart Fail* 2, 482-9

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PC195

Maternal high fat diet disrupts circadian rhythms in the adult mouse offspring heart and induces cardiac hypertrophy

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An increasing number of women are obese during pregnancy, which has an adverse effect on the cardiovascular health of their offspring. In mice, we have previously shown that a maternal high fat diet (mHFD) increased the offspring's risk to cardiovascular disease (1, 2). Nevertheless, the molecular mechanisms involved are unclear. Endogenous clock networks regulate near 24-hour or circadian rhythms in physiological processes, and the molecular components of this clock network are found in nearly all mammalian cells, including cardiovascular-related cells, and are also expressed in early life. Disrupting circadian clock function has been shown to increase cardiometabolic pathologies, including cardiovascular disease and the metabolic syndrome (3, 4). We therefore investigated whether mHFD could disrupt clock and metabolism-associated genes in the offspring heart, precipitating its abnormal development in adulthood. Female C57/BL6J mice were maintained under controlled conditions and fed either a HF diet (HF; 45% kcal fat) or standard chow diet (C; 21% kcal fat) 4 weeks prior to and during gestation and lactation. Weaned offspring were fed the HF or C diet, generating the dam-offspring groups: C/C, C/HF, HF/C, HF/HF. Hearts were taken from 15-week old male offspring killed at 6 time points over a 24h light-dark period ($n = 5-6$ per time point per treatment group). The left ventricle (LV) was dissected, weighed and processed for real-time-PCR quantification for the clock genes *Cry1* and *Cry2*, the metabolic markers *Sirtuin3* (*Sirt3*) and insulin receptor (*Insr*), and the apoptosis-associated gene *Foxo3a*. The LV mass were 1.4-fold heavier ($p < 0.01$, ANOVA) in offspring from HF-fed dams (HF/C and HF/HF groups) vs C/C group. Circadian rhythms in mRNA expression for all genes examined phase-shifted, except for *Sirt3*, in the HF/HF offspring heart vs C/C. The amplitude of peak expression in the HF/HF group was 1.5-fold and 1.2-fold lower for *Cry1* and *Cry2*, respectively, and 2.1-fold lower for both *Foxo3a* and *Insr* ($p < 0.05$), vs C/C. Although there was no shift in rhythms of *Sirt3* expression, amplitude of peak expression in the HF/HF group was 2.9-fold lower group vs C/C ($p < 0.05$). Mean mRNA levels over the 24h period was found to be 1.2-fold lower for both *Cry1* and *Cry2*, 1.7-fold lower for both *Foxo3a* and *Insr*, and 3.7-fold lower for *Sirt3* in HF/HF vs C/C offspring hearts. The results suggest that mHFD leads to

LV hypertrophy. Alterations in circadian expression patterns and levels of clock and metabolism-associated genes due to mHFD may contribute to the abnormal development of the offspring heart, leading to increase risk to cardiometabolic pathologies in later life.

Bruce et al. (2009) *Hepatology* 50:1796-808

Elahi et al. (2009) *Br J Nutri* 10:1-6

Turek et al. (2005) *Science* 308:1043-5

Anea et al. (2009) *Circulation* 119:1510-7

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PC196

Cardiac contractility modulation increases action potential dispersion and ventricular fibrillation

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Cardiac contractility modulation (CCM) is an electrical therapy under investigation for heart failure (HF) where electrical signals are applied during ventricular refractoriness[1]. We have previously shown the acute effects of CCM via stimulated norepinephrine (NA) release, β_1 -adrenoceptor activation causing shortening of monophasic action potential duration (MAPD) [2]. Adrenergic activation is known to increase arrhythmia vulnerability hence it is important to determine the effects of CCM on ventricular arrhythmia susceptibility.

The aims of this study were to determine the effects of CCM on ventricular fibrillation threshold (VFT). Experiments were conducted on New Zealand white rabbit hearts (2.5-3Kg, n=10). Animals were pre-medicated with ketamine (10mg/kg), medetomidine hydrochloride (0.2mg/kg) and butorphanol (0.05mg/kg) (i.m.) and killed by sodium pentobarbitone overdose (111mg/kg, i.v.) with heparin (1000IU). Hearts were excised and perfused under constant flow (40ml/min). CCM signals were applied to the left ventricle (LV) (amplitude=20mA, duration=20ms), timed to coincide with the plateau of a local MAP. The effects of CCM on LV basal (CCMB) and apical (CCMA) MAPD were assessed during pacing (200bpm). Susceptibility to ventricular fibrillation (VF) was assessed using VFT, defined as the minimum current required to induce sustained VF with rapid pacing (30x30ms). Protocols were repeated during perfusion of the β_1 -adrenoceptor antagonist metoprolol (MET, 1.8 μ M). Spatial effects of CCM on LV APD were assessed using optical mapping with di-4-ANNEPS. Data represent mean $\pm$ SEM.

CCM caused a shortening of MAPD (n=6) from baseline [BL] close to the site of delivery during both CCMB (105 $\pm$ 5[CCMB] vs. 127 $\pm$ 5[BL] ms, P<0.001) and CCMA (109 $\pm$ 4[CCMA] vs. 131 $\pm$ 5[BL] ms, P<0.05). In both cases this resulted in an increase in MAPD apico-basal (max-min) dispersion. VFT was reduced from 5.6 $\pm$ 0.4 mA to 4.3 $\pm$ 0.6 mA (P<0.01) and 4.6 $\pm$ 0.2mA (P<0.05) during CCMB and CCMA respectively. Decreased VFT was correlated with increased MAPD dispersion (r²=0.29, P<0.05, n=6). The effects of CCM on MAPD (131 $\pm$ 4[MET+CCMB] vs. 128 $\pm$ 2[MET] ms, P>0.05) and VFT (5.4 $\pm$ 0.8[MET+CCM(B)] vs. 5.4 $\pm$ 0.7[MET] ms, P>0.05) were abolished during MET perfusion (n=6). Optical mapping studies revealed a greater range

of CCM induced shortening with basal stimulation than with apical stimulation (Fig. 1, n=4).

We show that CCM increases susceptibility to ventricular fibrillation via a β_1 -adrenoceptor mechanism that is related to an increase in MAPD dispersion. These data are in keeping with the known pro-arrhythmic consequences of adrenergic activation. Differential responses to CCM may reflect regional distribution of sympathetic nerves, ion channels and/or coronary flow. The localised effects of CCM, promoting heterogeneities of repolarisation, raises concern over its clinical use.

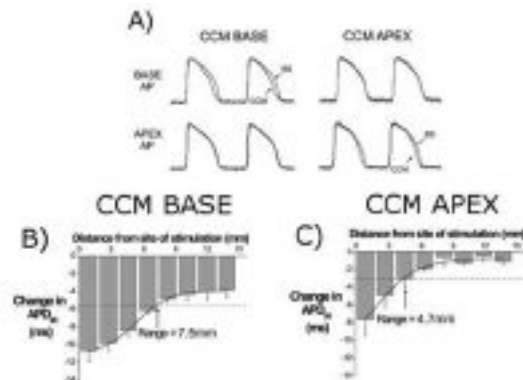


Fig 1. Spatial shortening of APD during CCM. A) Raw data B&C) Mean data demonstrating range of APD shortening during basal and apical CCM respectively.

Winter J, Brack KE, Ng GA. Cardiac contractility modulation in the treatment of heart failure: initial results and unanswered questions. *Eur J Heart Fail* 2010;13:700-710.

Winter J, Brack KE, Ng GA. The acute inotropic effects of cardiac contractility modulation (CCM) are associated with action potential duration shortening and mediated by beta1-adrenoceptor signalling. *JMCC* 2010; 51:252-262.

BHF and GWT

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC197

Pronounced reduction in parasympathetic innervation of brainstem vasculature in pre-hypertensive SHR rats

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Hypertension is a growing clinical problem. Understanding its aetiology could provide exciting new avenues for treatment and prevention. In humans and animals models, such as the Spontaneous Hypertensive Rat (SHR), it is commonly associated with high levels of sympathetic activity. Recent findings describe vertebrobasilar artery remodelling (wall thickening & lumen narrowing) in SHRs that occurs before the hypertension develops¹. We hypothesise this may be caused by increased sympathetic or decreased parasympathetic innervation of vertebrobasilar arteries. Thus, we examined these vessels in SH and Wistar-Kyoto (WKY) (normotensive) rats with focus on any changes in innervation that occurred with age and the development of hypertension in the SHR.

We used 7 pre-hypertensive (4-5w old) and 8 hypertensive (adult) SHRs and compared them to age-matched WKYs (N=4 and 8, respectively), all male. Rats were euthanised with sodium pentobarbital (200mg/ml) and transcardially perfused with 4% PFA. Ventral brainstem meninges containing the main

vasculature were labelled with the sympathetic noradrenergic marker α DBH and parasympathetic cholinergic marker α VAcHT. Images from the Vertebral Arteries (VA), Basilar Artery posteriorly (BAP) & Basilar Artery anteriorly (BAa) were sampled using fluorescent microscopy. For each the number of DBH or VAcHT stained fibres was summated within 3 'regions of interest' (0.15×0.15 mm). Fibre densities were calculated as fibres/ $(100 \mu\text{m})^2 \pm$ SEMs. 2way-ANOVAs and post-hoc Bonferroni (BFs) statistical tests were employed.

The biggest difference was observed in the parasympathetic innervation of SHRs compared to WKYs. Lower densities (-75%) occurred in both young (WKYs: 3.8-4.6 and SHRs: 1.0-1.3 ($\pm 0.1-0.6$)) and adult rats (WKYs: 3.2-4.3 and SHRs: 0.6-1.1 ($\pm 0.2-0.8$)) and hence was irrespective of hypertension. The effect was highly significant for both ages ($p < 0.0001$). All 3 areas were affected ($0.001 > p < 0.05$, BF). Sympathetic fibre densities were almost double that of the parasympathetic system, but the differences observed were more modest. Again SHRs had lower fibre densities than WKYs in both young (WKYs: 5.3-9.1 and SHRs: 4.9-5.3 ($\pm 0.4-1.2$)) and adults (WKYs: 5.0-6.8 and SHRs: 3.3-4.6 ($\pm 0.5-0.8$)). The effect was significant for both ages ($p < 0.05$ and $p < 0.0001$ respectively). Vessel position was also significant in young ($p < 0.01$). At both ages the BAP was significant in the BF ($p < 0.05$ and 0.01 respectively) so was the VA in adults ($p < 0.05$). Parasympathetic fibres mostly ran parallel to the sympathetic fibres.

We conclude that there is a paucity of parasympathetic innervation to the vertebrobasilar circulation and that this is present from early life in SHR. Given this finding, we propose that autonomically mediated vasodilatation of the vertebrobasilar arteries will be compromised in the SHR.

1. Cates MJ, Steed PW, Abdala AP, Langton PD, Paton JF. Elevated vertebrobasilar artery resistance in neonatal spontaneously hypertensive rats. *J Appl Physiol*. 2011 Jul; 111(1):149-56.

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PC198

Beta-adrenergic signalling and extracellular matrix remodelling: alterations in ovine ventricular cardiac fibroblasts

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Heart failure is characterised by an increase in circulating catecholamine levels and alterations to the composition of the cardiac extracellular matrix (ECM). Despite this association little is known regarding how beta-adrenergic signalling modulates cardiac fibroblast (CF) – dependent remodelling of the ECM. The aims of this study were; i) to develop a method to isolate and maintain ovine CFs in culture, ii) determine if beta-adrenergic stimulation of CFs alters production of matrix metalloproteinases (MMPs) and their endogenous inhibitors, the tissue inhibitors of MMPs (TIMPs) and, iii) determine if chronic exposure to catecholamines alters CF expression of the beta2-adrenergic receptor (beta2-AR).

Female Welsh Mountain ewes were killed by intravenous administration of 200 mg/kg-1 pentobarbitone sodium following 10,000 U of heparin. CFs were isolated from LV samples (~ 0.5 g) by incubation with 1,000 U collagenase type II

(Worthington) for 90 mins. Primary cultures were maintained at 37°C, 5% CO₂. All experiments were carried out on cells at passage 4. Confirmation that isolated cells were CFs was carried out by immunostaining. At passage 4, 2×10^6 cells were seeded in 225 cm² culture flasks, serum-starved for 24 h, and then media replaced with that containing either PBS, or 0.1 μ M ISO for 48 h. After which, conditioned-media was collected, concentrated with centrifugal filters (Millipore) and assessed for MMP activity (gelatin zymography) and TIMP proteins (immunoblotting). Cell pellets were collected and membrane preparations obtained for measurement of levels of beta2-AR by immunoblotting.

All cells isolated were positive for the fibroblast marker vimentin. Preliminary data ($n=2$ animals, minimum 3 repeats each) suggests that there is a trend towards an increase in MMP-2 activity with ISO compared to control. Furthermore there was also a trend towards an increase in protein levels of TIMP-1, TIMP-4 and membrane-bound beta2-AR with ISO.

These initial experiments suggest that beta-adrenergic signalling may play a role in ECM remodelling by altering levels of key players in matrix degradation. Further studies will be carried out to investigate changes in these proteins with increasing doses of ISO, and whether these changes occur to a similar degree in CFs isolated from aged sheep (>8 years) where cardiac ECM remodelling is different to that in young animals (1).

All procedures accord to The UK Animals (Scientific Procedures) Act, 1986.

1. Horn MA, Graham HK, Richards MA, Clarke JD, Greensmith DJ, Briston SB, Hall MCS, Dibb KM & Trafford AW. Age-related divergent remodeling of the cardiac extracellular matrix in heart failure: collagen accumulation in the young and loss in the aged. *JMCC* (2012). In press.

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PC199

Role of connexin36 in the electrotonic properties of sympathetic preganglionic neurones in the intermediolateral cell column of neonatal connexin36 null mutant mice

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The synchronisation of neuronal firing through electrotonic coupling via gap junctions is thought to play an important role in the coordination of sympathetic outflow. Sympathetic preganglionic neurones (SPN) within the intermediolateral cell column (IML) have previously been demonstrated to produce spontaneous activity in the absence of supraspinal innervations (1). Rhythmic population activity in spinal sympathetic nuclei which include these SPN can be abolished by gap junction blockers (2). The primary candidate for the formation of gap junctions within the IML is the connexin36 (Cx36) subunit (3). Using a Cx36-KO mouse we investigated the role of Cx36 in the electrotonic properties of SPN in the IML.

Experiments were performed using Cx36-CFP mice bred on a C57/Bl56 template and their wild-type litter mates (4). Neonatal mice (P7-14 days) were anaesthetised with i.p. sodium pentobarbital (60 mg/kg) before transcardial perfusion with ice-

chilled, oxygenated sucrose ACSF prior to decapitation. Whole cell recordings from SPN were performed in current clamp mode. Intracellular solution contained the sodium channel blocker, QX-314 bromide (2mM) to reveal the underlying coupled activity and Neurobiotin to allow anatomical identification of the neurone post-experimentally. Data was analysed by Fisher's exact test, one-way and two-way ANOVA as appropriate. All values are given as mean $\pm$ S.E.M.

Spontaneous coupled activity was observed in n=19/54 SPN from wild-type animals (Cx36^{WT/WT}) and in proportionally fewer SPN from heterozygote (Cx36^{WT/CFP}; n=16/78) and homozygote (Cx36^{CFP/CFP}; n=2/56) animals. There was a significant difference in the prevalence of spontaneous coupled activity between SPN from Cx36^{WT/WT} and Cx36^{CFP/CFP} animals (P<0.0001). Depolarisation by 5-HT (10 μ M) was observed in SPN from Cx36^{WT/WT} (n=17/28), Cx36^{WT/CFP} (n=22/42) and Cx36^{CFP/CFP} (n=15/34) animals with no significant difference between wild-type and homozygotes (P=0.213). Induction or augmentation of coupled activity by 5-HT (10 μ M) was significantly different between Cx36^{WT/WT} (n=16/28) and Cx36^{CFP/CFP} (n=6/34) SPN (P=0.0016). Quantification of the coupled activity showed a lower spontaneous frequency for Cx36^{CFP/CFP} SPN (0.1 $\pm$ 0.09 Hz, n=2) compared to Cx36^{WT/WT} (0.235 $\pm$ 0.05 Hz, n=12) and Cx36^{WT/CFP} (0.231 $\pm$ 0.067 Hz, n=15) SPN. Coupled activity in response to 5-HT (10 μ M) was induced or increased in SPN with a frequency of 1.12 $\pm$ 0.237 Hz (Cx36^{WT/WT}, n=15), 0.761 $\pm$ 0.136 Hz (Cx36^{WT/CFP}, n=21) and 0.298 $\pm$ 0.133 Hz (Cx36^{CFP/CFP}, n=6). Both 5-HT and genotype were significant contributors to these differences (P<0.0001 and P=0.0297 respectively).

We present here data showing the important contribution of Cx36 in both the prevalence and magnitude of rhythmogenesis in SPN in the IML.

Spannswick D & Logan SD (1990). *Neurosci* **39**(2):395-403.

Pierce ML, Deuchars J & Deuchars SA (2010). *Neurosci* **170**(3):827-38.

Marina N, Becker DL & Gilbey MP (2008). *Auton Neurosci* **139**:15-23.

Wellershaus K et al., (2008). *Exp Cell Res* **314**(5):997-1012.

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PC200

Age-related atrial remodelling and arrhythmogenesis in mice with atrial cardiomyocyte specific genetic deficiency of MKK4

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Fibrosis is a hallmark of atrial structural remodelling closely associated with ageing and atrial fibrillation (AF), however, its underlying molecular mechanisms are incompletely understood.

A pathway that is potentially involved in regulating the pathogenesis of AF is the mitogen activated protein kinase cascade (MAPK), since components of the MAPK pathway have been

shown to be down-regulated in patients with permanent atrial fibrillation (Kartmann et al. 2005). The MAPK pathway is involved in the regulation of fibrosis in the ventricle in aged/hypertrophied hearts (Lui W et al. 2011; Kyoi S et al. 2006) and fibrosis is linked to atrial fibrillation. To establish the role of the MAPK pathway in atrial function in age, we have developed a conditional knockout mouse model where a central component of this pathway; MKK4, has been specifically deleted from the atria (ACKO) (De Lange F et al. 2003; Liu W et al. 2009). Activity of MKK4 and its downstream effectors and associated changes in electrical, structural and intracellular signalling pathways and physiological consequences in the atrium will be investigated at cellular, tissue- and whole-heart and organism levels.

Hearts were assessed for atrial arrhythmia, both in vivo and ex vivo at 3 and 12 months of age (n $\geq$ 6 per group) and tissue was collected for molecular analysis. *In vivo* ECG analysis was carried out on mice anesthetized with isoflurane (2.5%) at 3 months of age showed abnormal atrial excitation with reduced P amplitudes (control 0.084mV $\pm$ 0.01 vs. ACKO 0.058mV $\pm$ 0.01 t-test p<0.05; mean $\pm$ SEM) but no arrhythmia; however as the mice aged they became more susceptible to atrial arrhythmia, such as atrial tachycardia (AT) and atrial ectopic beats. Ex vivo hearts from 12 month old MKK4 ACKO mice were more likely to develop AF/AT with electrical programme stimulation than old control mice (37% control mice vs. 100% ACKO mice). Conduction mapping revealed longer atrial conduction times in these mice, which could make the hearts more vulnerable to re-entry arrhythmias. In old ACKO mice an increase in fibrosis was detected by picro-sirius red stain of tissue sections (2.8% $\pm$ 0.2 in control vs. 6.1% $\pm$ 0.4 in ACKO t-test p<0.01; mean $\pm$ SEM). The expression of fibrotic pathway components was assessed using real-time PCR and western blot analysis and ACKO mice had altered patterns of expression of TGF- β 1, TGF- β receptors 1 and 2, MMP2/9 and TIMP2, compared to control mice.

Development of an atrial specific MKK4 knockout mouse therefore reveals a role for the MAPK pathway in protecting against atrial arrhythmia in age through regulation of fibrotic processes and provides insight into the design of more targeted therapies for atrial fibrillation.

The animal studies were performed in accordance with the Home Office and institutional guidelines.

De Lange F et al. (2003) *Genesis* **37**:1-4

Kartmann et al. (2005) *Circ Res*. **96**:1022-1029

Kyoi S et al. (2006) *Cardiovasc Res*. **69**:888-898

Liu W et al. (2009) *Circ Res*. **104**(7):905-14

Lui W et al. (2011) *J Mol Cell Cardiol*. **50**:702-711

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC201

Expression of protein markers of GABA transmission in the nucleus of the solitary tract of ovariectomized female rats in relation to the development of hypertension

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Many clinical and animal studies have demonstrated relationships between plasma oestrogen levels and blood pressure (BP), consistent with oestrogen acting centrally as a modulator of autonomic function(1). We have previously shown changes in the expression of oestrogen receptor (ER) subtypes in the nucleus of the solitary tract (NTS), an area of the brainstem regulating autonomic reflexes, associated with plasma oestrogen levels during the oestrous cycle in normal female rats and in ovariectomised (OVX) rats(2). It is well established that GABA transmission in the NTS is critical for baroreflex control of BP and since the promoter sequences for several GABA receptors, receptor associated proteins, synthetic enzymes and transporters include potential oestrogen response element consensus sites we have analysed levels of mRNA expression for a range of these proteins in the NTS of normal cycling and OVX female rats. Female Wistar rats (approx. 150 g) were fed a phytoestrogen free diet and were OVX or sham-OVX under anaesthesia (isoflurane, 5% in O₂) then radiotelemetry probes (DSI) were implanted with the cannula in the left carotid artery. BP, heart rate and activity were recorded for 3 weeks post surgery, at which point rats were killed under the same anaesthetic conditions. Brains were rapidly frozen on dry ice and micropunches of NTS tissue taken for reverse transcription of mRNA and analysis by real time PCR. Blood was collected for measurement of plasma oestradiol levels by ELISA. Percentage body weight gain in OVX rats ($22.8 \pm 0.6\%$; mean $\pm$ SE) was significantly greater than in sham-OVX rats ($14.3 \pm 0.9\%$), although food intake over the 3 weeks was no different. Mean BP after 3 weeks was significantly greater in all OVX rats compared to starting values (mean $+8.45\%$) whereas BP showed no change in the control rats (-1.29%). For 6 of the 14 GABA-A subunits analysed (alpha1; alpha3; beta1; gamma2, gamma3, delta) there were significant reductions in mRNA expression levels in OVX rats compared to shams in the low circulating oestrogen (metoestrus) phase of the cycle ($p < 0.05$; ANOVA; $n = 6$). In contrast, mRNA for the epsilon subunit was significantly up-regulated ($P < 0.05$). Expression levels of mRNAs for the GABA-B receptor B2 subtype and the vesicular transporter vGAT showed significant reductions ($p < 0.05$) in OVX, whereas expression levels for the GABA-A receptor associated protein GABARAPL1 and the GABA synthesising enzyme GAD67 were increased ($p < 0.05$) in OVX. The data are consistent with the hypothesis that circulating oestrogen acts on ERs in the NTS to modulate inhibitory GABAergic influences on autonomic reflexes controlling BP through affecting both GABA synthesis/release mechanisms and GABA receptor composition.

1. Saleh TM, Connell BJ. (2007). Role of oestrogen in the central regulation of autonomic function. *Clin.Exp.Pharmacol.Physiol.* 34:827-832.

2. Spary EJ, Maqbool A, Batten TFC. (2010). Changes in oestrogen receptor α expression in the nucleus of the solitary tract of the rat over the oestrous cycle and following ovariectomy. *J.Neuroendocrinol.* 22:492-502.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC202

Effects of changes in blood pressure and heart rate on indices of cardiac contractility

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Recently several drugs have been found to adversely affect cardiac contractility and there is a need to assess these risks in routine safety pharmacology studies. Left ventricular (LV) dP/dt_{max} and the QA interval (QAI, the interval between the onset of the Q wave of the ECG and the beginning of the rise in the following arterial blood pressure pulse) are used as indices of cardiac contractility, however, these have been reported to be influenced by changes in cardiac loading and heart rate. The aim was to determine the influence of heart rate and blood pressure (BP) on $LVdP/dt_{max}$ and QAI in the anaesthetised guinea pig.

Male Dunkin-Hartley guinea pigs (510-650 g) were anaesthetised with fentanyl ($50 \mu g kg^{-1}$ s.c.) followed by sodium (Na) pentobarbital ($50-60 mg kg^{-1}$ i.p.). The trachea was cannulated for ventilation with room air ($7-8 mL kg^{-1}$, 60 strokes min^{-1}) and ECG, arterial BP, and LV pressure (LVP) were recorded. BP was increased using phenylephrine (0.02 and $0.07 \mu mol kg^{-1}$) and decreased by Na nitroprusside (0.17 and $0.34 \mu mol kg^{-1}$) infused i.v. for 5 min each dose ($n = 6$). In a separate group of animals heart rate was increased via a pacing clip attached to the right atrial appendage and decreased by stimulation of the right vagus nerve ($n = 4$).

Phenylephrine increased mean BP from 48 ± 3 mmHg to a peak of 62 ± 2 mmHg with the first dose and 81 ± 8 mmHg with the second dose ($p < 0.05$, two-way ANOVA plus Dunnett's test). $LVdP/dt_{max}$ (2462 ± 371 to 2839 ± 285 and 3118 ± 342 mmHg sec^{-1}) and QAI (40 ± 2 to 44 ± 2 and 46 ± 2 msec) also increased. Heart rate was unchanged. Na nitroprusside decreased mean BP from 48 ± 3 to 30 ± 3 mmHg with the first dose and to 26 ± 3 mmHg with the second dose. Both $LVdP/dt_{max}$ and QAI also decreased (2462 ± 371 to 1947 ± 258 and 1800 ± 149 mmHg s^{-1} , and 40 ± 2 to 35 ± 3 and 34 ± 3 msec, respectively). Heart rate increased with the second dose of Na nitroprusside (258 ± 7 to 281 ± 8 beats min^{-1}).

Cardiac pacing and vagus nerve stimulation achieved a range of heart rates from 139 ± 4 to 321 ± 2 beats min^{-1} . At heart rates < 160 beats min^{-1} $LVdP/dt_{max}$ was 1295 ± 31 mmHg s^{-1} . This increased as heart rate increased peaking at 2860 ± 158 mmHg s^{-1} at heart rates of $220-249$ beats min^{-1} . As heart rate increased towards 310 beats min^{-1} $LVdP/dt_{max}$ decreased to 1532 ± 14 mmHg s^{-1} . QAI and BP were unchanged throughout.

In conclusion, $LVdP/dt_{max}$ and QAI were both altered by changes in BP, and $LVdP/dt_{max}$ by changes in heart rate. These parameters have limitations as reliable indices of cardiac contractility and care should be taken in their interpretation when simultaneous changes in heart rate and/or BP occur.

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PC203

Orthostatic test in diabetic patients. Results from a case-control study

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Introduction: OT is a simple, non invasive and reproducible test which allows the stimulation of the Autonomic Nervous System (ANS) and its exploration by measuring the BP and the HR variation during the upright posture. We aimed to describe the orthostatic test (OT) in diabetic patient and compare it with that of healthy subjects.

Patients and Methods: This cross-sectional case-control study included consecutive 20 diabetic patients suffering from type 2 diabetes (mean age 54.6 ± 7.6 years with extremes ranging from 31 to 72 years; duration of diabetes 48 [3, 240] months, 80% women) without degenerative complications which could interfere with ANS and 42 healthy subjects (mean age 53.5 ± 8.4 years with extremes ranging from 33 to 75 years, 81% women). The Medical research ethics comity of the faculty of medicine of Rabat approved the study, and all participants provided written consent. The two groups were matched for age ($p = 0.57$) and sex ($p = 0.99$). The basal BP and HR were measured at rest in both arms, every 5 minutes during at least 30 minutes. We then, proceeded to OT. We choose to measure HR and orthostatic SBP during upright posture of the rhythm of 3 measurements per minute during 10 minutes. The variations of the orthostatic HR (providing information on adrenergic alpha sympathetic activity impaired sympathetic if decrease more than 10 and sympathetic hyperreactivity if increase superior to 17) and orthostatic SBP (providing information on adrenergic beta sympathetic activity: orthostatic hypotension if decrease more than 20 and orthostatic hypertension if increase superior to 10) were compared between the diabetic patients and the healthy subjects using the Student's t-test for and the χ^2 test.

Results :

Diabetic patients have significantly more sympathetic hyperactivity than healthy controls (table 1).

Conclusion: This study suggests that diabetics tend to have a sympathetic hyper-reactivity during an orthostatic test. These results should be confirmed by larger studies.

parameters	Diabetic subjects (N=20)	Control subjects (N=42)	P
Abnormalities of the HR variations (ΔHR) (%)	80	54.5	0.2
Impaired Sympathic (%)	30	50	0.1
Sympathic Hyperactivity (%)	50	5.2	0.003
Abnormalities of the SBP variations (ΔPSA) (%)	25	26.2	0.5
Orthostatic Hypotension (%)	0	0	
Orthostatic Hypertension (%)	25	26.2	0.5

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC204

Low-level lead exposure and heart rate variability of urban children

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Previous studies report associations between lead exposure and cardiovascular morbidity. The cardiovascular effects of lead, however, are not limited to increased blood pressure and hypertension. It has been hypothesized that altered autonomic function may play a role. In this study we investigated the effects of low level lead environmental (background) exposure (assessed in hair by X-ray spectrophotometry) on autonomic function measured by changes in heart rate variability (HRV) in twenty-seven 15-16 year old children. HRV was evaluated from analysis of three-lead electrocardiography 5-minute recordings, which met the standards of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Lead concentration in hair was 5.62 ± 0.57 $\mu\text{g/g}$.

The mean total power (TP), very low frequency (VLF), low frequency (LF), high frequency (HF) and LF/HF ratio were 9826.07 ± 3303.30 ms^2 , 2168.37 ± 721.88 ms^2 , 2958.04 ± 886.98 ms^2 , 4699.74 ± 1999.97 ms^2 and 0.83 ± 0.08 in the frequency domain spectral analysis at supine rest. The mean standard deviation of the normal-to-normal interval (SDNN), the square root of the mean squared differences of successive NN intervals (RMSSD) and RRNN were 87.04 ± 11.33 ms, 94.41 ± 14.43 ms and 887.81 ± 21.76 ms in the time domain analysis at supine rest. In addition, HRV parameters in short-term recordings were examined in orthostatic position, after exercises and during deep breathing. There was a significant decrease of RRNN, pNN50 and HFn, accompanied by increase of LF/HF and LFn during active orthostasis and after exercises ($p < 0.05$). While the opposite changes were observed during deep breathing with significant increase of SDNN ($p < 0.05$). No significant correlations were found between lead concentration and HRV parameters assessed during the supine rest. However, statistically significant negative associations between LF/HF, LFn and lead and positive for HFn and lead ($0.36 < r_s < 0.44$; $p < 0.05$) were observed during exercises, showing that exposure to lead may be one of multiple factors that influence HRV.

These results suggest that altered cardiac autonomic function may play a role in pathogenesis relating to the harmful effects of lead exposure on human health.

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PC205

Effect of long term traditional meditation practice (Raja Yoga) on cardiovascular autonomic functions

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Traditional meditation technique (Raja Yoga) is a mind-body practice which affects psychological, behavioral and autonomic functions and is considered as an altered state of consciousness differing from wakefulness and relaxation at rest. It is

being used in some parts of the world as a therapeutic strategy for a various disorder of autonomic functions in complementary and alternative medicine (CAM). The present study was aimed to investigate the effects of duration of meditation on cardiovascular autonomic functions in long term traditional meditation (Raja Yoga) practicing healthy adults aged between 30 to 60 years. The case control study enrolled 12 males (age 46 ± 13.24 years) and 17 females (age 49.45 ± 10.10 years) with a history of 5 to 20 years (male, 11.16 ± 6.10 and female, 10.1 ± 4.9 years) of traditional meditation practice of at least 1 hour every day and cardiovascular autonomic parameters were compared with their healthy controls of same age group (male $n=30$, age $=45.91 \pm 11.9$ years, female $n=40$, age $=51.21 \pm 13.11$ years) without experience of any kind of meditation. After 15 minutes of awake supine rest for physical and mental relaxation, systolic blood pressure (SYS), diastolic blood pressure (DIA), heart rate (HR) were recorded in power lab from each participant following standard procedure. After recording at rest was complete, deep breathing test (6 cycles/ min) (DBT) in the supine position and lying to standing test (within 3 seconds) was done. To avoid masking effect of previous test, the valsalva maneuver and isometric exercise tests were administered at interval of every 15 minutes of the supine rest. From the record of Electrocardiogram, Expiration to Inspiration of R-R interval (E/I) and Valsalva Ratios were determined, and the values (mean $\pm$ SD) for SYS, DIA and HR were analyzed in SPSS 17 for windows and Pearson's correlation and Paired sample T test were used to observe the effect of duration of meditation on autonomic cardiovascular function and to determine the gender differences. Cardiovascular autonomic functions were not found significantly changed following different tests with meditating group. However, we observed a negative correlation of duration of meditation with SYS blood pressure ($r=-0.380$, $p \leq 0.05$) and mean arterial blood pressure ($r=-0.374$, $p \leq 0.05$) recorded during DBT. Comparison between the sexes showed that the practice meditation caused significant ($p \leq 0.05$) decrease in HR during DBT in females than in males. These findings suggest that the long term traditional meditation technique Raja Yoga reduces systolic and mean arterial blood pressures and it also enhances parasympathetic activities during relaxation Deep Breathing Test.

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PC206

Physiological performance profiles of Turkish Olympic water canoe team

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This study evaluated the physiological measures and objective performance parameters by incremental exercise test in elite canoeists. Eight male national level canoeists (mean age: 18.8 ± 2.8 years; height: 176 ± 6.4 cm; body mass index: 24.3 ± 3.2) competing at the international level for Turkey, volunteered and gave their written informed consent to participate in this study, which was approved by the institutional Ethics Committee. The athletes underwent symptom limited incremental (starting at a power output of 20 W for 5 min then increasing by 15 W every 2 min until exhaustion 15 W) cardio-pulmonary exercise testing on a cycle ergometer. Peak oxy-

gen consumption ($\dot{V}O_2$), peak workload, heart rate reserve (obtained from age predicted heart rate maximum) at exhaustion. Exhaustion was deemed to have occurred when the participant could no longer maintain the test. Data are presented as means $\pm$ standard deviations. They had $\dot{V}O_2$ of 46.21 ± 6.02 ml/kg/min and peak workload of 224.4 ± 27.6 Watt. The athletes have used $<90\%$ of their maximum heart rate reserve at exhaustion. During the test the athletes spent total exercise duration time (including 5 min of warming up) of 18.9 ± 1.9 min until exhaustion. This study revealed similar results for the Turkish Olympic Water Canoe Team to those of the international competitive canoeing athletes. This study was informative for the basal physiological performance parameters among these athletes and also would be useful for monitoring performance improvement through training.

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PC207

Long term enhancement of cerebral vascular resistance in spontaneously hypertensive rats produces a short term pressor response but long term re-modeling of the cerebral circulation

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Our recent evidence suggests that elevating cerebral vascular resistance in normotensive rats produces hypertension (Paton et al (2011)). We tested whether elevating cerebrovascular resistance by permanent occlusion of 3 cerebral arteries supplying the brain (3 vessel occlusion, 3VO: right carotid and both vertebral arteries) in spontaneously hypertensive rats (SHR) would produce a larger elevation in arterial pressure as they already have high cerebrovascular resistance.

Male SHR (≥ 250 g) were anaesthetized with ketamine (60mg/kg) and metadomidine (250 μ g/kg) i.p. The level of anaesthesia was frequently checked during the procedure using the pinch reflex. Body temperature was maintained at 37°C using a heat blanket. The animals were recovered from anaesthetic with a subcutaneous injection of atipamezole (1mg/kg) and given buprenorphine (0.15mg/kg) for pain relief as needed. Post surgery animals were housed individually, given standard rat chow and water ad libitum and kept in a climate controlled room on a 12 hour light/dark cycle. For BP monitoring, a DSI radiotelemetry implant was placed in the descending aorta. Following surgery rats were allowed to recover for at least 7 days before starting baseline BP recordings. For 3VO, the right common carotid and both vertebral arteries were occluded. Sham animals underwent the same surgery without occlusion of vessels. Cerebral vessels were studied 15-31 days after 3VO.

3VO elicited a rapid and significant increase in systolic BP (17 ± 2 (SEM) mmHg) from baseline compared to sham controls (3VO $n=7$, sham control $n=6$, $p<0.001$, unpaired T-test) lasting approximately 3 days after which BP returned towards baseline. The peak pressor response in SHR was larger than that seen in normotensive rats (~ 15 mmHg, Paton et al (2011)). Histological examination revealed that the diameter of the left vertebral artery, ventral medial spinal artery and right posterior communicating artery were increased significantly in the 3VO group by 19.3% (3VO $n=9$ vs. sham $n=10$, $p<0.05$), 56%

(3VO n=6 vs. sham n=8, $p<0.01$) and 74.7% (3VO n=6 vs. sham n=4, $p<0.01$) respectively compared to the shams.

In conclusion, our studies have provided further support of a neurovascular/cerebrovascular control of BP as occlusion of cerebral arteries feeding the brain results in a marked increase in BP, which is greater in SHR than normotensive rats. We propose that the vessel re-modelling is responsible for the lowering of BP after the initial pressor response post 3VO and permits an attempt to restore cerebral perfusion.

J. Paton, S. Guild, S. Malpas, C. Barrett (2011) Is cerebral blood flow a determinant of a long term arterial pressure set-point in the rat? Physiological Society Meeting, Oxford: PC132

MRC

Pfizer

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PC208

Drosophila Bteb2, a Kruppel-like transcription factor, specifies and maintains adult pericardial nephrocytes

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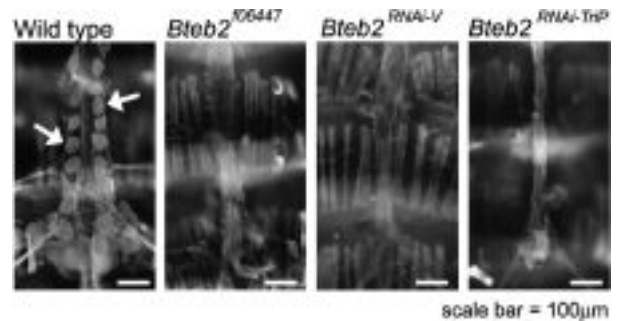
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Drosophila Bteb2 (dBteb2) is a Kruppel-like transcription factor (KLF) with no known function but which has mammalian orthologs that regulate cardiac hypertrophy and kidney fibrosis. dBteb2 expression initiates during embryogenesis and is sustained in the adult heart, which comprises contractile cardiomyocytes and pericardial nephrocytes (PNs), cells that represent important models of mammalian cardiac and kidney podocyte function.

In this work we explored the role of dBteb2 in *Drosophila* using flies carrying a P-element insertion in the dBteb2 gene and RNAi-mediated silencing of dBteb2 during development and adulthood, in heart cells and nephrocytes.

We found that the flies with the P-element insertion had negligible dBteb2 expression and that this was associated with the absence of PNs in adults and a slow heart rate. RNAi-mediated silencing of dBteb2 using two independent RNAi lines under the control of the *Hand* driver also prevented PN development. Temporal and regional control of gene expression (TARGET) was used to silence dBteb2 in the adult heart and PNs and this led to a profound impact on PN morphology and endocytic function but not viability.

These data establish that dBteb2 regulates the development of pericardial nephrocytes in *Drosophila*. Secondly, sustained expression of dBteb2 is required to maintain the functional integrity of the adult pericardial nephrocyte lineage. We propose that the dBteb2 transcriptional pathway represents an important model of KLF activity within mammalian kidney podocytes.



The adult *Drosophila* heart was stained with wheat germ agglutinin (red, cell surfaces) and phalloidinFITC (green, cytoskeleton). Large pericardial nephrocytes (arrows) can be seen in their characteristic position adjacent to the heart tube in wild type (CaS is shown) but not in the dBteb2^{T06447} mutants or Hand-driven dBteb2 RNAi silencing experiments using two independent RNAi lines, one from Vienna (RNAi-V), the other from Harvard (RNAi-Trip). Anterior is uppermost.

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PC209

Apelin and the APJ receptor – pharmacology and role in pulmonary arterial hypertension

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Introduction: The G protein-coupled receptor APJ was paired with its endogenous ligand apelin. Apelin has been shown to produce endothelium-dependent vasodilatation, endothelium-independent vasoconstriction, and to increase force of contraction in the heart *in vitro* (Maguire et al., 2009). Pulmonary arterial hypertension (PAH) is a serious condition in which pulmonary arterial pressure is increased with death resulting from right heart failure. Evidence suggests that vasodilatation and positive inotropy may be beneficial in PAH (Andersen et al., 2011) and therefore we hypothesised that APJ may be a therapeutic target. The endogenous apelin isoform (Pyr¹)apelin-13 has a short half-life, so we have generated a cyclic analogue, MM07, which is equipotent with (Pyr¹)apelin-13 in functional studies but more resistant to degradation. The objective was firstly to investigate the expression of apelin and APJ in PAH heart and lung to look for changes with disease. Secondly, to determine the pharmacology of MM07 compared with two endogenous apelin isoforms, (Pyr¹)apelin-13 and apelin-36 using a β -arrestin recruitment assay.

Methods: Heart and lung tissues were obtained from male Sprague-Dawley rats that had previously been treated with monocrystalline (MCT, s.c. 60mg/kg) (n=4), as a model of PAH, or saline (n=4). 30µm Tissue sections were used for peroxidase-anti-peroxidase and dual-labelling fluorescent immunocytochemistry using apelin and APJ-specific antibodies. Chinese hamster ovary cells artificially expressing the apelin receptor were used to investigate agonist-induced β -arrestin recruitment in a β -galactosidase fragment complementation assay. Concentration-response curves were constructed for

(Pyr¹)apelin-13 and apelin-36 (both 10⁻¹¹-10⁻⁶M), and MM07(10⁻⁸-10⁻³M). Values of pD₂ (-log₁₀ of the agonist concentration producing 50% of the maximum response) were obtained.

Results: APJ-like immunoreactivity (LI) was detected in the vascular endothelium and smooth muscle and the myocardium; while apelin-LI localised exclusively to the endothelium in saline and MCT-treated tissues. Specific staining of apelin and APJ appeared to be reduced in endothelium in PAH compared to healthy tissue. Receptor pharmacology revealed that MM07 (pD₂=5.8±0.1 SEM, n=6) was less potent than the endogenous agonists (Pyr¹)apelin-13 (pD₂=8.6±0.01, n=15) and apelin-36 (pD₂=8.5±0.01, n=3) at inducing β-arrestin recruitment.

Conclusions and Implications: Apelin and the APJ expression was identified in specific cell types of PAH rat heart and lungs. Quantitative measurements are necessary to confirm down-regulation of the apelin system in PAH. Compared to the endogenous apelin isoforms, MM07 was significantly less potent (p<0.05, Student's t test) as an agonist in the β-arrestin assay. This indicates that MM07 may be a biased agonist as these peptides were equi-effective in vasoconstriction studies. Maguire JJ *et al.* (2009). *Hypertension* **54**, 598-604.

Andersen CU *et al.* (2011) *Pulm Circ* **1**, 334-46.

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PC210

Carotid body responses to hypoxia and hypercapnia in a model of type I diabetes

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The consequence of type I diabetes upon alterations in ventilatory chemosensitivity is unresolved. *In vivo* observations in streptozotocin (STZ)-induced, chronically hyperglycaemic rats, have reported a decrease in tidal volume and minute ventilation in normoxia and a reduction in the ventilatory response to hypoxia and hypercapnia (Hein *et al.* 1994; Yamazaki *et al.* 2002). We aimed therefore to assess directly the effect of chronic hyperglycaemia upon carotid body (CB) function.

Diabetes was induced in male Wistar rats with streptozotocin (60mg.kg⁻¹ body mass) by intraperitoneal injection on two consecutive days. Initial starting weight of all rats (n=7) receiving STZ was 250-275g and tended to decline over the subsequent 2 wk period. Experiments were also performed on age-matched controls (n=6). CBs were isolated from terminally-anaesthetized adult rats (4% isoflurane in O₂ administered via face mask at a flow rate of 2 ml.min⁻¹), 2-3 weeks after STZ administration and single fibre recordings of chemoafferents were made from the carotid sinus nerve as described previously (Pepper *et al.* 1995). Precision flow meters (Cole Palmer Instruments) were used to set the HCO₃⁻ buffered superfusate with a desired PO₂ and PCO₂ to measure the CB response to hypoxia and hypercapnia respectively. Data was expressed as mean ± S.E.M and significance determined with unpaired t-test or ANOVA (Statview, Abacus Concepts) and taken as P<0.05.

The basal, single fibre chemoafferent frequency measured at ~300 mmHg PO₂ and 40 mmHg PCO₂ was significantly depressed in CBs from STZ animals compared with control CBs

(0.18±0.02 Hz vs 0.4±0.09 Hz, respectively). Reduction of the superfusate PO₂ caused an exponential increase in single fibre frequency with no significant differences being observed in the shape or position of the hypoxic response curves between groups. Peak hypoxic frequency was also not altered between the STZ and the control group; 18.4±4.3 Hz vs 25.2±1.5 Hz. CB activity in both groups increased in response to hypercapnia (80 mmHg PCO₂ in ~300 mmHg PO₂), but in contrast to hypoxia, the hypercapnic sensitivity of the CBs from the STZ group was significantly diminished, measuring 0.005±0.002 Hz.mmHg⁻¹ PCO₂ vs 0.015±0.003 Hz.mmHg⁻¹ PCO₂ in the control group.

The results from the present study suggest that basal CB activity is depressed in STZ animals, which may contribute to the decrease in minute ventilation in normoxia as previously described (Hein *et al.* 1994). Hypoxic sensitivity of the CBs from STZ animals appears to be well maintained after 2-3 weeks of hyperglycaemia. However, we suggest that the depression of the *in vivo* response to hypercapnia may have a significant peripheral component that is a consequence of a decreased CB sensitivity to changes in CO₂/H⁺.

Hein M, Schlenker E & Patel K (1994). Altered control of ventilation in streptozotocin-induced diabetic rats. *Proc Soc Exp Biol Med.* **207**, 213-219.

Yamazaki H, Okazaki M, Takeda R & Haji A (2002). Hypercapnic and hypoxic ventilatory responses in long term streptozotocin-diabetic rats during conscious and pentobarbital induced anaesthetic states. *Life Sciences.* **72**, 79-89.

Pepper D, Landauer R & Kumar P (1995). Postnatal development of CO₂-O₂ interaction in the rat *in vitro*. *J. Physiol.* **485**, 531-541.

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PC211

How do systolic blood pressure and heart rate change during Muslim's prayer?

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Objective: we aimed to explore changes of systolic blood pressure (SBP) and heart rate (HR) during muslim's prayer.

Material and methods: In this cross-sectional study, were included 32 adult persons without any Pwch could disturb autonomic nervous system (ANS). All participants underwent a standardized prayer test including 3 positions effected in the same following order: standing, bowing and prostrating. The positions' durations were respectively 60, 15 and 15 seconds to simulate the Muslim's prayer. SBP was measured using a sphygmomanometer. HR and SBP were recorded at supine position before the test (basal measures) and during the prayer test. Paired sample tests were used to compare HR and SBP values obtained at the 3 positions of the prayer test. P values were 2 sided and were considered statistically significant if less than 0.05.

Results: Basal measures of HR and SBP were respectively 63.4 bat/min and 111.8 mmHg. HR increase significantly at standing position of the prayer test (71 vs 63.4; $p < 0.01$) whereas SBP increase remains not significant (114 vs 111.8). HR and SBP decrease significantly during bowling ($p < 0.01$) and prostrating positions ($p < 0.01$).

Conclusion: This data illustrates changes of HR and SBP during Muslim's prayer. After a slight increase of HR and SBP at the standing position, a significant decrease of the 2 parameters occurs in both bowling and prostrating positions. Those results should be verified by larger studies.

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PC212

Centrally administered minocycline prevents desensitization of baroreflex induced by chronic intracerebroventricular infusion of hyperosmotic saline in Sprague-Dawley rats

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A diet containing high load of salt is considered as one of the important contributing factors to the development of essential hypertension. One of the possible mechanisms linking salt intake with hypertension is an increased concentration of sodium ions in the cerebrospinal fluid, which rearranges central control of the cardiovascular system (1). Recently proinflammatory cytokines (PICs) acting in the central nervous system were recognized as an important player in the pathogenesis of hypertension (2). Hyperosmotic conditions are known to induce synthesis of PICs (3). In the present study we sought to find out if centrally released PICs contribute to changes in the control of circulatory system induced by intracerebroventricular (ICV) infusion of hyperosmotic saline. To inhibit PICs production we decided to use a tetracycline antibiotic, minocycline, which inhibits release of PICs from microglial cells.

The study was performed on adult Sprague-Dawley male rats. The animals were implanted with L-shaped cannulae connected to osmotic mini-pumps for 2-week ICV infusion of either isoosmotic saline (0.9% NaCl, 5 μ l/hr), hyperosmotic saline (5% NaCl, 5 μ l/hr) or minocycline (5 μ g/hr) together with hyperosmotic saline (5% NaCl, 5 μ l/hr). After 14 days catheters were inserted into femoral artery and femoral vein for measurement of BP and HR and for intravenous infusions. All surgical procedures were performed under ketamine (100 mg/kg *i.p.*) and xylazine (10 mg/kg *i.p.*) anesthesia. 24-48hr after implantation of catheters we conducted measurements according to the following protocol: after baseline measurement of BP and HR, acute stressor was applied in the form of standardized air jet directed on the rat's head. Then baroreflex was pharmacologically tested by administration of phenylephrine *i.v.* (from 20 up to 200 μ g/kg/min) in order to produce a ramp increase of BP. After return of BP and HR to resting values nitroprusside was infused *i.v.* (from 20 up to 200 μ g/kg/min) to generate a ramp decrease of BP. Subsequently autonomic ganglia were blocked with hexamethonium *i.v.* (20 mg/kg) and measurements of BP and HR continued.

The increase of BP in response to air jet stress differed insignificantly between treatments. The blockade of autonomic ganglia resulted in similar decrease of BP and increase in HR in all groups. Baroreflex was blunted in rats receiving ICV infusion

of hyperosmotic saline in comparison to control group. Treatment with minocycline restored sensitivity of baroreflex. In conclusion these results show that centrally administered minocycline prevents desensitization of baroreflex caused by chronic ICV infusion of hyperosmotic saline.

Huang BS, Wang H, Leenen FH. Enhanced sympathoexcitatory and pressor responses to central Na⁺ in Dahl salt-sensitive vs. -resistant rats. *Am J Physiol Heart Circ Physiol.* 2001; 281(5):H1881-9.

Shi P, Diez-Freire C, Jun JY, Qi Y, Katovich MJ, Li Q, Sriramula S, Francis J, Summers C, Raizada MK. Brain microglial cytokines in neurogenic hypertension. *Hypertension.* 2010; 56(2):297-303.

Schwartz L, Guais A, Pooya M, Abolhassani M. Is inflammation a consequence of extracellular hyperosmolarity? *J Inflamm (Lond).* 2009; 23;6:21.

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PC213

Metabolic remodelling in mouse hearts with chronic coronary artery disease

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Background:

Atherosclerosis in the coronary arteries is a gradual, progressive and chronic process that may result in significant occlusion and subsequent myocardial infarction. However, prior to occlusive coronary disease, parts of the myocardium will experience reduced blood flow. This low-flow ischaemia is likely to trigger significant metabolic remodelling.

Aim:

To determine the changes in myocardial energetics in a murine model of chronic coronary heart disease (CHD).

Methods:

18 male apolipoprotein E knockout mice (ApoE^{-/-}) were fed either a western-type high-fat diet (n=12; 21% lard and 0.15% cholesterol) or chow diet (n=6) for 24 weeks from weaning. High-fat diet ApoE^{-/-} mice develop progressive atherosclerosis including in their coronary arteries (CHD). In contrast, littermates fed a chow diet exhibit no coronary disease (control). Ex-vivo hearts were perfused on a Langendorff apparatus with oxygenated Krebs solution. After 20 minutes of stabilization (pre-ischemia) hearts were snap frozen. The concentration of adenine nucleotides (ATP, ADP, AMP), inosine, hypoxanthine was determined using high pressure liquid chromatography (HPLC). Myocardial lactate was measured using a commercially available kit. Data are presented as Mean $\pm$ SEM and significance assessed using an unpaired Student's t-test.

Results:

There were no significant differences between ATP and ADP levels between groups. AMP nucleotide increased in coronary heart disease tissue compared with non-diseased control mice (CHD, 1.07 ± 0.11 ; control, 0.62 ± 0.045 ; $p = 0.013$). Subsequently, the phosphorylation potential as measured by ATP/AMP ratio was significantly lower in the disease group (1.7 ± 0.2 vs. 2.6 ± 0.38 ; $p < 0.05$). Additionally, the adenylate energy charge (AEC) was significantly lower in the diseased group (0.57 ± 0.02 vs. 0.65 ± 0.02 , $p = 0.019$). There were no differences in the tissue levels of lactate, inosine and hypoxanthine.

Conclusion:

The data obtained in this work suggests that hearts with progressive, chronic coronary disease are metabolically stressed compared to their control. This "ischaemic" stress is likely to trigger cellular remodelling including survival signalling which will impact upon their ability to respond to ischaemia and reperfusion injury.

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PC214

Using *Drosophila* to study functional relevance of conserved heart genes

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Drosophila melanogaster is increasingly utilised as a model of heart development and function due to its unparalleled genetic toolbox and fast experimental turnaround. We utilised the Fly Atlas database (the fruit fly gene expression atlas) to identify genes enriched in the adult fly heart that have high sequence homology with human genes. We are screening these genes experimentally to establish if they have a role in heart development and/or function. We identified 59 genes that have clear human orthologues. Genes identified included well-known 'heart' genes, e.g. Tinman, Neuromancer, Pannier, Seven Up - thus validating our *in silico* strategy.

One candidate heart gene identified, Fermitin 1 (*Fit1*) - an integrin-associated protein with 47% identity with its human orthologue (Kindlin 2) - was shown to be upregulated in the adult fly heart. Kindlin-2^{-/-} null mouse embryos die before cardiogenesis, making it very difficult to investigate how Kindlin-2 is involved in heart development and function. We have created a *Fit1* gene deletion line via imprecise P-element excision that displays semi-lethality during larval development - probably compensated for by its sister homologue in flies, *Fit2*. We will look at adult heart function in these mutants and, therefore, anticipate the study of *fit1* animals will shed additional light on the function of this gene during development and tissue morphogenesis. With our tools, the fruit fly will be an excellent model to study the function of these genes in the adult heart and will lead to further enlightenment of their function in the mammalian heart.

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PC215

The effects of temperature on cardiac E-C coupling and intracellular Ca²⁺ buffering in trout cardiomyocytes

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To investigate the effects of acute temperature change on cardiac excitation-contraction in fish heart, atrial and ventricular myocytes were isolated from rainbow trout acclimated to 12°C and studied using voltage clamp recording while simultaneously recording [Ca²⁺]_i at 7°C, 14°C, and 21°C. Cells were stimulated in the presence or absence of sarcoplasmic reticulum (SR) inhibitors and SR Ca²⁺ load and intracellular Ca²⁺ buffering were determined with caffeine. Atrial and ventricular cells showed increased *I*_{Ca} density, faster *I*_{Ca} inactivation, and smaller gain at 14°C and 21°C compared to 7°C. SR inhibition was most effective at 7°C, where it decreased [Ca²⁺]_i rise slope and amplitude by 58% and 44%, respectively, and prolonged recovery of [Ca²⁺]_i by 1.6-fold and decreased gain by 40%. An opposite trend emerged in ventricular cells in which SR inhibition had its largest effect at 21°C, where it decreased [Ca²⁺]_i rise slope and amplitude by 60% and 75%, respectively, resulting in a 56% decrease in gain. Bmax for Ca²⁺ buffering did not differ between atrium and ventricle but was higher at 7°C (1392 ± 274 μM) than at 21°C (451 ± 274 μM) while Kd was unaffected by tissue or temperature (0.27 and 0.30 μM for atrium and ventricle, respectively). Thus, atrial myocytes show a particular sensitivity to cold and increased reliance on the SR to maintain [Ca²⁺]_i in the face of reduced *I*_{Ca}, while ventricular myocytes do not. Maintained [Ca²⁺]_i at cold temperatures in both cell types is maintained by a larger SR Ca²⁺ load.

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PC216

Effect of Khat (*Catha edulis*) on bronchial asthma in Jimma University Specialized Hospital, Adult Chest Clinic, Jimma, Ethiopia

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Introduction:- Asthma is a chronic inflammatory disorder of the airways. About 300 million people worldwide were affected by asthma leading to approximately 250,000 deaths per year. The active chemical present in Khat could have a bronchodilator effect like that of catecholamine. The present study aims at determining the effect of khat chewing on bronchial asthma.

Methods:- A comparative cross sectional study was conducted in JUSH Adult Chest Clinic on 170 asthmatic patients with a 1.4 to 1 ratio of non-chewer to chewer between November 2010 and January 2010. Interviewer administered questionnaire, patient history and pulmonary function test using Spirometer was used to collect the data.

Results:- of 170 asthmatic patients, 72 were khat chewers and 98 were non chewers. Frequent asthmatic symptoms was seen

on 23(31.9%) of chewers and 43(43.9%) of non chewer ($\chi^2=2.488$, $p=0.11$). A less frequent use of β_2 agonist was observed on 42(58.3%) of chewers and 53(54.1%) of non chewer patients ($\chi^2=12.678$, $p=0.12$). Less frequent night time awake and chewing status was found to be positively associated [AOD=2.633, CI(1.778, 3.059)]. The mean predicted personal best of forced expiratory volume in one second (FEV1%) for chewers and non chewer was 62% and 46% respectively while their PEFR% was 40% and 26% respectively.

Conclusion:- In conclusion, apart from psycho stimulating prosperities, khat has moderate potential benefit for the improvement of episodes of asthma attack. This study showed that chewer asthmatic patients had relatively better PEFR and also relatively lesser recurrent night time awake due to asthmatic attack.

Bruce D. Where psyche meets soma in asthma. *Psychiatric times* 2002;19(1)

Wenzel S. Factor determining the severity of asthma. *Clinical and experimental allergy* 1998; (28); Supp 5; 119-125

Phal A. Szelenyi I. Asthma therapy in the new millennium. *Inflammation research*. 2002; 51(6):273-282

Damiani M, Dixon J. Managing the pressure: emergency hospital admissions in London, 1997-2001. London: Kings Fund, 2002

Matthias W, Daniel B. Asthma in Africa. *PLOS Med*. 2007;4(2);72

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PC217

Mitochondrial plasticity may determine the development of oxygen-sensitivity in rat carotid body

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Carotid body (CB) responses to hypoxia are low at birth and increase over time to mature responses. Using an in vitro rat CB-carotid sinus nerve (CSN) preparation, Kholwadwala and Donnelly (1992) demonstrated that the CSN activity in response to hypoxia increased from low levels to a robust adult response after two weeks. This time course of maturation was paralleled by an increase in TASK channel sensitivity to hypoxia in the O_2 -sensing Type I cells (Kim *et al*, 2011). The mechanism(s) which underpins this increase in O_2 -sensitivity has not been addressed. However, a body of evidence suggest mitochondria in Type I cells are critical for O_2 -sensing by the CB. Here the hypothesis that changes in mitochondrial organization and rate of oxidative phosphorylation may account for the development of O_2 -sensitivity in CBs was examined.

Type I cells were isolated from juvenile (4-6 days) and mature (14-16 days) rat carotid bodies, surgically removed during isoflurane (4%) anaesthesia. Cells were stained with Mito-tracker and Celltracker, imaged using a DeltaVision system (Applied Precision) and analyzed for volume using the program Imaris XT (Bitplane). Total mitochondrial volume in juvenile Type I cells was $100.3 \pm 13.6 \mu m^3$ (S.E.M, $n = 23$) and this was significantly smaller in mature cells, $22.6 \pm 3.4 \mu m^3$ (S.E.M, $n = 17$, $P < 0.00003$, unpaired Students t-test). This decrease in mitochondrial volume was not observed in non-oxygen sensing cells (superior cervical ganglion). Rate of oxidative phos-

phorylation was assessed by measuring the conversion of C_{12} -resazurin to fluorescent resarufin using an Olympus FV1000MPE 2-photon confocal microscope. Resarufin was excited at 870nm and emission recorded at 587 nm. No significant differences were observed in the rate of production of resarufin between juvenile ($n = 142$) and mature ($n = 57$) Type I cells. Resarufin production was abolished in the presence of cyanide (2mM).

These data demonstrate significant reductions in the mitochondrial content of Type I cells during development. This decrease in number does not cause a reduction in the rate of production of resarufin indicating that the smaller number of mitochondria may have increased their rate of oxidative phosphorylation. It has been observed that the K_m for oxygen in mitochondria is dependent on energy state (Petersen *et al*, 1974; Krab *et al*, 2011). Thus by increasing the rate of oxidative phosphorylation it is possible that mitochondria in mature Type I cells become more susceptible to inhibition by hypoxia than their juvenile counterparts. Consequently, the development of O_2 -sensitivity in rat carotid body Type I cells may be attributed to mitochondrial plasticity during the first two weeks of life.

Kholwadwala, D and Donnelly, D. F (1992) *J. Physiol.* **453**, 461-473

Kim, D *et al* (2011) *Resp Physiol and Neurobiol.* **117(3)**, 278-235

Krab, K *et al.* (2011) *Biochem. Biophys. Acta.* **1807**, 348-358

Petersen, L. C., Nicholls, P and Degn H (1974) *Biochem. J.* **142**, 247-252

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PC218

A modelling approach to assess the contribution of vascular remodelling to increased resistance in hypoxic pulmonary hypertension

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Chronic exposure to hypoxia such as at high altitude causes pulmonary hypertension (PH). PH is characterised by remodelling of pulmonary vessels and sustained increases in pulmonary vascular resistance (PVR)(1, 2). Vasoconstriction was previously viewed as playing a minor role in chronic PH, with remodelling of the pulmonary vessels accounting for the majority of the elevation in PVR. More recently studies have shown that in the rat increased PVR in the chronically hypoxic lung is almost entirely due to rho kinase dependent vasoconstriction (3). Knockout and transgenic mice are increasingly commonly used to investigate of PH. Therefore we developed an approach combining the isolated perfused lung technique and stereology to determine the contributions of vasoconstriction and remodelling to PVR in the hypoxic mouse.

Wild type mice were exposed to hypoxia (10% O_2) or normoxia (21% O_2) for three weeks following which they were deeply anaesthetized (sodium pentobarbital 60mg.kg⁻¹, i.p.) and exsanguinated. The lungs were removed and resistance assessed using an isolated ventilated perfused preparation ($n=17-20$ per group). In a subset of lungs ($n=8$ per group), the rho kinase inhibitor Y27632 (10-5M) was added to the perfusate and resistance assessed pre- and post-vasodilator addition. The increase in PVR in the hypoxic group was expressed

as a fraction of the mean normoxic resistance. Separate groups of lungs were isolated and fixed at standard airway and vascular pressures for quantitative stereological assessment of vascular structure as previously described(4). The reduction in lumen diameter in each hypoxic lung was expressed as a fraction of the mean normoxic value and the resultant change in PVR calculated using Poiseuille's equation.

In chronic hypoxic mice the mean increment in PVR was $0.85(\pm 0.032)^*$ of the mean normoxic value in control lungs. The rho-kinase inhibitor Y27632 significantly ($P<0.01$) reduced PVR by $0.37(\pm 0.04)^*$, but did not restore it to normoxic levels. The remaining increment above the mean normoxic value was taken to be due to structural changes (0.47 ± 0.04)^{*}. Total intracinar vessel length was unchanged following chronic hypoxia and therefore the structurally determined increase in PVR was modeled as solely due to a reduction in lumen diameter. This calculated structural component (Poiseuille's equation) was $0.54(\pm 0.089)$, which was not significantly different from the hemodynamically determined value.

In conclusion, two mechanisms contribute approximately equally to the elevation in PVR in the chronically hypoxic mouse lungs. Firstly, rho-kinase mediated vasoconstriction and secondly, structural lumen narrowing of the pulmonary vessels.

* significantly different from 0 ($P<0.01$)

Hopkins N, McLoughlin P. The structural basis of pulmonary hypertension in chronic lung disease: remodelling, rarefaction or angiogenesis? *J Anat.* 2002 Oct;201(4):335-48.

Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, et al. Survival in patients with idiopathic, familial, and anorexia-associated pulmonary arterial hypertension in the modern management era. *Circulation.* Jul 13;122(2):156-63.

Hyvelin JM, Howell K, Nichol A, Costello CM, Preston RJ, McLoughlin P. Inhibition of Rho-kinase attenuates hypoxia-induced angiogenesis in the pulmonary circulation. *Circ Res.* 2005 Jul 22;97(2):185-91.

Howell K, Costello CM, Sands M, Dooley I, McLoughlin P. L-Arginine promotes angiogenesis in the chronically hypoxic lung: a novel mechanism ameliorating pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol.* 2009 Jun;296(6):L1042-50.

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PC219

Identification of lung-selective microRNAs that contribute to the pathophysiology of chronic hypoxic lung disease

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Chronic lung diseases are among the major causes of death and disease worldwide. Pulmonary hypoxia is a common complication of chronic lung diseases leading to the development of pulmonary hypertension. The underlying sustained increase in vascular resistance in hypoxia is a response unique to the lung, suggesting that there are genes whose expression is selectively modulated in the lung. MicroRNA (miRNA) modulation of gene expression is emerging as an important regulatory mechanism in human disease. The aim of the study was to identify the miRNA profile underlying lung selective gene expression in hypoxia. Primary human microvascular endothelial cells from lung and cardiac tissue were cultured in normoxia

or hypoxia (1% O₂) for 3hr, 24hr or 48hrs (n=6 experiments for each time-point). Total RNA was extracted using the mir-Vana RNA Isolation kit (ABI, USA) and hypoxic conditions confirmed by TaqMan analysis using the hypoxic responsive gene VEGF-A. miRNA microarrays (n=48; LC Sciences-AS1001), which allow the simultaneous analysis of 1,719 human miRNAs, were used. RNA was reverse-transcribed to cDNA using Superscript II RNase H-Reverse Transcriptase kit (Invitrogen, UK) or Taqman microRNA Reverse Transcriptase kit (ABI, USA). Hypoxic conditions were confirmed by Taqman analysis using hsa-miR-210 (ABI, USA). The Eukaryotic 18S rRNA and RNU6B were used as endogenous controls (ABI, USA). Using a subtractive miRNA strategy, a cohort of 238 miRNA probes were identified which were differentially regulated in response to hypoxia in the pulmonary ($p<0.05$), but not the cardiac cells. Of these, 227 miRNAs were uniquely altered in the lung endothelium only and included miR-424 (previously reported as up-regulated in hypoxic human endothelial cells). Nine miRNAs were down-regulated in the lung and up-regulated in the heart, while two miRNAs (miR-18b and miR-19b) were up-regulated in the lung and down-regulated in the heart. Our subtractive approach also revealed 7 miRNAs that were up-regulated in the lung cells > 2-fold but showed no significant increase of expression in cardiac cells (miR-10b, miR-19b, miR-30b, miR-125a-5p, miR-20b, miR-466 and miR-568). These findings supported the hypothesis that miRNA expression was modulated by hypoxia in the pulmonary endothelium in a manner that was specific to that cell type and was different from the pattern of gene response observed in cardiac endothelial cells. Little has been reported of many of the hypoxic responsive lung-selective miRNAs that were identified in the microarray, highlighting novel targets for in-depth studies in our future work. Further analysis of miRNA/mRNA targets could reveal a role for these miRNAs in hypoxic lung disease, suggesting that therapeutic manipulation of these miRNAs may represent novel treatment strategies.

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PC220

Respiratory plasticity following chronic intermittent hypoxia during early neonatal life

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The respiratory control system is subject to considerable developmental plasticity. Perturbations during vulnerable periods of development can induce persistent maladaptive changes. Exposure to intermittent hypoxia (IH) is a common feature of several neonatal respiratory disorders, such as apnoea of prematurity. It has been established that chronic intermittent hypoxia (CIH) induces muscle dysfunction in adult animal models of sleep-disordered breathing. Any impairment in respiratory muscle function may have detrimental effects on respiratory performance and could exacerbate conditions characterised by IH. The major aims of this study were to determine the long-term effects of CIH, during development, on respiratory muscle function and basal breathing.

Litters of Wistar rats, together with their dams, were placed from birth or postnatal day 7 in hypoxia chambers. The CIH lit-

ters received alternating cycles of 90 sec hypoxia (5% O₂ at the nadir) and 210 sec normoxia for 8hr/day for 7 days. Sham litters were exposed to circulating normoxic gas for 7 days. After gas treatments, ventilation and the frequency of apnoeas were measured in sham and CIH treated animals using whole-body plethysmography. Following this sternohyoid and diaphragm muscles were excised and functional properties were examined *in vitro*. Littermates from sham and CIH groups were returned to normoxia for 21 days, after which ventilation and respiratory muscle function were studied. (n=8 all groups)

CIH treatment significantly decreased peak tetanic force in sternohyoid (4.2 ± 0.8 vs. 1.6 ± 0.3 N/cm²; sham vs. CIH, $P=0.009$, Student's *t* test) and diaphragm (12.5 ± 1.2 vs. 8.0 ± 0.7 N/cm², $P=0.006$) muscles from PD7 rats, but had no effect on PD14 respiratory muscles. CIH increased minute ventilation (209 ± 21 vs. 261 ± 19 ml/min/100g, $P=0.09$) and apnoea index (9.3 ± 1.9 vs. 14.0 ± 2.3 n/hr, $P=0.0674$), but these changes did not reach statistical significance. The CIH-induced negative inotropic effects on PD7 respiratory muscles persisted into early adulthood following recovery in normoxia, in sternohyoid (12.9 ± 0.7 vs. 11.0 ± 0.6 N/cm², $P=0.0685$), but not diaphragm muscles. There was no long-lasting effect of CIH on normoxic ventilation or apnoea index.

We conclude that early neonatal life represents a particularly vulnerable period for IH-induced respiratory plasticity. The combination of CIH-induced respiratory muscle weakness and increased susceptibility to apnoeas could have significant implications for respiratory performance in infants with immature respiratory control systems. Upper airway dilator, but not diaphragm, muscle dysfunction persisted into early adulthood, this mismatch in respiratory muscle performance could increase the risk of airway collapse *in vivo*. This is of clinical significance as episodic hypoxia could have detrimental long-term consequences for respiratory homeostasis later in life.

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PC221

Endogenous hydrogen sulfide mediates ventilatory response induced by hypoxia

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Background/Aims: Exposure to hypoxia triggers a series of responses including hyperventilation and anapnoea. Hydrogen sulfide (H₂S) synthesis is catalyzed by cystathionine β-synthase (CBS) or cystathionine γ-lyase (CSE). The presence and participation of CBS and CSE in response to hypoxia have been described in the carotid body but not yet in brain regions involved in hypoxia-induced hyperventilation and anapnoea. Therefore, we aimed at investigating the putative role of H₂S in ventilatory and thermoregulatory responses to hypoxia. **Methods:** Surgical procedures were performed under ketamine-xylazine anesthesia (100 and 10 mg/kg, respectively; 1 ml/kg, i.p.). Antibiotics (160,000 U/kg benzylpenicillin, 33.3 mg/kg streptomycin, and 33.3 mg/kg dihydrostreptomycin, i.m.; prophylactically) and analgesic medication (Flunixin; 2.5 mg/kg, s.c.) were provided immediately after the end of surgeries. Male Wistar rats (270-300g, n=45) were implanted with a guide cannula toward the third ventricle (3V; for microinjection i.c.v.) and with a temperature datalogger capsule in

the peritoneal cavity (to record body temperature, Tb). The animals were bolus microinjected into the 3V with a CBS inhibitor (Aminooxyacetate (AOAA), 100 pmol/2 μl, n=15), or a CSE inhibitor (Propargylglycine (PAG), 160 pmol/2 μl, n=16) or saline (2 μl, n=14). After the microinjection, ventilation and Tb of the animals were measured at 5, 10, 20, 30, 40, 50 and 60 minutes in normoxia or hypoxia (7% O₂). The results were statistically analyzed by linear mixed model followed by Duncan post hoc test. **Results:** During normoxia, microinjection of AOAA or PAG did not change ventilation and Tb compared with saline ($p>0.05$). When microinjected with PAG and exposed to hypoxia, the rats showed a significant increase ($p<0.05$) in tidal volume (12.57 ± 0.35 ml/kg) and ventilation (1674.57 ± 38.24 ml/kg/min) compared with saline (10.90 ± 0.34 ml/kg and 1461.78 ± 46.61 ml/kg/min). Interestingly, this increase was even higher when the animals were microinjected with AOAA and exposed to hypoxia, showing a significant increase ($p<0.05$) in tidal volume (13.90 ± 0.35 ml/kg) and ventilation (1971.12 ± 32.57 ml/kg/min) not only compared with saline (11.30 ± 0.46 ml/kg and 1497.47 ± 31.65 ml/kg/min) but also with PAG (12.57 ± 0.35 ml/kg and 1674.57 ± 38.24 ml/kg/min). **Conclusion:** The present results are consistent with the notion that endogenous H₂S plays an important role in modulating ventilatory response to hypoxia in rats.

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PC222

The novel cyclin-dependent kinase inhibitor, AT7519, overrides neutrophil survival mediated by a major gram positive bacterial cell wall component, lipoteichoic acid

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Neutrophils are a central component of the innate immune system and are beneficially recruited to the lungs in response to infection or injury. However, if neutrophil activity persists inappropriately this can perpetuate inflammation leading to disease. Apoptosis ensures potentially injurious inflammatory cells are recognised for removal by macrophages resulting in successful resolution of inflammation(1). Recently, cyclin-dependent kinase inhibitors (CDKi) such as Roscovitine have been shown to induce neutrophil apoptosis leading to improved resolution in animal models of inflammation(2). Neutrophil lifespan can be prolonged by the gram negative membrane component lipopolysaccharide (LPS)(3). Therefore we investigated the gram positive bacterial component (lipoteichoic acid (LTA)) on prolonging neutrophil survival by delaying apoptosis and whether this could be overcome by the novel CDKi, AT7519(4). We also investigated the effect of AT7519 on the key endogenous neutrophil survival protein, Mcl-1. Human neutrophils were isolated from peripheral blood and cultured over time in the presence of increasing LTA concentrations ($0-30 \mu\text{g/ml}$) ± AT7519 (Astell Therapeutics). Neutrophil viability and apoptosis were assessed by flow cytometry (annexinV and propidium iodide staining) and morphological analysis while Mcl-1 levels were analysed by western blotting(5). Values are mean ± SEM with statistical analysis performed using two-way ANOVA with a Bonferroni multiple comparison post hoc test.

LTA enhanced neutrophil viability in a time- and concentration-dependent manner by inhibition of apoptosis. For example,

by 20h in culture neutrophil survival was $16.2 \pm 2.2\%$ which more than doubled in the presence of $30 \mu\text{g/ml}$ LTA ($36.6 \pm 7.1\%$, $n=4$; $p<0.001$). AT7519 induced a time dependent induction of neutrophil apoptosis which was able to override the survival effects of LTA. For example, at 8h rates of apoptosis were $23.2 \pm 3.5\%$ in control, $11.5 \pm 3.1\%$ in LTA ($30 \mu\text{g/ml}$), $70.8 \pm 2.4\%$ in AT7519 ($1 \mu\text{M}$) and $64.4 \pm 2.4\%$ in combined LTA and AT7519 ($n=4$, $p<0.01$ for control vs. AT7519 and LTA). Induction of apoptosis by AT7519 was associated with down-regulation of Mcl-1, even in the presence of LTA, as assessed by western blotting ($n=3$).

This study showed that LTA enhances neutrophil longevity in vitro in a concentration and time-dependent manner by preventing apoptosis whereas the CDKi, AT7519, induces neutrophil apoptosis and overcomes the anti-apoptotic effect of LTA by a rapid down-regulation of Mcl-1. CDKi drugs can effectively "turn-off" neutrophils by driving apoptosis despite the presence of a gram positive pro-inflammatory stimulus. As such, CDKi drugs like AT7519 may have pro-resolution, anti-inflammatory therapeutic potential in disease states such as pneumonia where gram positive infections predominate.

Savill J, Wyllie AH, Henson J, Walport M, Henson PM, Haslett C (1989). Macrophage phagocytosis of aging neutrophils in inflammation. Programmed cell death in the neutrophil leads to its recognition by macrophages. *Journal of clinical investigation* 83: 865-875

Rossi AG, Sawatzky DA, Walker A, Ward C, Sheldrake TA, Riley NA, et al. (2006). Cyclin-dependent kinase inhibitors enhance the resolution of inflammation by promoting inflammatory cell apoptosis. *Nat Med* 12(9): 1056-1064

Lee A, Whyte MK, Haslett C. (2007). Inhibition of apoptosis and prolongation of neutrophil functional longevity by inflammatory mediators. *J Leukoc Biol.* Oct;54(4):283-8

Santo L, Vallet S, Hideshima T, Cirstea D, Ikeda H, Pozzi S, Patel K, Okawa Y, Gorgun G, Perrone G, Calabrese E, Yule M, Squires M, Ladetto M, Boccadoro M, Richardson PG, Munshi NC, Anderson KC, Raje N. (2010). AT7519, A novel small molecule multi-cyclin-dependent kinase inhibitor, induces apoptosis in multiple myeloma via GSK-3beta activation and RNA polymerase II inhibition. *Oncogene.* Apr 22;29(16):2325-36

Leitch AE, Riley NA, Sheldrake TA, Festa M, Fox S, Duffin R, Haslett C, Rossi AG. (2010). The cyclin-dependent kinase inhibitor R-roscovitine down-regulates Mcl-1 to override pro-inflammatory signalling and drive neutrophil apoptosis. *Eur. J. Immunol.* 40: 1127-1138

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC223

Effect of chronic intermittent hypoxia on reflex recruitment of sternohyoid EMG during airway obstruction in the anaesthetised rat

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Intermittent hypoxia elicits plasticity in sensory and motor pathways involved in the control of breathing with potentially adaptive and maladaptive consequences for respiratory homeostasis. We and others have shown that chronic intermittent hypoxia (CIH) – a major feature of sleep-disordered breathing – has deleterious effects on rat upper airway dilator muscle contractile function and motor control (1-3). In the present study, we sought to test the hypothesis that CIH impairs reflex

recruitment of sternohyoid (pharyngeal dilator) EMG during obstructive airway events.

Adult male Wistar rats were exposed to 20 cycles of normoxia and hypoxia (5% O₂ at nadir) per hour, 8 hours a day for 7 days (CIH, N=7). The sham group (N=7) were exposed to normoxia in parallel. Following gas treatments, rats were anaesthetised with an i.p. injection of urethane (1.5g/kg; 20% w/v). Fine concentric needle electrodes were inserted into the sternohyoid (pharyngeal dilator) and the costal diaphragm. Discriminated sternohyoid motor unit potentials and whole EMG, together with arterial blood pressure, tracheal pressure and O₂ saturation were recorded during quiet basal breathing and during nasal or tracheal airway occlusion. Arterial blood samples were taken intermittently to determine arterial PO₂, PCO₂, pH and haematocrit.

During basal breathing, the commonest discharge frequency of individual sternohyoid motor units determined from auto-correlograms was decreased in CIH rats but this did not achieve statistical significance ($53 \pm 6\text{Hz}$ vs. $36 \pm 7\text{Hz}$; sham vs. CIH; mean $\pm$ SEM, Student's t test, $p=0.09$). Area under the curve analysis of the integrated EMG recordings revealed that basal sternohyoid EMG activity was increased in CIH-treated rats but this did not achieve statistical significance ($18 \pm 5\text{a.u.}$ vs. $37 \pm 13\text{a.u.}$; $p=0.2$). Airway obstruction increased sternohyoid EMG activity in all animals; there was no difference in the reflex response to airway occlusion between sham and CIH-treated animals ($+47 \pm 3\%$ vs. $+49 \pm 6\%$, % increase from baseline, $p=0.75$).

We conclude that neither basal nor reflexly evoked motor discharge to the sternohyoid is affected by CIH. This is surprising since we have previously established that CIH causes sternohyoid muscle weakness – an effect blocked by antioxidant supplementation (3). Of interest, upper airway muscle activity is enhanced in sleep apnoea patients compared to controls, but this adaptation is not seen in this rat model. Though we have recently demonstrated that 7 days of CIH is sufficient to alter the control of breathing in sleeping rats (4), longer durations of CIH may be necessary to elicit plasticity in the neural pathways regulating upper airway calibre (1, 2).

O'Halloran KD *et al.* (2002) *Chest* 122: 269-275.

Veasey S *et al.* (2004) *Am J Respir Crit Care Med* 170: 665-672.

Skelly JR *et al.* (2012) *Am J Respir Cell Mol Biol* 46: 139-148.

Edge D *et al.* (2012) *Adv Exp Med Biol*, in press.

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PC224

Characterisation of noradrenaline and neuropeptide Y containing vesicles in brainstem of normo- and hypertensive rats

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We previously reported exocytotic release events of noradrenaline and adrenaline (N/A) in organotypic brainstem slice cultures from young (pre-hypertensive) spontaneously hypertensive rats (SHR) and normotensive Wistar (WR) controls. The distribution of quantal N/A sizes suggested diameters of up to 300 nm or more for a minority of vesicles, decidedly larger

than described in earlier publications (Chiti & Teschemacher, 2007). Release quanta were significantly larger in the rostral ventro-lateral medulla (RVLM) of SHR as compared to the nucleus of the solitary tract (NTS) of SHR; quantal events were also significantly larger in RVLM of SHR than in NTS or RVLM of WR (Teschemacher et al., 2008).

In the present study, we employed transmission electron microscopy in combination with immunogold labelling for dopamine- β -hydroxylase (DbH) or vesicular monoamine transporter-2 (VMAT2) to measure diameters of N/A-containing (NAergic) vesicles in neurones in the NTS and RVLM of adult WR (n=3) and SHR (n=3). Experiments were conducted according to the UK Animals (Scientific Procedures) Act 1986 and associated guidelines. 129 NAergic vesicle cross sections were evaluated and the distribution of vesicle diameters was extrapolated by linear regression analysis. The data indicate that the majority (>95%) of labelled vesicles have diameters of more than 150 nm and some measure up to 500 nm. Vesicle size distributions were neither significantly different between NTS and RVLM of WR, nor between NTS in WR and NTS in SHR (Student unpaired t-test). In contrast, the vesicle diameter distribution in RVLM of SHR (median 294 nm) was right shifted as compared to RVLM of WR (median 204 nm; $p < 0.01$) and as compared to NTS of SHR (median 215 nm; $p < 0.001$).

Double immunogold labelling showed that, in NTS and RVLM of WR, around 20% of NAergic vesicles also contained neuropeptide Y (NPY), while in SHR, in NTS over 50% and in RVLM over 60% of NAergic vesicles co-labelled for NPY. In RVLM of SHR co-labelling was found in significantly larger vesicles than in RVLM of WR or NTS of SHR ($p < 0.001$).

These results suggest that NAergic vesicles in brainstem areas involved in central cardiovascular control are larger than previous literature reported. Consistent with our earlier data on quantal release, vesicle sizes are increased in the RVLM of SHR. Co-localisation of N/A and NPY in the same vesicles, suggestive of co-transmission, is more pronounced in the RVLM of SHR, again in larger vesicles. Therefore, larger quantal sizes in combination with NPY co-release may be contributing to elevated sympathetic outflow promoting hypertension.

Chiti Z & Teschemacher AG (2007). Exocytosis of norepinephrine at axon varicosities and neuronal cell bodies in the rat brain. *FASEB Journal* 21, 2540-2550.

Teschemacher AG, Wang S, Raizada MK, Paton JF, & Kasparov S (2008). Area-specific differences in transmitter release in central catecholaminergic neurones of spontaneously hypertensive rats. *Hypertension* 52, 351-358.

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PC225

Optoactivation of the rat locus coeruleus bidirectionally modulates the excitability of nociceptive dorsal horn neurons in vivo

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Pontospinal noradrenergic (NA) neurons are believed to play a role in the descending control of nociception and stimula-

tion of the locus coeruleus (LC) can produce an antinociceptive effect at a spinal level through α_2 adrenoreceptors. However these experimental approaches employed local electrical stimulation of the LC which introduces potential confounds and it is not known whether these effects are mediated by postsynaptic actions of NA. We aimed to use selective, optoactivation of the LC to examine its effect on the excitability of lumbar dorsal horn (DH) neurones using patch clamp recording in vivo.

Rats (160g, n=8) were anaesthetised for recovery surgery with ketamine (50mg/kg) and medetomidine (300 μ g/kg) i.p. for stereotactic viral vector injection to the right LC. A lentiviral vector expressing ChR2 under the control of the PRS promoter was injected into the LC (300nl/site x 3) and a guide cannula was implanted. Four weeks later the animals were anaesthetised with urethane (1.5g/kg, i.p.) and a 200 μ m diameter optical fibre was placed above the LC for optoactivation (470nm, 10-30mW). Whole-cell recordings were made from DH neurons of the L3 spinal cord segment. Cells were characterised as being either nociceptive - high threshold (HT), low threshold (LT) or wide dynamic range (WDR) by responses to pinch or brush stimulation. Data are mean $\pm$ SEM or median[IQR].

Recordings were obtained from 70 DH neurons (Vm-64.8 $\pm$ 0.9mV, n=53, at depths from the surface of 230 [166-300] μ m) of which 18 were identified as HT neurons of 57 tested. LC stimulation evoked changes in excitability with latency from stimulus onset of 15s [11-22.5] and lasting 45s [30-100] (n=19). LC activation excited 41%, inhibited 25%, had biphasic actions 8% and was without effect in 25% of DH neurons. The change in excitability was mediated by postsynaptic membrane polarisations (32%), changes in synaptic activity (23%) or both (34%). A similar picture was seen in HT cells with 5/17 inhibited and 8/17 excited with both membrane polarisations and changes in synaptic input. These actions could significantly alter the behaviour of the spinal neurons either leading to spike discharge or attenuating their synaptic inputs. Post-hoc histology confirmed successful vector transduction of the LC in all cases, with evidence of expression of ChR2-mCherry in DBH containing fibres in the spinal cord.

These findings show that LC has multiple actions on sensory neurons in the DH. Far from observing a uniform inhibitory action (even on HT neurons) we were able to observe both pre and post-synaptic actions that were of both polarities. There is specificity of action of the LC at a spinal level and its engagement exerts bidirectional effects on sensory circuit excitability.

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PC226

Hypermetabolic *Gnasxl* knock-out mice have increased sympathetic control of heart rate variability

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The sympathetic nervous system (SNS) plays a crucial role in control of many physiological systems, including metabolism

and the cardiovascular system. It is often deregulated in disease states, for example chronic heart failure is associated with increased SNS activity. We are investigating changes in SNS control of the cardiovascular system in a hypermetabolic mouse model, the *Gnasxl* knock-out (1). *Gnasxl* encodes the signalling protein XL α s, which activates cAMP signalling pathways (2). It is expressed in brain regions crucial for SNS control, including the paraventricular and dorsomedial nuclei of the hypothalamus, the nucleus of the solitary tract and the Raphe Pallidus (3). Here we show, using heart rate variability analysis, that mice lacking XL α s have increased sympathetic stimulation of the cardiovascular system. We used radio telemetry to record ECG in conscious freely-moving adult knock-out (KO) mice and wild-type (WT) siblings. Telemetric transmitters were implanted subcutaneously under isoflurane anaesthesia; following recovery ECG was recorded continuously at 5kHz. After assigning beats using a custom program, heart rate power spectra were produced using Fast Fourier Transform of 3-minute sections of clean and stable heart rate data, using Welch's periodogram with 32-second windows and 50% overlap. Low frequency (LF) and high frequency (HF) bandings were determined empirically following inhibition of sympathetic and parasympathetic activity using reserpine and atropine, respectively. LF and HF bandings were assigned as 0.15-1.0 Hz and 1.0-5.0 Hz, respectively and validated by LF, HF and LF/HF responses to reserpine and atropine; LF/HF ratio was used as an indicator of SNS activity. Analysis of circadian heart rate and activity showed the most active period to be between 20:00 and 01:00; analysing heart rate variability during this time period showed elevated LF/HF ratio in KO compared to WT (1.73 ± 0.20 vs 1.17 ± 0.15 , $p < 0.05$ by t-test). Following SNS inhibition by reserpine, KO had a more substantial drop in LF/HF (1.39 ± 0.26 to 0.26 ± 0.06 , $p < 0.001$) than WT (1.25 ± 0.26 to 0.47 ± 0.09 , $p < 0.05$). Mild stress, caused by handling and scruffing of animals, elicited an increase in LF/HF in WT (1.23 ± 0.13 to 1.86 ± 0.20 , $p < 0.05$ by t-test), but no increase was seen in KO (1.77 ± 0.44 to 1.66 ± 0.44). Together, these results confirm previous data from the lab indicating that *Gnasxl* knock-outs have increased SNS stimulation of the cardiovascular system, and that the knock-outs may be chronically stressed. This indicates a global increase in SNS activity, and that XL α s must act centrally to inhibit SNS activity. The pathways involved will be investigated in future experiments, through targeted intracranial injections and brain slice electrophysiology.

Xie T., et al. (2006), J. Biol. Chem. 281: 18989-18999

Klemke M., et al. (2000), J. Biol. Chem. 275: 33633-33640

Krechowec S., et al. (2012), PLoS ONE 7: e29753

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PC227

The IUPHAR Database: Tools to navigate pharmacological space

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IUPHAR-DB (www.iuphar-db.org) is an open access database providing detailed, expert-driven annotation of the pharmacology of drug target systems from peer-reviewed primary literature sources. The database is maintained by a team of skilled curators, with guidance from the IUPHAR Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) and an international network of ~700 expert contributors. Here, we present several recent developments that significantly increase the scope and enhance the utility of IUPHAR-DB. The coverage of targets in IUPHAR-DB currently stands at 627 genes encoding G protein-coupled receptors, voltage- and ligand-gated ion channels, nuclear hormone receptors and the 10 enzymes of the lanosterol biosynthesis pathway. A joint initiative between NC-IUPHAR and the British Pharmacological Society (BPS) has led to the recent launch of a new open access portal, www.guidetopharmacology.org, which integrates IUPHAR-DB and the BPS Guide to Receptors and Channels (GRAC) and currently documents quantitative pharmacological information on over half of the targets of current licensed drugs. The database now contains over 3800 distinct ligand molecules, ranging from synthetic organic chemicals to natural products and peptides; an important recent addition is the curation of the sequences and post-translational modifications of ~500 endogenous peptide ligands. Information provided about ligands includes 2D structures, calculated physical-chemical properties, synonyms, selectivity data at targets and links to external chemical structure databases and to co-crystallised 3D structures in the Protein Data Bank. The database search interface has also been enhanced, allowing for navigation of the ligand chemical structure space covered by IUPHAR-DB and GRAC through text, identity, similarity, substructure and SMARTS-pattern queries. As an established internationally-recognised resource for pharmacologists, these recent developments to IUPHAR-DB further extend its usability, facilitate exploratory research in pharmacology and drug discovery, educate the next generation of biomedical and clinical scientists, and provide the general public with accurate information on how drugs work.

NC-IUPHAR (International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification)

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PC228

Enkephalinase inhibitors attenuates adenosine triphosphate-induced increase of intracellular calcium in cultured rat dorsal root ganglion neurons

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Several lines of evidence indicates that endogenous enkephalinergic system is involved in antinociceptive response in the spinal cord. However enkephalins are rapidly broken-down by endogenous enzymes, and therefore enkephalin-degradation enzyme inhibitors are considered as potential analgesics. Purinergic agonists adenosine triphosphate (ATP), a non-selective agonist for several ionotropic P2X and metabotropic P2Y receptor subtypes, function as peripheral pain mediator through mechanism involving increased sensitivity of peripheral nociceptive sensory fibres. By using intracellular calcium as a key nociceptive signal we investigated the effects of two endogenous enkephalinase inhibitors, opiorphin and spinorphin, on ATP-induced increase of intracellular calcium in cultured rat dorsal root ganglion (DRG) neurons. Following enzymatic digestion and mechanical agitation the DRG neurons were cultured on coated coverslips and loaded with 5 μ M Fura-2 AM. Standard fura-2 ratiometric technique was utilised for quantifying $[Ca^{2+}]_i$ responses in individual DRG neurons using fluorescence imaging system consisting of CCD camera coupled to an inverted microscope. For each experiment cells were consecutively stimulated twice with consistent duration of ATP application. Effects of opiorphin and spinorphin were tested on response to the second stimulus with ATP. To avoid variations in the $[Ca^{2+}]_i$ responses to the stimulation with ATP across experiments, the responses were normalized. All data were analyzed by using unpaired t test, $P < .05$ defining statistical significance.

The rise in $[Ca^{2+}]_i$ in response to ATP was significantly smaller after its second application in the presence of opiorphin than its first control application [(100 $\pm$ 0% (n=16, basal response to ATP) vs. 54.7 $\pm$ 4.4% (n=16, $P > 0.001$ for application of ATP with 10 μ M opiorphin); and 100 $\pm$ 0% (n=16, basal response to ATP) vs. 5.2 $\pm$ 3.9% (n=70, $P > 0.0001$ for application of ATP in the presence of 100 μ M opiorphin), respectively]. Application of spinorphin also attenuated Ca^{2+} responses to ATP in a dose dependent manner [(100 $\pm$ 0% (n=46, basal ATP response) vs. 97.9 $\pm$ 4.4% (n=46, $P < 0.05$) for application of ATP with 10 μ M spinorphin; and 100 $\pm$ 0% (n=51, basal response to ATP) vs. 58.6 $\pm$ 4.8% (n=51, $P > 0.001$) for application of ATP in the presence of 100 μ M spinorphin, and 100 $\pm$ 0% (n=47, basal response to ATP) vs. 58.6 $\pm$ 4.8% (n=47, $P > 0.0001$) for application of ATP in the presence of 300 μ M spinorphin, respectively].

In conclusion the study demonstrate that enkephalinase inhibitors, opiorphin and spinorphin, attenuates ATP-induced increase of intracellular calcium in cultured rat DRG neurons.

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PC229

The Nrf2 pathway is epigenetically silenced in neurons

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We recently demonstrated that Nrf2, a transcription factor and master regulator of Phase II antioxidant defences, has a key role in neuronal ischemic preconditioning *in vitro*. Neuronal protection originated from astrocytes not neurons, as no Nrf2 activation occurred in pure neuronal cultures. We currently aim to determine whether the Nrf2 pathway is indeed paradoxically inactive in neurons. Specific overexpression of Nrf2 *in vitro* in neurons affords significant protection from ischemia and upregulates Nrf2 target products, suggesting the pathway can functionally respond when Nrf2 is present. Basal Nrf2 mRNA expression levels are significantly higher in astrocytes than in neurons *in vitro*, and Bach-2 a major repressor of Nrf2 activity is significantly increased in neurons, possibly suggesting that the inactivity within neurons stems from a decreased availability of Nrf2, as opposed to impaired downstream pathway function. ChIP experiments reveal a significantly lower association of acetylated histone H3 (a marker of transcriptional activity) at the Nrf2 promoter in neurons versus astrocytes in culture. Application of histone deacetylase inhibitor TSA increases the association of acetylated histone H3 at the Nrf2 promoter in neurons and boosts neuronal Nrf2 mRNA expression. Fluorescent assisted cell sorting of mouse cortical tissue is currently underway to determine whether Nrf2 levels also differ in adult cell populations *in vivo*. Given that oxidative stress is a key pathological feature of numerous neurodegenerative diseases and stroke, an improved understanding of Nrf2 regulation in neurons is relevant.

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PC230

Natriuretic peptides decrease calcium transient in cardiac sympathetic neurons

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Natriuretic peptides (NPs) have recently been reported to elicit calcium dependent noradrenaline exocytosis from PC12 cells (sympathetic neuron phenotype) via a particulate guanylyl cyclase -cGMP-PKG-mediated inhibition of PDE3-induced hydrolysis of cAMP(1). However, others have shown that the nitric oxide coupled soluble guanylyl cyclase -cGMP pathway can inhibit cardiac noradrenaline release(2). Therefore we revisited the effect of natriuretic peptides on depolarization evoked calcium influx in cardiac sympathetic neurons in an attempt to resolve this discrepancy. 4-5 week old SD rats were humanely killed by an approved Home Office schedule 1 method, and neurons from the stellate ganglia were enzymatically isolated. Intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured by ratiometric fluorescence imaging using fura-2/AM in neurons(3). Western blot analysis was used to demonstrate NP receptor type A and B protein expression.

The evoked $[Ca^{2+}]_i$ transient was evaluated by 30 sec exposure to 50 mM KCl in the Tyrode solution. Fura-2/AM was excited alternately at 350 nm and 380 nm and the emitted fluorescence measured at 510 nm. Brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) were tested in this study. BNP and CNP significantly reduced the depolarization evoked $[Ca^{2+}]_i$ transient (100 nM BNP: $-12.73 \pm 2.80\%$, $n=18$, $P<0.01$; 250 nM BNP: $-21.22 \pm 7.46\%$, $n=11$, $P<0.05$; 100 nM CNP: $-16.09 \pm 4.19\%$, $n=6$, $P<0.01$) in cardiac sympathetic neurons. The cell-permeable cGMP analogue, 8-Bromoguanosine 3',5'-cyclic monophosphate (8-Br-cGMP, 100 μ M), also significantly decreased the $[Ca^{2+}]_i$ transient ($-14.71 \pm 5.78\%$, $n=13$, $P<0.05$). In contrast to the results of Chan et al., we show that BNP and CNP reduce cardiac sympathetic Ca^{2+} transients, an effect mimicked by increasing intracellular cGMP.

Chan NY, Seyedi N, Takano K, Levi R. An unsuspected property of natriuretic peptides: Promotion of calcium-dependent catecholamine release via protein kinase G-mediated phosphodiesterase type 3 inhibition. *Circulation*. 2012;125:298-307

Li D, Wang L, Lee CW, Dawson TA, Paterson DJ. Noradrenergic cell specific gene transfer with neuronal nitric oxide synthase reduces cardiac sympathetic neurotransmission in hypertensive rats. *Hypertension*. 2007;50:69-74.

Li D, Lee CW, Buckler K, Parekh A, Herring N, Paterson DJ. Abnormal intracellular calcium homeostasis in sympathetic neurons from young prehypertensive rats. *Hypertension*. 2012;59:642-649

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PC231

Novel optogenetic tools for control of astrocytic $[Ca^{2+}]_i$

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Astrocytes regulate the strength of synaptic networks and various aspects of neuronal activity (1). Neurotransmitters can activate astrocytic Gq-coupled receptors which results in accumulation of InsP3. InsP3 binds to its receptor located on endoplasmic reticulum (ER) to release Ca^{2+} . Ca^{2+} may also enter astrocytes from the extracellular space via various mechanisms. In order to use optogenetics to trigger $[Ca^{2+}]_i$ elevations in astrocytes previously we expressed in these cells the channelrhodopsin-2 mutant, ChR2(H134R) (2),(3). However, Ca^{2+} permeability of ChR2(H134R) is relatively low. Therefore, a recently published Ca^{2+} translocating channelrhodopsin (CatCh) reported to have up to 6-fold higher Ca^{2+} permeability (4) is of interest as a tool for optogenetic control of astrocytes. Native ChR2 and similar opsins such as CatCh are normally targeted to the plasma membrane but many important signalling events in astrocytes are triggered by release of Ca^{2+} from the ER.

As a step towards simulating this process, we have generated a fusion of CatCh, via a linker, to the transmembrane domains 1 and 2 of the InsP3 receptor 1 to achieve ER-specific targeting/retention. Enhanced yellow fluorescent protein (EYFP) was also fused into the construct to aid visualization. In HEK 293 cells, ER-CatCh-EYFP showed clear preference to localization within endomembranes, in contrast to native CatCh-EYFP, which is plasma membrane targeted.

We evaluated CatCh-EYFP and its ER-CatCh-EYFP as optogenetic tools for control of astrocytic $[Ca^{2+}]_i$. Primary astrocytes from neonatal rats were transfected with 0.5 μ g DNA plasmids to express the relevant constructs under control of cytomegalovirus (CMV) promoter using the TransIT-293 reagent (Mirus). After 48h, transfected astrocytes loaded with Rhod-2 AM were placed in a chamber mounted on a confocal microscope and perfused with Hank's Balanced Solution (HBS) at 34°C. Light-stimulation of the ER-CatCh-EYFP astrocytes triggered increases in $[Ca^{2+}]_i$ ($130\% \pm 1$; $n=80$; $p<0.001$; Student's paired t-test) and of the CatCh-EYFP ($135\% \pm 2$; $n=68$; $p<0.001$; Student's paired t-test). The latency of response was ≤ 2 s for both ER-CatCh-EYFP and CatCh-EYFP and >5 s for ChR2(H134R). The $[Ca^{2+}]_i$ peaked within ~ 30 -35s with CatCh in contrast to >60 s with ChR2(H134R) under comparable conditions.

Thus, both constructs are superior to previously used ChR2(H134R) as tools for optogenetic control of astrocytic $[Ca^{2+}]_i$ (5). Currently, we are working to establish exact contributions of extracellular and intracellular Ca^{2+} to the observed elevations of $[Ca^{2+}]_i$ in astrocytes. Viral constructs to express these tools specifically in astrocytes are also being generated. This approach opens a wide range of opportunities for enquiries into the physiology and signalling properties of these cells.

M. Nedergaard, et al., "New roles for astrocytes: redefining the functional architecture of the brain", *Trends Neurosci* 2003 Oct;26(10):523-30.

G. Nagel, et al., "Light activation of channelrhodopsin-2 in excitable cells of *Caenorhabditis elegans* triggers rapid behavioral responses", *Curr Biol* 2005 Dec 20;15(24):2279-84.

V. Gradinaru, et al., "Targeting and readout strategies for fast optical neural control in vitro and in vivo", *J Neurosci* 2007 Dec 26;27(52):14231-8.

S. Kleinlogel, et al., "Ultra light-sensitive and fast neuronal activation with the Ca^{2+} -permeable channelrhodopsin CatCh", *Nat Neurosci* 2011 Apr;14(4):513-8.

M. Figueiredo, et al., "Optogenetic experimentation on astrocytes", *Exp Physiol* 2010 Nov 1.

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PC232

Kisspeptin-evoked calcium signals in cultured rat trigeminal ganglion neurons

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Kisspeptin, the peptide product of KISS-1 gene, is potent secretagogue of gonadotropin-releasing hormone and has important role in puberty onset. Previous studies have shown that kisspeptin receptor, GPR54, is expressed in the dorsal root ganglia and dorsal horns of the spinal cord in addition to hypothalamus, amigdala and some different peripheral tissues. There is quite only limited data on possible involvement of kisspeptin system in peripheral nociceptive transmission. The aim of this study was to investigate the effects of kisspeptin on intracellular calcium ($[Ca^{2+}]_i$) levels in isolated rat trigeminal ganglion (TG) neurons.

TG neurons were isolated from neonatal rats, plated on poly-D-lysine-coated coverslips and maintained in neurobasal medium supplemented with B27. These neurons were loaded with 1 μ M Fura-2 AM and Ca²⁺ responses were assessed by using the fluorescent ratiometry. Fura-2 loaded cells were excited at 340 and 380nm, and emission was recorded at 510nm by using calcium imaging system. Changes in free [Ca²⁺]_i were determined by the 340/380nm ratio in each neurons. All data were analyzed by using unpaired t test, with a 2-tailed P level of <.05 defining statistical significance.

The increases in [Ca²⁺]_i as % of preceding control (baseline) levels was 141.9 $\pm$ 5.7 % (p < 0.001, n=28) produced by 1 μ M kisspeptin treatment. We performed same experimental procedure in extracellular Ca²⁺ free conditions. Similarly, kisspeptin significantly elevated [Ca²⁺]_i in TG neurons (100.0 $\pm$ 0.0 % and 116.9 $\pm$ 4.8 % in baseline and 1 μ M kisspeptin, respectively, n= 25 cells, p<0.01).

Results from this study for the first time indicates that kisspeptin increases [Ca²⁺]_i in isolated TG neurons in normal Ca²⁺ concentrations and Ca²⁺ free conditions. We can suggest from these data that kisspeptin may have a role for nociceptive transmission. Further investigations are needed to clarify the mechanism(s) of kisspeptin action on calcium signaling in TG neurons.

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PC233

The physiology of mitochondrial quality control is limited by mTSPO

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Mitophagy is a crucial process that preserves normal cell function in mammals by selective removal of defective mitochondria. In dissecting the molecular pathways that mediate its efficiency we have now identified an important role for the mitochondrial Translocator Protein (mTSPO), an 18-kDa protein localized on the outer mitochondrial membrane and over-expressed in pathologies such as brain inflammation and cancers (Gatliff and Campanella, 2012). In human, murine and canine cell lines, mTSPO was transiently over-expressed or silenced via mTSPO cDNA (+mTSPO) or siRNA (-mTSPO) using Lipofectamine and Ca²⁺ Phosphate transfection techniques. An empty vector (C) or non-silencing siRNA was used in control cells. Cells were harvested 36 h post transfection with modulation confirmed via western blot analysis (n>5). Fluorescence (F) or luminescence-based assays were employed to monitor alterations in core aspects of mitochondrial physiology (Seneviratne et al, 2012). Values are means $\pm$ S.E.M., compared by ANOVA. +mTSPO led to reduced mitochondrial Ca²⁺ uptake after challenge with an InsP₃-generating stimulus, ATP (100 μ M) (maximum Rhod-2 F (arbitrary units (A.U.): C 0.51 $\pm$ 0.02, +mTSPO 0.29 $\pm$ 0.03, -mTSPO 0.76 $\pm$ 0.03; p<0.001; n>10); lower mitochondrial membrane potential ($\Delta\Psi_m$) (tetramethylrhodamine methyl ester (TMRM) F (A.U.): C 1.00 $\pm$ 0.1 +mTSPO 0.84 $\pm$ 0.09 -mTSPO 1.93 $\pm$ 0.17; p<0.001; n>15); increased reactive oxygen species (ROS) production (rate of increase of oxidized dihydroethidium F: C 2.37 $\pm$ 0.19 +mTSPO 4.7 $\pm$ 0.34 -mTSPO 0.42 $\pm$ 0.05; p<0.001; n>10) and limited ATP generation. The opposite was observed across parameters in -mTSPO cells. To examine if these effects were

due to changes in mitophagy, 20 μ M carbonylcyanide-4-trifluoromethoxyphenylhydrazone (FCCP), an uncoupler, was applied for 2 h to stimulate mitophagy in cells transiently co-transfected with light-chain III-green fluorescent protein (LC3-GFP), an autophagosome (AP) marker, and mitochondria-targeted red fluorescent protein (mtRFP). Co-localization of the signals was quantified using Velocity software. Mitochondrial ubiquitination –pivotal in removal of defective mitochondria via the PINK1/Parkin pathway [Narendra et al, 2010]- was explored by immunolabeling in transfected cells and confirmed via immunoblotting of mitochondrial fractions (n=2). In summary +mTSPO cells had limited mitochondria containing APs (C 0.404 $\pm$ 0.034 +mTSPO 0.102 $\pm$ 0.024 -mTSPO 0.586 $\pm$ 0.065; n>20; p<0.05) by preventing ubiquitination of mitochondrial proteins (% cells with mitochondrial ubiquitination: C 25.4 $\pm$ 3.2 +mTSPO 4.9 $\pm$ 2.4 -mTSPO 53.4 $\pm$ 3.7 p<0.05 n>3) while in -mTSPO cells basal and activated mitophagy were augmented. These data suggest mTSPO may limit mitochondrial quality by restricting the efficiency of mitophagy. Where mTSPO levels surpass normal, unphysiological accumulation of defective mitochondria may cause and sustain cellular and tissue degeneration.

Gatliff J and Campanella M (2012) *Curr. Mol. Med.*

Seneviratne MS et al. (2012) *Curr. Mol. Med.*

Narendra DP et al. (2010) *PLoS Biol.* 8(1)

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PC234

Effect of body mass index on peripheral nerve conduction

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Nerve conduction study (NCS) assesses peripheral nerve functions and its parameters are known to vary with anthropometric measurements. This cross sectional normative study was done in Electro-diagnosis Lab II of department of Basic and Clinical Physiology, BPKIHS, Nepal. It was aimed to study the effect of BMI on NCS variables of the peripheral nerves of upper and lower limbs. The study was done in 34 consenting healthy human adults of either sex. The anthropometric factors, compound muscle action potential (CMAP) and sensory nerve action potential (SNAP) were recorded using standard technique. The relation of BMI with NCS variables were analyzed using Pearson's correlation test. After the adjustment of other anthropometric factors, BMI (21.8 $\pm$ 2.11 Kg/m²) showed a negative correlation with the CMAP duration of most of the motor nerves: right median (r= -0.388, p<0.005), left median (r= -0.342, p<0.05), left ulnar (r= -0.375, p<0.005), left tibial and right common peroneal (r= -0.347, p<0.05). The CMAP amplitudes of the right median (r= -0.341, p<0.05), left median (r= -0.456, p<0.01) and right common peroneal (r= -0.361, p<0.05); CMAP latencies of bilateral ulnar, left radial and right common peroneal were also negatively correlated. However, a positive correlation was seen with the SNAP amplitude of the right sural (r= 0.441, p<0.01) and a negative correlation with conduction velocity of left median sensory nerve (r= -0.420, p<0.05). The SNAP duration, latency and CMAP F-waves latency did not show any correlation. BMI showed a significant correlation with the NCS parameters of most of the motor and few

sensory nerves. Diagnostic conclusions made from the nerve conduction data without corrections for the BMI may be invalid in patients who are at its extreme. This must be also considered while developing standard/reference normative data for different nerves.

We would kindly like to acknowledge the technical staffs and the participating volunteers for supporting the research.

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PC235

Effect of Hexabromocyclododecane on TH-mediated action in the developing brain

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Thyroid hormones (THs) are critical for normal cerebellar development and function. Hypothyroidism has consistently resulted in abnormal growth, development, and function of the cerebellum including reduced growth, branching of the Purkinje cell dendrites, and extension of granule cell neurites. Hexabromocyclododecane (HBCD) is a brominated flame retardant used in a variety of household and commercial products including thermoplastic polymers and textile. It is currently a global environmental contaminant with detectable levels in both biotic and abiotic samples. Recent studies have shown that perinatal exposure to HBCD may alter spontaneous motor activity, disrupt learning and memory. Previously, we have shown that T4 (10 nM) treatment led to extensive dendrite arborization by Purkinje cells and low dose HBCD (10 pM) remarkably suppressed TH-induced Purkinje cell dendrite arborisation. In the present study, using primary cerebellar culture derived from new born rats, we show that time of withdrawal of T4 treatment is essential in Purkinje cell dendritogenesis. New born Wistar rats were decapitated under diethyl ether anesthesia on post natal day 1. The cerebella were digested with papain and dissociated cells were suspended in a serum-free medium without TH and plated in wells of chamber slides at a density of 2.5×10^5 cells / 0.2 ml. The slides were pre-coated with 0.1 mg/ml poly-L-lysine. Next day after cell plating, the culture was treated with T4, and then subsequently withdrawn in a time-dependent manner. Half of the culture medium was replaced with fresh medium every 2-3 days for 17 days. Also, we show that HBCD suppresses TH-mediated Purkinje cell dendrite arborization in a time-dependant manner *in vitro*. Furthermore, we also showed that low dose HBCD significantly impaired TH-mediated granule cell neurite extension *in vitro* in Wistar rats. Taken together, our study shows that exposure to low dose HBCD can lead to impaired TH-mediated morphogenetic events in the developing Cerebellum and may consequently disrupt normal brain development and functions.

Ministry of the Environment, Japan.

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PC236

Experience-dependent, layer-specific development of divergent thalamocortical connectivity

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The main sensory pathway from the whiskers to layer 4 (L4) of the cortex is shaped by experience during a critical period in the first postnatal week. The thalamocortical axons that comprise the final stage of this pathway also make a much weaker input to layer 6 (L6). The developmental profile of the relative input strengths to these 2 layers is currently unknown. To study this we made whole-cell recordings (perforated patch for LTP experiments) from thalamocortical slices from mice aged postnatal day (P) 3-9.

Using simultaneous recordings we show that thalamocortical input strength to L4 and 6 is similar in neonates (EPSC amp L4 36 ± 13 pA, L6 36 ± 14 pA, $n = 10$) and the dominance of the L4 input is established during the first postnatal week (EPSC amp L4 67 ± 19 pA, L6 28 ± 7 pA, $n = 7$). This relative change is associated with the selective expression of thalamocortical LTP in L4 (EPSC amp after pairing $206 \pm 46\%$ baseline, $n = 12$), but not L6 ($98 \pm 14\%$ $n = 18$). This increased input to L4 is mediated not by an increase in the input strength of each thalamocortical axon (P3-5 37 ± 6 pA, $n = 18$, P 8-9 37 ± 6 , $n = 26$), but by an increase in the proportion of L4 cells contacted by each axon (P3-5 9%, $n = 11$, P 8-9 55%, $n = 11$). Furthermore this increase in thalamocortical connectivity is experience-dependent in that it is absent in whisker trimmed mice (P8-9 whisker trimmed 9%, $n = 11$). Thus early experience acts via synaptic plasticity and leads to a layer specific increase in thalamocortical input strength. All values mean $\pm$ s.e.m.

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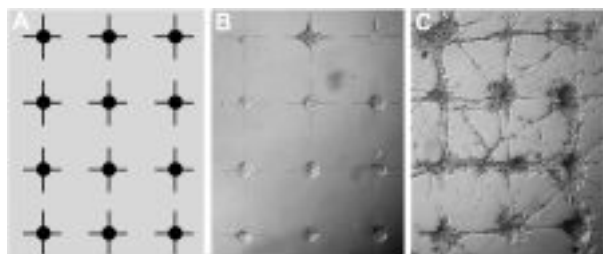
Patterning human dopaminergic neurons on photolithographically engineered silicon dioxide wafers functionalized with pre-adhered HEK293 cells

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The ability to interface neurons with silicon semiconductors remains a significant challenge but promises to allow bidirectional control of neural circuits and computers. A fundamental pre-requisite is the ability to define the spatial organization of patterned cells on a silicon chip. Parylene-C, a polymer used commercially to coat printed circuit boards, has previously been used to pattern primary murine hippocampal cells (1). Photolithographically defined arrays of parylene-C (on a silicon dioxide background) are activated by immersion in fetal calf serum; after which cells show preferential adhesion to parylene and repulsion from bare SiO₂. However, the underlying patterning mechanism is as yet uncharacterized. Moreover, these cultures are restricted by rapid glial overgrowth

which overwhelms patterned neurons. We sought to pattern a purified source of neurons, questioning whether functional neurons can remain viable in this isolated 'on-chip' context or whether they require a supporting cell substrate. The LUHMES (Lund Human Mesencephalic) cell line was chosen as a source of homogenous post-mitotic neurons. Two indices were derived to assess cell patterning. Parylene Adhesion Index (PAI) was calculated by dividing the surface area of cell material on parylene by total surface area of parylene within a given Region Of Interest (ROI). Each ROI consisted of an iteration of the parylene geometric pattern (figure A) surrounded by a square area of SiO₂. A SiO₂ Repulsion Index (SRI) was calculated by dividing surface area of cell material on SiO₂ by total area of SiO₂ in a given ROI, and subtracting the result from 1. 'Perfect' cell patterning on parylene results in a PAI of 1 (complete cell coverage of all parylene) and SRI of 1 (complete absence of cell material from SiO₂). Data reported are means $\pm$ S.D. Both undifferentiated and pre-differentiated LUHMES failed to adhere, nor show any morphological signs of differentiation, when cultured directly on-chip (undifferentiated LUHMES: PAI 0.02 $\pm$ 0.05, SRI 1.0 $\pm$ 0.01; differentiated LUHMES: PAI 0.0 $\pm$ 0.0, SRI 1 $\pm$ 0.001). We therefore sought a different cell type with which to pre-pattern parylene areas. HEK 293 cells pattern with high fidelity (PAI 0.46 $\pm$ 0.2, SRI 0.98 $\pm$ 0.01, figure B). Subsequent application of pre-differentiated LUHMES cells resulted in their adhesion to pre-established HEK 293 cell clusters (figure C). Moreover, this co-culture environment promoted morphological differentiation with neurites extending between islands of adherent cell somata. HEK 293 cells appear to fulfill a role analogous to glia, dictating cell location and generating a pro-neuronal niche. By refining our protocol, we aim to improve the resolution of this patterning platform and to gain control of the direction of neurite outgrowth.



A: Schematic illustrating photolithographically generated parylene pattern (black, 100 μ m diameter node with 300 μ m 'cross hair'. B: Following application of HEK 293 cell suspension. C: Four days following secondary co-culture with pre-differentiated LUHMES cell suspension.

Delivopoulos E, Murray AF, MacLeod NK, Curtis JC. Guided growth of neurons and glia using microfabricated patterns of parylene-C on a SiO₂ background. *Biomaterials* 2009;30:2048-2058.

Scholz D, Pöhl D, Genewsky A, Weng M, et al. Rapid, complete and large-scale generation of post-mitotic neurons from the human LUHMES cell line. *Journal of Neurochemistry* 2011;119(5):957-951.

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PC238

Role of Disrupted-in-Schizophrenia 1 (DISC1) in cortical inhibitory neurons

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Schizophrenia is a relatively poorly understood, debilitating psychiatric disorder affecting around 0.5% of the population worldwide. Recently, it has been suggested that DISC1 might be one of the main genetic risk factors for this disease. DISC1 has been implicated in brain development, in neurite outgrowth, neural precursor proliferation/differentiation, integration of newborn neurons and neuronal migration (1). DISC1 function in radial neuronal migration has been studied in some detail but there is little evidence implicating DISC1 in tangential migration and generation of cortical interneurons. In this study, two mouse lines with point mutations in the DISC1 sequence were used: the L100P and Q31L N-ethyl-N-nitrosourea (ENU) mutant mice previously characterized as 'schizophrenic-like' and 'depressive-like' respectively (2). The brain tissue was collected from 21 days old mice (both sexes, 8-12g, n=4-8 of each strain and genotype). Mice were sacrificed by injecting an overdose of anaesthetic (40mg/kg sodium pentobarbital) and perfused with 10ml PBS and 5ml 4% paraformaldehyde (PFA). The brains were fixed overnight in 4% PFA, cryoprotected in 30% sucrose and cut on a Leica CM3050 S cryostat. The number and relative distribution of cortical interneuron subclasses was analysed in five 500 μ m wide cortical regions: frontal and barrel-field primary somatosensory (fpSS and pSS respectively), visual (Vis), ventral auditory (vAUD) and primary/ventral auditory (p/vAUD) cortices. There was a significant decrease in the number of parvalbumin positive interneurons in fpSS, pSS and vAUD cortices of L100P mice when compared to their wild-type (WT) littermates, but no reduction in the total number of interneurons (cells expressing glutamate decarboxylase GAD67) (Table.1). A minor disruption in the relative distribution of the GAD67-positive cells in pSS and vAUD cortices was observed. No such differences were observed in the Q31L line. Furthermore, there was no significant difference in the total number calretinin and somatostatin expressing interneurons in L100P mice when compared to the WT control (Table.1). These findings implicate DISC1 in the generation of particular interneuron subclasses within the cortex.

cortical region	PV WT	PV L100P	GAD67 WT	GAD67 L100P	STT WT	STT L100P	CLR WT	CLR L100P
	n=8	n=4	n=5	n=5	n=5	n=3	n=5	n=5
fpSS	67.75 $\pm$ 4.76	50.00 $\pm$ 2.07	154.80 $\pm$ 7.47	142.67 $\pm$ 11.42	62.50 $\pm$ 4.51	66.83 $\pm$ 5.92	18.80 $\pm$ 1.09	20.67 $\pm$ 0.33
pSS	81.50 $\pm$ 3.69	35.15 $\pm$ 5.55	141.50 $\pm$ 8.97	155.33 $\pm$ 5.27	58.90 $\pm$ 3.75	51.67 $\pm$ 3.34	23.20 $\pm$ 4.04	18.33 $\pm$ 4.41
vAUD	33.81 $\pm$ 2.83	23.88 $\pm$ 1.91	127.00 $\pm$ 3.87	128.58 $\pm$ 5.85	59.83 $\pm$ 3.43	51.33 $\pm$ 1.17	21.50 $\pm$ 1.68	16.17 $\pm$ 5.67

Table.1 Total number of cells expressing selected interneuron markers: parvalbumin (PV), GAD67, somatostatin (STT) and calretinin (CLR); in regions of the cerebral cortex showing significant decrease in the number of parvalbumin expressing cells in L100P mice when compared to WT control (*Student's T-test; fSSp p<0.01; pSS, vAUD: p<0.05). Results presented as a mean $\pm$ SEM.

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Ect2, an ortholog of *Drosophila* pebble, regulates formation of growth cones in primary cortical neuronsT. Tsuji^{1,2}, C. Higashida-Tsuji^{1,2} and H. Higashida¹¹Department of Biophysical Genetics, Kanazawa University Graduate School of Medical Science, Kanazawa 920-8640, Japan, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan and ²Neurophysiology, Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK

To identify genes required for the brain development, we previously performed in vivo RNAi screening in *Drosophila* embryos. We identified pebble as a gene that disrupts development of the *Drosophila* nervous system. Although pebble has been shown to implicate in the neuronal development of *Drosophila* in the several screenings, involvement of Ect2, a mammalian orthologue of pebble, in the mammalian neuronal development has not been addressed.

To examine the involvement of Ect2 in the neuronal differentiation, we performed Ect2 RNA interference in the mouse neuroblastoma x rat glioma NG108-15 cell line. Silencing endogenous Ect2 mRNA with double-stranded RNA induced depletion of Ect2 and resulted in increased proportion of binucleate cells and morphological differentiation of NG108-15 cells characterized by the outgrowth of neuritis. These morphological changes were correlated with an increased level of acetylcholine esterase mRNA. In addition, expression of Ect2 was decreased in differentiated NG108-15 cells induced by dibutyl cyclic AMP. We also confirmed in another adrenergic clone, PC12 cells, that inhibition of Ect2 expression by RNAi stimulated neurite outgrowth. These findings indicate that Ect2 negatively regulates the morphological and functional differentiation in neuronal cell line.

Next, the effects of Ect2 depletion were studied in primary cultures of mouse embryonic cortical neurons. In the mouse embryonic cortex, Ect2 was accumulated throughout the ventricular and subventricular zones, which contain neuronal progenitor cells, from immunohistochemistry analysis. Loss of Ect2 did not affect the differentiation stages of neurogenesis, the number of neurites, or axon length, while the numbers of growth cones and growth cone-like structures were increased. Our results suggest that Ect2 contributes to neuronal morphological differentiation through regulation of growth cone dynamics in mouse embryo neuron.

Taken together, our results show that ECT2 may play a role in neuronal differentiation through regulation of cell cycle and cytoskeleton reorganization.

Koizumi K et al. (2007). PNAS 104, 5626-5631

Islam MS, Tsuji T et al. (2010). J Neuroendocrinol 22,477-82

Tsuji T et al. (2011). Cell Mol Neurobiol 31,663-668

Tsuji T et al. (2012). Neurochem Int [Epub ahead of print]

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PC240

Deficit in long-term potentiation after acoustic overexposure explained by a higher release probability

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The dorsal cochlear nucleus (DCN) in the auditory brainstem integrates auditory and non auditory inputs within its molecular layer. Exposure to loud sound (acoustic overexposure: AOE) has been shown to modulate synaptic excitability within the DCN. Furthermore, hyperexcitability in the DCN has been reported to underlie tinnitus (Zhang & Kaltenbach 1998). The mechanisms responsible for those excitability changes are still poorly understood, particularly in the molecular layer where synaptic plasticity has been reported. Here we investigated whether AOE altered synaptic plasticity within DCN slices of P17-P22 Wistar rats, 4 to 7 days after AOE. Rats were anaesthetized (with fentanyl 0.15mg/kg; fluanisone 5mg/kg, hypnovel 2.5mg/kg, i.p.) and exposed to a loud (110dB SPL) single tone (15kHz) for 6 hours. Control rats were anesthetized (but unexposed). Hearing threshold shifts of 30-40 dB SPL were observed 4 to 7 days after AOE at frequencies exceeding 15kHz ($p < 0.05$, $n = 5$). In unexposed condition, 0.3Hz stimulations of the DCN molecular layer triggered post synaptic field potentials in the DCN fusiform layer that were blocked by the glutamatergic AMPA receptor antagonist, NBQX. Furthermore, high frequency stimulations (HFS; 30 seconds 50 Hz) of the DCN molecular layer triggered an increase of the postsynaptic field potential peak amplitude from 0.35 ± 0.04 mV to 0.50 ± 0.06 mV ($n = 21$, $p < 0.05$). This long term potentiation (LTP) persisted for over 30mins. However after AOE, HFS were unable to elicit LTP in the DCN ($-4 \pm 6\%$; $n = 20$, NS). Paired pulses performed at 50ms showed a paired pulse facilitation (PPF: 1.28 ± 0.11 , $n = 10$, $p < 0.05$) in the unexposed condition by contrast to AOE where PPF was absent (0.97 ± 0.07 ; $n = 8$, $P > 0.05$). This latter indicated that the absence of LTP after AOE was due to higher release probability at DCN multisensory synapses. This was further confirmed by changing extracellular $[Ca^{2+}]_e$ to increase or decrease the release probability. In the unexposed condition, there was no PPF (0.92 ± 0.07 ; $n = 5$, $P > 0.05$) when tests were carried out in 3mM $[Ca^{2+}]_e$ (instead of 2mM $[Ca^{2+}]_e$). Increasing $[Ca^{2+}]_e$ from 2 to 3mM in the unexposed condition stopped the induction of LTP after HFS ($-4 \pm 7\%$; $n = 7$, $P > 0.05$). By contrast after AOE, decreasing $[Ca^{2+}]_e$ from 2 to 1mM reproduced the induction of LTP previously observed in the unexposed condition (field potentials increased from 0.37 ± 0.05 mV to 0.32 ± 0.06 mV; $n = 11$, $p < 0.05$). Paired pulse tests carried out in 1mM $[Ca^{2+}]_e$ after AOE also showed PPF (2.7 ± 0.5 ; $n = 4$, $p < 0.05$). In conclusion, after AOE, the absence of LTP at glutamatergic DCN multisensory synapses is due to an increased release probability and can be reversed by lowering extracellular calcium. This finding is an important step to finding selective therapeutic targets against tinnitus after AOE. (All values are mean $\pm$ sem and Paired T tests are used).

Zhang JS, Kaltenbach JA. 1998. Increases in spontaneous activity in the dorsal cochlear nucleus of the rat following exposure to high-intensity sound. Neuroscience letters 250:197-200

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PC241

Altered maturation of the primary somatosensory cortex in a mouse model of fragile X syndromeS. Till^{1,2}, L. Wijetunge^{1,2}, C. Bagni³, A. Contractor⁴, T. Gillingwater^{1,2} and P. Kind^{1,2}¹Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK, ²Patrick Wild Centre, University of Edinburgh, Edinburgh, UK, ³VIB Dept of Molecular and Developmental Genetics, K.U.Leuven, Leuven, Belgium and ⁴Department of Physiology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability, and is caused by mutations in the *FMR1* gene that lead to loss of the protein it encodes, FMRP. Many fragile X-related cognitive and behavioral features emerge during childhood and are associated with abnormal synaptic and cellular organization of the cerebral cortex. Yet, how the loss of FMRP influences the developmental trajectory of cortical maturation remains unclear.

To monitor cortical maturation, we exploited the stereotyped development of the organization of anatomical structures in layer 4 of the murine primary somatosensory cortex (S1), called barrels, which recapitulate the pattern of whiskers on the snout. Immunohistochemistry revealed FMRP throughout S1 in wild-type (wt) mice (n=3-4) during postnatal development, with its highest expression corresponding to times critical to barrel formation and synaptogenesis. To determine the effect of the loss of FMRP on S1 development, we compared cortical maturation in *Fmr1* knockout (*Fmr1*^{-/-}) and wt mice. Whereas cortical patterning and dendrite complexity in layer 4 are not altered in *Fmr1*^{-/-} mice compared to wt, loss of FMRP leads to a delay in the emergence of fine features of barrel cytoarchitecture and a decrease in the synaptic levels of proteins involved in glutamate receptor signaling at times corresponding to the highest levels of FMRP expression. Quantification of barrel segregation expressed as the ratio of the density of layer 4 cells in barrel walls to barrel hollows revealed a reduction in *Fmr1*^{-/-} mice compared to wt at postnatal day 7 (P7) [wt: 1.56 ± 0.04 (SEM), n=14; *Fmr1*^{-/-}: 1.42 ± 0.04, n=14; p=0.03 by unpaired Students t test], but not at P14. To look at the role of FMRP in dendritic spine shape, we classified spine morphology on individual fluorescent dye-filled S1 layer 4 spiny stellate neurons in *Fmr1*^{-/-} and wt mice at P14. Loss of FMRP led to an immature dendritic spine shape profile, with a greater proportion of filopodia [wt: 16.58 ± 2.70 (SEM), n=6; *Fmr1*^{-/-}: 26.56 ± 2.25, n=4; p=0.042 by MANOVA], and a decrease in the proportion of mushroom shaped dendritic spines [wt: 54.30 ± 3.77 (SEM), n=6; *Fmr1*^{-/-}: 42.71 ± 2.34, n=4; p=0.042 by MANOVA] in *Fmr1*^{-/-} mice compared to wt. Consistent with this, Western blotting of homogenates and synaptosomes from P7 and P14 *Fmr1*^{-/-} and wt neocortex (n=3-6) indicated that loss of FMRP causes a transient decrease in synaptic levels of proteins involved in glutamate receptor signaling. The specificity of the developmental delay in *Fmr1*^{-/-} mice indicates that loss of FMRP does not result in a general stalling of cortical maturation. Instead, our results suggest that inaccurate timing of developmental processes caused by the loss of FMRP may lead to changes in neural circuitry that underlie behavioral and cognitive dysfunctions associated with FXS.

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PC242

Normal time-course of synaptogenesis in a mouse model of Fragile X Syndrome

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Fragile X Syndrome (FXS) is the most common inherited form of intellectual disability. It is characterised by developmental delay and cognitive impairment and many affected individuals have autistic-like behaviours. The predominant cause of FXS is the transcriptional silencing of the fragile X mental retardation gene (*FMR1*) and loss of the corresponding protein (FMRP). The cognitive and behavioural features of FXS first emerge over the first two years of life, correlating well with the rapid phase of synaptogenesis in the cerebral cortex.

In mouse models of FXS the critical period for the maturation of glutamate receptor signalling at thalamocortical synapses is delayed (1) and there is an increase in dendritic filopodia in layer IV of the primary somatosensory cortex (S1). We hypothesized that genetic deletion of *Fmr1* in mice (2) causes a general delay in synaptogenesis.

To test this hypothesis, we compared the morphological features of synapses in layers II-III and IV of S1 at P14 and P35 by electron microscopy (3). No differences were detected between genotypes (*Fmr1*^{-/-} [P14: N=7, P35: N=5] and *Fmr1*^{+/+} [P14: N=8, P35 N=4]) in synaptic density, PSD length, number of presynaptic vesicles/μm of PSD or number of docked vesicles/μm of PSD at either age. Furthermore, age-dependent changes in these parameters were not significantly different in the *Fmr1*^{-/-} mice compared to controls indicating a similar time-course of synaptic maturation. We next examined mRNA levels in S1 for a selection of synaptic proteins at P7 and P14 [N=5 per genotype and age] using qPCR (3). Again, no differences were detected between *Fmr1*^{-/-} and *Fmr1*^{+/+} at either age or in the magnitude of the age-dependent changes in expression.

This study suggests that the loss of FMRP does not cause a general developmental delay of synaptogenesis in primary somatosensory cortex in a mouse model of FXS.

Harlow, EG et al. (2010) *Neuron* 65: 385-98Bakker, C & Reyniers, E (1994) *Cell* 78: 23-33Till, SM et al. (2012) *Hum Mol Genet* 1-14, doi: 10.1093/hmg/dds030

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PC243

Electrophysiological characterisation of human cortical neurones derived from stem cellsM. Livesey¹, B. Bilican², G. Haghi², S. Chandran², G.E. Hardingham¹ and D.J. Wyllie¹¹Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK and ²Euan MacDonald Centre, University of Edinburgh, Edinburgh, UK

The ability to generate regionally defined neuronal populations from human embryonic stem cells (hESCs) provides an important new experimental resource for the investigation of human neuronal physiology and disease. In order to realise this promise there is a need to address fundamental, unanswered questions pertaining to the ability of such hESC-derived neurones to exhibit multiple functional properties relating to the regionally defined population. In this regard, we have performed a detailed electrophysiological study of stem cell-derived neurones with a human cortical identity. H9 hESCs were neuralised in suspension using chemically defined medium. Such an approach was based upon the default model of neurogenesis that minimizes extrinsic and intrinsic signals that lead to alternative cell fates. Analysis of the resulting neural stem cells (NSCs) revealed a forebrain identity as determined by FoxG1 and Otx2 expression. NSCs that are plated as a monolayer culture subsequently generates predominantly Pax6-positive precursors that terminally differentiate into a mixed population of cortical neurones showing CTIP2, SATB2 or Reelin expression. The electrophysiological properties of neurones were sampled weekly up to Week 5 (inclusively) after plate down of neural stem cells. From Wk1 to Wk5, whole-cell capacitance measurements increased ($p < 0.001$) from 6.5 ± 0.4 pF to 17.6 ± 1.4 pF, resting membrane potential hyperpolarizes from -43.8 ± 2.1 mV to -56.9 ± 2.1 mV ($p < 0.001$) and the input resistance of the neurones decreased insignificantly from 2361 ± 185 M Ω to 1486 ± 101 M Ω . By Wk 5 nearly all cells patched fired TTX-sensitive action potentials in response to current input ($>95\%$), where the majority of these responses (60%) were in the form of trains of action potentials. Correspondingly, there was a developmental increase in the current density of voltage-gated sodium and potassium channels. AMPA-, GABA- and NMDA-evoked currents were recorded from the neurones, and at Wk 5 the current densities (in pA/pF) were found to be 87.8 ± 7.5 ($n = 30$), 3.2 ± 0.5 ($n = 30$) and 1.2 ± 0.3 ($n = 30$) respectively. Perforated-patch-clamp experiments using gramicidin demonstrated that GABA mediated a depolarising response ($n = 8$) that had a reversal potential of -46.1 ± 4.0 mV ($n = 5$) consistent with an immature phenotype. In addition neurones also display tetrodotoxin-insensitive mEPSCs that are blocked by CNQX. Further pharmacological assessment of NMDA and AMPA-evoked currents also indicated the presence of receptor complexes associated with early-stage development. Taken together our data indicate these hESC-derived neurones possess an embryonic-like phenotype compatible with immature cortical neurons. The data suggests the protocol employed is a model of human corticogenesis in vitro.

This work is supported by The Wellcome Trust and MRC.

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PC244

White Matter Abnormalities in the *Fmr1* Knockout MouseC. Chiang^{1,2}, S.M. Till^{1,2}, A. Domanski^{1,2} and P.C. Kind^{1,2}¹Centre for Integrative Physiology, The University of Edinburgh, Edinburgh, UK and ²The Patrick Wild Centre, The University of Edinburgh, Edinburgh, UK

The autism spectrum disorders (ASDs) and fragile X syndrome (FXS) share many symptoms such that many fragile X males will meet autism diagnostic criteria at some point in their lives. Whereas other forms of autism appear to involve multiple genetic factors, FXS is caused by mutations in a single gene, *FMR1*, that lead to loss of the protein it encodes, FMRP. Thus, genetic models of FXS facilitate the study of the role of FMRP in addition to offering insights into mechanisms underlying symptoms shared with the ASDs.

While most studies have focused on the effect of FMRP deletion on neuronal physiology, very little is known about its role in glial development. Two studies suggest that FMRP suppresses the synthesis of myelin basic protein (MBP), indicating that loss of FMRP may lead to alterations in myelination. Consistent with this possibility, human imaging studies have revealed abnormalities in the microstructure of white matter tracts in FXS and the ASDs. One possible consequence of abnormal myelination is the impairment of action potential propagation, potentially leading to alterations in the timing of synaptic signaling, a key regulator of spike-timing dependent plasticity (STDP). In support of this notion, global *Fmr1* knockout (*Fmr1*^{-/-}) mice have alterations in this form of synaptic plasticity.

Myelination in the rodent CNS begins around postnatal day 14 (P14) and reaches its maximum around P35. To determine whether loss of FMRP affects the development of forebrain myelinated fibres, we compared the progression of myelination in *Fmr1*^{-/-} mice and wild-type littermates over this period. We used immunohistochemistry for MBP to determine the gross morphology of major white matter tracts at P15 and P21. To get a functional and structural index of axon myelination, we quantified the g-ratios from myelinated axons in high-power electron micrographs through the corpus callosum at P14 and P35. Finally, to understand how loss of FMRP might affect the basic physiological properties of axons, we simulated axon conduction velocity using measures derived from our quantification of electron micrographs.

Our preliminary data suggest that loss of FMRP does not affect the gross morphology of white matter tracts or g-ratio measures. However, axon diameter is significantly larger in *Fmr1*^{-/-} mice ($n = 5$) compared to controls ($n = 4$) at P35 ($p = 0.03$; Student's t-Test). We will further use our computer simulations to test whether this increase in axon diameter would eventually lead to altered signal transduction or not. Together, our current data suggest a potential mechanism underlying the disrupted STDP associated with the loss of FMRP, and offer a new perspective on the cause of intellectual disabilities in FXS and related disorders.

Hagerman R, Hoem G, Hagerman P (2010). *Mol Autism* **1**, 12.

Wang H et al. (2004). *Hum Mol Genet* **13**, 79-89.

Barnea-Goraly N et al. (2003). *Am J Med Genet B Neuropsychiatr Gene* **118B**, 81-88.

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PC245

Early glucocorticoid negative feedback alters electrical activity of anterior pituitary corticotroph cells

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Corticotroph cells from the anterior pituitary are an important component of the hypothalamic-pituitary-adrenal (HPA) axis, which controls the neuroendocrine response to stress. HPA axis dysfunction can have many consequences on health. In response to a stressor, the hypothalamic neuropeptides corticotrophin-releasing hormone (CRH) and AVP stimulate ACTH release from corticotrophs. ACTH, in turn, releases glucocorticoids (corticosterone in rodents) from the adrenal gland which negatively feedback to inhibit ACTH secretion. Corticotroph cells are electrically excitable and fire single-spike action potentials as well as showing complex bursting patterns. The aim of this project was to establish whether glucocorticoid negative feedback changes the electrical properties of murine corticotroph cells.

Corticotrophs were cultured from male mice (aged 2-5 months) constitutively expressing GFP under control of the POMC promoter (POMC-GFP). Electrophysiological recordings were obtained using the perforated patch clamp technique in the current clamp configuration. Under basal conditions, cells had a resting membrane potential of -53.7 ± 1.5 mV ($n = 7$, Data are Means $\pm$ SEM) and showed low frequency spontaneous action potentials (0.34 ± 0.14 Hz). CRH and AVP (0.2 nM and 2 nM respectively) caused a significant ($p < 0.01$, ANOVA) depolarisation of the resting membrane potential to -47.4 ± 0.74 mV. There was also a significant ($p < 0.01$) increase in firing frequency from 0.34 ± 0.14 Hz to 0.99 ± 0.27 Hz. The increase in firing frequency was associated with a transition from a predominantly single-spike firing pattern to a bursting-like behaviour. Cells pre-treated for 1.5 hours with corticosterone (100 nM) were significantly ($p < 0.01$) hyperpolarised compared with controls (-62.9 ± 2.2 mV) under basal conditions ($n = 8$). Although CRH and AVP could depolarise resting membrane potential this was still significantly ($p < 0.05$) hyperpolarised (-55.7 ± 2.6 mV) compared with controls treated with CRH and AVP. Basal firing rate was lower in cells treated for 1.5 hours (0.12 ± 0.1 Hz) and although CRH/AVP was still able to increase firing frequency (0.49 ± 0.13 Hz), it was significantly ($p < 0.05$) reduced compared with control cells exposed to CRH and AVP. Furthermore, in corticosterone pre-treated cells, CRH and AVP failed to induce a significant transition from single spikes to bursting behaviour.

These results demonstrate that physiological concentrations of CRH/AVP produce a robust membrane depolarisation and increase in firing frequency of murine corticotroph cells. Treatment with corticosterone causes an overall suppression of both spontaneous and CRH/AVP-evoked firing frequency. Thus glucocorticoid negative feedback involves modulation of the electrical excitability of native murine corticotrophs. The mechanisms and molecular targets for corticosterone action remain to be defined.

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PC246

Photoperiodic regulation of retinoic acid signalling in the adult hypothalamus

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Retinoic acid (RA) is a powerful morphogen which is critically important to many diverse processes including embryonic development of the central nervous system. RA signalling has also been found in the adult brain, most notably in the regulation of neurogenesis in the dentate gyrus region of the hippocampus (McCaffery et al. 2006).

The hypothalamus is a region of the brain important in the maintenance of homeostasis. Feeding behaviours, the sleep/wake cycle, and reproduction are all co-ordinated by nuclei associated with this area of the adult brain (Morris et al. 2012). The hypothalamus is now known to express several of the proteins necessary for RA signalling. In particular, hypothalamic tanycytes lining the third ventricle express the RA synthetic enzyme RALDH2 and the retinoic acid receptors RAR α , RAR β , and RAR γ . The expression of these proteins in the hypothalamus is regulated by seasonal changes in day length, or photoperiod (Shearer et al. 2010).

A diverse array of organisms ranging from single cell bacteria, to plants, and more advanced species including mammals have all developed an endogenous timing system that links behaviour to external light cues. By this means, physiological processes are optimally synchronised with both the solar day and changes in photoperiod throughout the year. The Suprachiasmatic Nucleus (SCN), located in the anterior hypothalamus is the central pacemaker of the circadian clock which is entrained by external light cues to day length. The aim of the present study was to elucidate whether components of the RA signalling pathway are present in the SCN, and whether levels of expression of these proteins are regulated by photoperiod. Adult male F344 photoperiodic rats were exposed to long day (14:10 Light:Dark) or short day (10:14 Light:Dark) light conditions for a duration of 3 months. The SCN was then subject to analysis for the RA synthetic enzymes (RALDHs) and retinoic acid receptors (RARs) by both immunohistochemistry, and quantitative PCR.

The present study describes the expression of the RALDHs and RARs by the SCN in the adult rat hypothalamus. The SCN expresses RALDH2, but not RALDH1 or RALDH3, and RAR α , RAR β , and RAR γ suggesting that it has the ability to both synthesise, and respond to RA signalling. Given the importance of the SCN in regulating physiological responses to seasonal and daily changes in light conditions, RA signaling may be important in the hypothalamus to the generation of the circadian rhythm in the adult rat

McCaffery P., Zhang J., Crandall J. E. (2006) Retinoic acid signaling and function in the adult hippocampus. *Journal of Neurobiology*. 66: 780–791.

Morris C.J., Aeschbach D., Scheer F.A.J.L. (2012) Circadian system, sleep and endocrinology. *Molecular and Cellular Endocrinology*. 349: 91–104.

Shearer K.D., Goodman T.H., Ross A.W., Reilly L., Morgan P.J., McCaffery P.J. (2010) Photoperiodic regulation of retinoic acid signalling in the hypothalamus. *Journal of Neurochemistry*. 112: 246–257

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PC247

Negative regulation of angiotensinogen by angiotensin receptors in astrocytes *in vivo*E.L. O'Callaghan^{1,3}, A.M. Allen³ and W.G. Thomas²¹Physiology and Pharmacology, University of Bristol, Bristol, Avon, UK, ²Physiology and Pharmacology, University of Queensland, St Lucia, QLD, Australia and ³Physiology, University of Melbourne, Melbourne, VIC, Australia

The renin-angiotensin system potently regulates blood pressure and fluid homeostasis. Whilst originally described as a circulating, humoral system, several tissue RAS exist, including those of the heart and brain. Physiological studies have shown that the predominant actions of angiotensin II (Ang II) in the brain involve cardiovascular regulation, consistent with the circulating RAS. Some studies suggest that the brain RAS functions and is regulated independently of the circulating system. The only known precursor of angiotensin peptides is angiotensinogen (AGT) and, in the brain, it is produced predominantly by astrocytes. Local upregulation of AGT in astrocytes of the brain has been observed in response to steroid hormones and osmotic stimulation. New evidence in cultured astrocytes suggests that activation of angiotensin type 1A receptors (AT_{1A}Rs) in astrocytes does not increase AGT gene expression. In this study, the hypothesis that AT_{1A}Rs activation negatively regulates AGT was tested *in vivo*. Recombinant adenoviral vectors were used to express the wild type or a constitutively active mutant (N111G) version of the AT_{1A}Rs in rat astrocytes. Primary astrocyte cultures from neonatal C57Bl6 mice were used to first validate the use of this virus *in vitro*. When cultured astrocytes were transduced with [N111G]AT_{1A}Rs, they exhibited a decrease in AGT gene expression after 72 hours (44.9% ± 13.4%, P=0.02, n=6). AGT gene expression was further reduced (24.5% ± 8.7%, P<0.01, n=6) 24 hours after administration of AngII (100nM). To determine whether AGT was similarly regulated by AT_{1A}Rs *in vivo*, male rats (250-350g, n=3) were anaesthetised with ketamine (60mg kg⁻¹) and medetomidine (250µg kg⁻¹, both i.m) and 1.3 × 10⁷ viral particles of either the recombinant adenovirus encoding [N111G]AT_{1A}Rs or the wild type receptor control, were microinjected into the nucleus of the solitary tract. The rats were then allowed to recover for 5-6 days before histological analysis. A marked reduction in AGT immunoreactivity and gene expression was observed in astrocytes that expressed the constitutively active AT_{1A}Rs, but not in astrocytes expressing the wild type receptor. This demonstrated for the first time *in vivo* that AT_{1A}Rs signalling pathways can negatively regulate AGT production in astrocytes. An important consideration of these data is that by over-expressing [N111G]AT_{1A}Rs one has, in effect, transduced astrocytes with a G-protein coupled receptor that couples to the InsP₃/Ca²⁺ signalling pathway. These results could, therefore, be extrapolated to any receptor present on astrocytes that couples to the same intracellular signalling pathways. Thus, whilst regulation of AGT may not occur via Ang II in astrocytes, a pathway that negatively regulates AGT expression both *in vitro* and *in vivo* has been identified.

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Inactivation of androgen receptor impairs myelin repair in mouse brainR. Hussain^{1,2}, A. Ghomari¹, M. Schumacher¹ and S. Ghandour³¹UMR 788, INSERM and Université Paris-Sud, Le Kremlin Bicetre, France, ²Department of Animal Sciences, Quaid-i-Azam University, Islamabad, Pakistan and ³UMR 7237, CNRS and Université de Strasbourg, Strasbourg, France

Prevalence of multiple sclerosis (MS) is two times higher in females than in males and significant low levels of serum testosterone in male patients are indicative of a potential role of testosterone in MS. We designed experiments to investigate the role of testosterone in rodent models of demyelination. Chronic demyelination (*in vivo*) in mice was achieved by feeding them on cuprizone added powdered food (0.2%) for 12 weeks while acute demyelination was induced in brain slices (*in vitro*) by addition of lysolecithin in mice and rats cerebellar slice cultures. After cuprizone mediated demyelination, we treated one group of mice with testosterone to induce remyelination. Testosterone administration was through placement of testosterone containing subcutaneous implants (size 10mm) dorsally in the shoulder region, for 6 weeks. The constant release of steroid was insured by analyzing brain and serum in a pre-trial. We observed that testosterone promotes robust remyelination in the above mentioned two models. Remyelinating effects of testosterone were assessed by direct counting of oligodendrocytes in corpus callosum as well as estimation of myelin basic protein (MBP) and proteolipid protein (PLP) levels. Our *in vitro* studies of lysolecithin mediated demyelination show that remyelination is dependent on androgen receptor (AR) as cerebellar slice cultures failed to recover in the presence of flutamide (an antagonist of AR). The AR-dependent remyelinating effects were also evident in transgenic mice that express an inactive form of the AR, because they showed an impairment of remyelination. We also provide evidence that testosterone promotes proliferation and differentiation of oligodendrocyte progenitor cells (OPC) into mature myelin forming cells.

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The hypothalamic Paraventricular nucleus: The hungry rat, the thirsty rat and the hypertensive ratC.C. Hindmarch¹, S. Yao¹, M. Fry², A. Ferguson², J.F.R. Paton¹ and D. Murphy¹¹University of Bristol, Bristol, Bristol, UK and ²Department of Physiology, Queens University, Kingston, ON, Canada

We have recently published microarray data from the subfornical organ (SFO; [3]) and the area postrema (AP; [1]) that represent catalogues of gene expression from two circumventricular organs (CVO). In these papers, we have identified the populations of ion channels and G protein-coupled receptors (GPCR) that are expressed in the AP and SFO of the male Sprague Dawley rat under basal conditions and established the transcriptional trends that accompany homeostatic challenge in these tissues. Additionally, we have in the AP, compared the spontaneously hypertensive rat (SHR) to the Wis-

tar Kyoto (WKY) rat control. Here, we shift attention from those neural structures that detect physiological challenge onto a structure that is responsive to such challenge; the paraventricular nucleus (PVN), an integrative hypothalamic brain structure that exhibits a wide functional range and a diverse pattern of innervation and termination; a signal integrator in the brain [2]. Sprague Dawley rats (n=25) were either dehydrated for 72-hours (SD-DH) or fasted for 48-hours (SD-F) or left as controls (SD-CT). Spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rat PVN were also taken. Animals were stunned prior to decapitation and the PVN was removed in an RNase free manner using a dissecting microscope and stored in RNAlater (Ambion) for <1 month before hybridisation onto Affymetrix 230 2.0 microarrays. Initially, catalogues of genes that are flagged as Present in all microarrays from the PVN for SHR [14,835], WKY [15,428], SD-CT [16,144], SD-DH [15,678] and SD-F [16,136] were established. These represent, with a high degree of confidence mRNA populations expressed in the PVN. When combined, this experiment resulted in 18,355 genes that are under investigation in this experiment which are expressed in at least one of these comparisons. Comparison (ANOVA, $p < 0.05$, Benjamini & Hotchberg, Tukey HSD) between these groups reveals 100-genes that are differentially expressed following dehydration and 3007-genes differentially expressed following fasting; this pattern of over-responsiveness to fasting compared to dehydration was noticed in both the SFO and AP data. In the hypertensive brain, 363-genes were differentially regulated. Comparison between the AP, SFO and PVN reveals very little overlap in terms of commonly regulated genes for the SD-DH regulated gene lists, and no overlap between the two CVO's in the SD-F lists but nearly half of all SFO regulated genes in the SD-F list are also regulated in the PVN following fasting. In the hypertensive brain (AP and PVN) nearly half of those genes in either tissue are commonly regulated. We present a comprehensive catalogue of data from the rat PVN and attempt to unravel physiological significance from these large databases using both conventional molecular validation techniques novel mathematical protocols.

Charles Hindmarch, Mark Fry, Pauline Smith, Song Yao, Georgina Hazell, Stephen Lolait, Julian Paton, Alastair Ferguson & David Murphy (2011) The transcriptome of the medullary area postrema: The hungry rat, the thirsty rat and the hypertensive rat. *Experimental Physiology* – 96(5): 495-504

Charles Hindmarch, David Murphy (2010) The Transcriptome and the Hypothalamo-Neurohypophyseal System, Loche S, Cappa M, Ghizzoni L, Maghnie M, Savage MO (eds): *Pediatric Neuroendocrinology*. Endocr Dev. Basel, Karger, 2010, vol 17, pp 1–10

Charles Hindmarch, Mark Fry, Song Yao, Pauline M. Smith, David Murphy and Alastair Ferguson (2008) Microarray Analysis of the Transcriptome of the Subfornical Organ of the Rat: Regulation by Fluid and Food Deprivation – *American Journal of Physiology* – 295(6):R1914-20

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PC250

Evaluation of salt appetite in brain specific 11 β hydroxysteroid dehydrogenase type 2 knockout mice (11 β HSD2-/-)

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Male brain specific 11 β HSD2-/- mice and floxed controls were individually housed in metabolic cages and basal water

turnover assessed over 4 days. In separate studies mice were given access to 2 bottles containing water and 1.5% NaCl and salt preference measured over 7 days. The role mineralocorticoid receptor (MR) in salt appetite was evaluated using spironolactone s.c., 60mg per mouse. Urinary sodium was measured by flame photometry. Blood was collected from a cannula in the carotid artery under terminal anaesthesia (Inactin hydrate). Plasma sodium was measured by electrolyte analyzer.

Basal water and sodium turnover and plasma sodium concentrations were not altered by the deletion of 11 β HSD2 in the brain. When given access to NaCl brain specific 11 β HSD2-/- mice displayed a significant salt preference, 70% of total fluid intake was accounted for by NaCl. Salt appetite was significantly reduced by MR blockade. Sodium was not retained in the null mice; increased salt intake resulted in significant natriuresis and polyuria.

Deletion of 11 β HSD2 in the brain, which is specifically expressed in the nucleus of the solitary tract (NTS) in mice (1), resulted in an increased salt appetite. Activation of MR is implicated since spironolactone significantly reduced NaCl intake. These data are the first demonstration of salt appetite occurring in the absence of sodium depletion and with normal renal function. They suggest that activation of MR on 11 β HSD2 positive neurons in the NTS increases salt appetite.

HOLMES, M. C., SANGRA, M., FRENCH, K. L., WHITTLE, I. R., PATERSON, J., MULLINS, J. J. & SECKL, J. R. (2006) 11 β -Hydroxysteroid dehydrogenase type 2 protects the neonatal cerebellum from deleterious effects of glucocorticoids. *Neuroscience*, 137, 865-73.

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PC251

Source reconstruction of MEG human brain responses to retinotopic visual stimuli

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Retinotopic maps are an established feature of early visual areas and are useful for evaluating the source localization of neuroimaging techniques. Magnetoencephalography (MEG) is a physiological technique that allows recording of human brain activity at high temporal resolution. However, the accurate localization of brain sources of the MEG signal remains a challenge, as the signal alone cannot constrain a unique solution without additional assumptions. We evaluated three MEG source reconstruction methods by comparing retinotopic activity obtained with MEG with retinotopic maps obtained with functional magnetic resonance imaging (fMRI).

Six participants placed in a MEG scanner (Elekta Neuromag) viewed black/white checkerboard stimuli, located in visual field quadrants and annular rings to map angular and eccentric locations respectively. Brain sources were reconstructed with three contrasting approaches: i) an assumption of multiple sparse priors (MSP); ii) a beamformer (spatial filter); and iii) a minimum norm estimate (MNE) that assumes independently distributed sources of minimal amplitude. We calculated the per-

centage of MEG sources localized to the subregions of early visual areas V1, V2 and V3, defined retinotopically with fMRI in the same individuals.

For quadrants, all reconstruction methods localized sources better than chance level ($p < 0.05$, t-test), but the MNE approach was significantly more accurate than the beamformer or MSP ($F = 7.3$, $p < 0.01$, ANOVA). For complete rings, no reconstruction method localized activity better than chance. However, when ring stimuli were shown in quarters, eccentricity of lower field stimuli was accurately represented by MNE and beamformer but not MSP.

Our results suggest that MNE and beamformer approaches are the most suitable for localizing early visual activity, but that there are limitations to using MEG to localize stimuli that transect quadrant boundaries. This may be due to the folding and orientation of the cortical surface of early visual areas.

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Estimating human contrast-dependent visual delay: a new approach using saccadic competition

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The study of reaction times has become a fundamental tool for studying neural decision-making, and quasi-Bayesian models such as LATER¹ can give a good account of latencies in response to high-contrast targets. But when contrast is nearer threshold, the time taken for local detection of the target must also be taken into account; a complete model of visual reaction time requires two stages: a detection stage embodying a random walk, and a decision stage that follows LATER in having a linearly-rising decision signal².

To test this idea, we need more information about the time taken in the detection stage. Previous work, looking at simple reaction time as a function of target contrast, found that the average time taken for detection at contrast C was of the form $K/\log(1 + C/C_0)$, where K and C_0 are respectively latency-scaling and contrast-scaling factors^{3,4}. However, this method is imprecise and slow to generate data.

Here, we show that an alternative approach can provide quantitative data of this kind more quickly and more precisely. Subjects completed a precedence task⁵, in which stimuli appear to both the left and right, and the subject chooses which to look at, whilst their eye movements were recorded and saccades registered, using standard techniques³. The two targets appear asynchronously, with the first-appearing being lower contrast than the second (which was 75% contrast), making it harder - and hence slower - to detect. Fig. 1a shows how increasing the interstimulus delay increases the probability of one subject looking at the first target to appear but reducing the contrast does the opposite. Our graph allows us to 'titrate' this balance: there is a '50% point', at which the subject is equally likely to look at either stimulus, which can be estimated by linear interpolation. Comparing these at different contrasts allows us to quantify the extra decision time taken by the detection stage as a result of the reduced contrast. Using a precedence task exaggerates these differences because there are two LATER units which come close to threshold: they therefore inhibit one another. For each subject we can estimate K and C_0 from such data, and then see how well the latency-predicting formula models the observed data. The average value

of C_0 across all subjects ($4.94 \pm 0.024\%$) was similar to what had previously been reported^{3,4}; the average K value ($77.6 \pm 8.1\text{ms}$) is substantially higher, reflecting the enhancing influence of lateral inhibition in this competitive task. The technique appears to generate reasonably consistent results: Fig. 1b shows a comparison of observed and predicted reaction times across all subjects (Pearson $R = 0.962$, $p < 0.0001$); but they suggest that the original formula may need some reconsideration. We hope to use this method to examine the discrepancy in more detail.

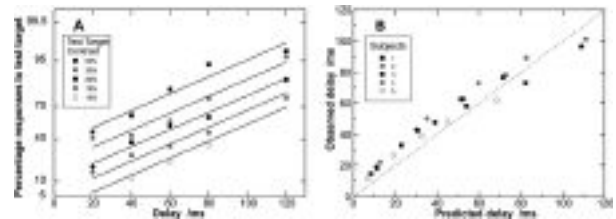


Figure 1. A: Relation between proportion of responses made in the direction of the first, lower-contrast, target as a function of the delay of the second target, for one subject at different contrast levels; the lines are best linear fits (maximum likelihood), constrained to be parallel. B: Observed delays, for all subjects, as estimated from data as shown in A, plotted as a function of the delay predicted for the corresponding contrast, calculated from the existing formula (see text) using best-fit values of the parameters K and C_0 for each subject. The dashed line shows the expectation for perfect prediction.

1. Carpenter, R.H.S. & Williams, M.L.L. (1995) *Nature* **377**, 59-62.
2. Reddi, B.A.J. (2001) *Current Biology* **11**, 603-606.
3. Carpenter, R.H.S., Reddi, B.A.J. & Anderson, A.J. (2009) *Journal of Physiology* **587**, 4051-4062.
4. Taylor, M.J., Carpenter, R.H.S. & Anderson, A.J. (2006) *Journal of Physiology* **573**, 741-751.
5. Leach, J.C.D. & Carpenter, R.H.S. (2001) *Vision Research* **41**, 3437-3445.

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PC253

'Self-Occlusion' during a manual control task

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Introduction:

There is evidence to suggest that sensory information is gated during, and immediately following movements. We wished to test this hypothesis using a novel paradigm in a manual control task.

Methods:

With ethical permission and their informed consent, subjects ($n = 20$) sat in a self selected position holding a small uniaxial joystick and wore a pair of PLATO visual occlusion spectacles. A computer screen in front of them displayed both a red circle, which represented the angle of a virtual inverted pendulum, and a white cross which marked the centre of the screen. Subjects were required to maintain the position of the circle as close to the centre of the screen as possible via gentle taps of the joystick (Loram et al., 2010). As subjects performed the task, their vision was occluded for a period of 200 ms following each contact with the joystick. In different trials, the delay between joystick contact and the 200 ms visual occlusion was varied among the following values: 1 ms, 50 ms, 100 ms, 150

ms, 200 ms, 250 ms and 2000 ms. There was also a control condition where no occlusions were presented throughout the whole trial.

Results:

In the control condition, and in the 2000 ms condition, the modal tapping intervals were 398 and 384 ms respectively. In the 1, 50, 100 and 150 ms conditions the modal tapping intervals consistently occurred at a relatively fixed interval following the end of the occlusion (263 ± 10 ms, mean $\pm$ SD). In the 200 and 250 ms conditions, there were two common tapping intervals. The modal tapping intervals were 381 and 376 ms respectively, and there was a secondary peak at 258 and 257 ms following the end of the occlusion. Performance in the task was least impaired compared to the control condition when the occlusion was presented after only a 1 ms delay.

Discussion:

These observations suggest that, in this particular task, subjects were able to compute, select and execute a corrective response (a tap of the joystick) within ≈ 260 ms of the presentation of visual information. However, when subjects were not occluded, or when the delay between the tap and the occlusion was long (2000 ms), subjects elected to make corrective responses at longer intervals (approximately 390 ms). We suggest this discrepancy may be explained if each tap is immediately followed by a brief period of 'self-occlusion' where subjects make little use of sensory information. This may also explain why performance was least impaired when the visual occlusion was presented immediately following each tap because in this case, the visual occlusion is partly concurrent with the self-occlusion.

Loram ID, Gollee H, Lakie M & Gawthrop P (2010). Human control of an inverted pendulum: Is continuous control necessary? Is intermittent control effective? Is intermittent control physiological? *The Journal of physiology* 2, 307–324.

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PC254

Electrophysiological mapping of cerebellar - striatal pathways

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The cerebellum and the basal ganglia are major subcortical structures crucial for motor control, which are generally thought to have little or no direct interactions with one another. However, recent tracing studies provide an anatomical route by which the two structures could communicate [1–3]. The aim of the present study was to use electrophysiological techniques to investigate whether a functional link also exists between the cerebellum and the basal ganglia. Local field potentials (LFPs) and single units were recorded in the striatum following electrical stimulation of the cerebellar dentate nucleus (DN). Experiments were carried out in accordance with UK Animals (Scientific Procedures) Act 1986. Adult male Wistar rats ($n=6$) were anaesthetised with urethane (1.4mg/kg, i.p.) and maintained with supplementary doses of a ketamine and xylazine mix (25mg/kg and 2.5mg/kg respectively, i.p.). Craniotomies were performed to allow the placement of a bipolar stimulating electrode in DN and a recording electrode in the contralateral striatum. Stimulation of DN (single pulses,

0.2ms duration, current range: 18–600 μ A) evoked a localised field potential in the striatum with a mean onset latency of $4.4\text{ms} \pm 0.24$ s.e.m ($n=4$). LFP peak-to-peak amplitude was measured across the dorsal-ventral extent of the striatum at three medial-lateral co-ordinates relative to midline. LFP amplitude was significantly larger in the dorsolateral striatum (dorsolateral vs dorsomedial striatum $P<0.001$, dorsolateral vs dorsocentral striatum $P<0.001$; Kruskal-Wallis with Dunn's post-test, $n=4$). In separate experiments DN stimulation evoked spike activity in individual striatal neurons with a high probability of occurrence and at a mean latency of $6.6\text{ms} \pm 0.49$ s.e.m ($n=3$ cells). Overall, these data reveal that a powerful, short latency pathway connects the cerebellar DN with the dorsolateral striatum in the rat, providing a substrate for rapid interactions between these two major motor control structures. Further investigations are underway to investigate the behavioural significance of this projection in awake adult Wistar rats during performance of a goal-directed operant task.

1. Ichinohe, N., F. Mori, and K. Shoumura, A di-synaptic projection from the lateral cerebellar nucleus to the laterodorsal part of the striatum via the central lateral nucleus of the thalamus in the rat. *Brain Research*, 2000. 880(1-2): p. 191-197.

2. Ichinohe, N., H. Iwatsuki, and K. Shoumura, Intrastriatal targets of projection fibers from the central lateral nucleus of the rat thalamus. *Neuroscience Letters*, 2001. 302(2-3): p. 105-108.

3. Hoshi, E., et al., The cerebellum communicates with the basal ganglia. *Nature Neuroscience*, 2005. 8(11): p. 1491-1493.

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PC255

Cerebellum: a mediator of fear behaviour

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Cerebellum: a mediator of fear behaviour

Stella Koutsikou, Viren Ahluwalia, Emma Earl, Bridget M. Lumb & Richard Apps

The ventrolateral sector of the midbrain periaqueductal grey (vlPAG) has a well-established role in conditioned fear (Carrive et al. 1997); characterised by clearly-defined autonomic and sensory changes that are accompanied by freezing behaviour (Carrive 2000). To date, attention has focused on neural pathways underlying autonomic and sensory control from the vlPAG and almost nothing is known about the pathways and mechanisms that link vlPAG to motor control centres. We have previously shown that a neural pathway exists that links the vlPAG with the cerebellar vermal lobule VIII, suggesting that this link may be involved in coordinating behavioural responses associated with vlPAG function (Leith et al. 2009). Here we test the hypothesis that the cerebellum is a key supraspinal structure that links the vlPAG to spinal motor circuits mediating fear-conditioned freezing behaviour.

Experiments were carried out in adult male Wistar rats ($n=9$) and in accordance with the UK Animals (Scientific Procedures) Act 1986. All animals were subjected to a fear conditioning paradigm at least 24hrs prior to either lesion of cerebellar lobule VIII ($n=6$) using the neurotoxic tracer CTb-Saporin, or sham microinjections (PBS; $n=3$). All surgical operations were performed under a mixture of ketamine (0.6ml; Vetalar, Pfizer UK) and medetomidine hydrochloride (0.25ml; Domitor, Orion Pharma Finland) mixed in 1ml of 0.9% saline (final anaesthetic dose: 1.8mg.kg⁻¹; i.p.).

Lesions of vermal lobule VIII input-output pathways, using CTb-Saporin, significantly disrupted fear-conditioned freezing behaviour (the time animals displayed freezing was reduced by 49.29% compared to sham animals, Mann-Whitney, $P < 0.05$). Foot gait analysis and vertical grid test (Seoane et al. 2005) were used to assess changes in locomotor activity and muscle tone, respectively. No significant changes in these motor functions were observed in both groups of animals (Wilcoxon matched pairs, $P > 0.05$). However, in the open field test animals treated with CTb-Saporin spent more time in the centre of the open field arena (33.6%) than the Sham animals (17.9%). This is consistent with a reduction in anxiety-like behaviour. Finally, in naïve animals, chemical excitation of vIPAG (with dl-homocysteic acid; 0.05M) exerts a facilitatory effect on the H-reflex, which might reflect the contribution of vIPAG to the tense postures and freezing immobility associated with fear (Koutsikou et al. 2009, 2011). This facilitatory effect is abolished, in alpha-alone-anaesthetised (i.v. Alfaxan, Jurox UK; 25mg.kg⁻¹.hr⁻¹) rats previously injected with CTb-Saporin in cerebellar vermal lobule VIII ($n=4$, pre-vIPAG vs vIPAG activation; Kruskal-Wallis, $P > 0.05$).

Overall, these data provide evidence for a pivotal role for vermal aspects of the olivocerebellar system in linking vIPAG to spinal motor circuits for expression of the affective component of fear behaviour.

Carrive P (2000). *Brain Res* **858**, 440-445.

Carrive et al. (1997). *Neurosci Lett* **223**, 33-36.

Koutsikou et al. (2009). *Proc Physiol Soc* **17**, C14.

Leith et al. (2009). *Proc Physiol Soc* **17**, PC20.

Seoane et al. (2005). *Eur J Neurosci* **22**, 880-894.

BBSRC and The Physiological Society

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PC256

Sensory feedback during interpersonal light fingertip contact stabilises human balance

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Lightly touching a fixed point of reference has been shown to reduce body sway compared to normal standing (Jeka and Lackner, 1994). When touching another person the point of reference is not fixed, but constantly moving due to body sway. Despite this, recent studies have shown interpersonal light fingertip contact to be effective in stabilising balance (Johannsen et al., 2009; 2012). However, the underlying mechanisms remain unclear. Here sixteen subjects (3 all-male, 5 all-female pairs) stood facing a partner who was positioned in front and slightly to their right, such that their right shoulders were approximately aligned. Both stood in narrow bipedal stance on a separate force plate. We measured their body sway in a number of interpersonal contact (shoulder, light fingertip, no contact) and visual (both eyes closed, one subject eyes closed) conditions. Sway speed was significantly affected by the form of interpersonal contact between the pair ($F_{2,28} = 20.9$, $p < 0.001$; repeated measures ANOVA). For instance, extending their right arms and grasping their partner's shoulder led to a $15 \pm 12\%$ ($n=16$, mean $\pm$ SD in all cases) reduction in sway, when both subjects had eyes closed. Furthermore, reaching out with

their right forearms and lightly touching fingertips reduced sway by $9 \pm 7\%$ ($n=16$). But how is this stabilisation achieved? Cross-correlations were used to analyse the relationship between subjects' anteroposterior body motions at delays of up to ± 1 s. When both subjects had eyes closed (see Figure, black lines) the results were as follows. During shoulder contact the pairs motion was highly correlated at approximately zero lag (peak correlation at -70 ± 115 ms, $n=8$), demonstrating they were strongly linked. This suggests that the reduction in sway in this condition involved mechanical stabilisation. In contrast, during light fingertip contact correlation magnitude was low at zero lag. It was, however, increased either side of zero, with two distinct peaks occurring at -478 ± 202 ms and 366 ± 117 ms ($n=8$). This demonstrates that each subjects' sway was correlated with their partner after a short delay, in a reciprocal manner. This suggests that stabilisation in this condition involves sensory feedback mechanisms. When one subject was permitted to open their eyes (see Figure, grey lines), thus causing asymmetrical visual conditions, the effects of light fingertip contact were also asymmetrical. That is, the relationship of the eyes-closed subject's sway leading their partner was enhanced. This was also the case in the no-contact condition, suggesting interpersonal visual inputs modulate body sway. Nonetheless, with light fingertip contact the average peak correlation continued to occur at a delay of around ± 300 -500ms. We therefore conclude that mechanisms of sensory feedback underlie the reduction in body sway during interpersonal light fingertip contact.

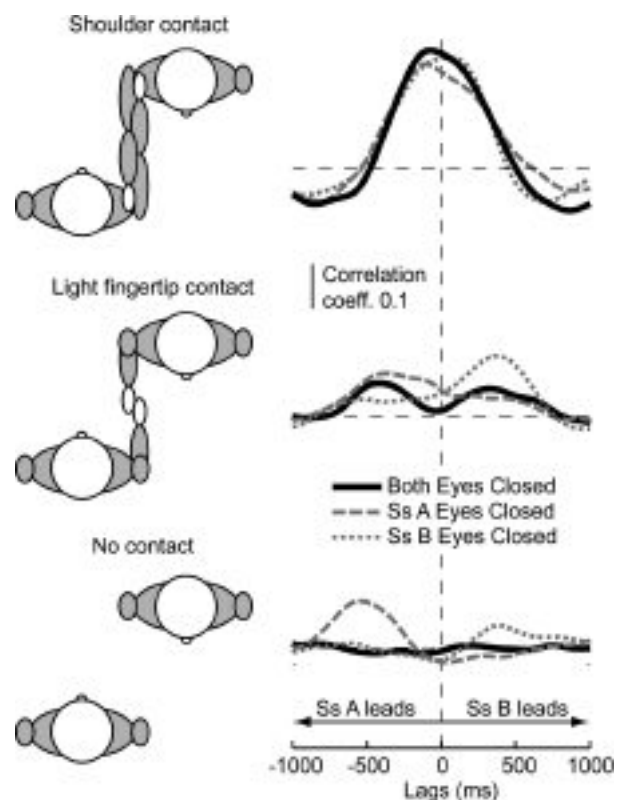


Figure - Group average cross-correlations ($n=8$) show the relationship between anteroposterior centre of pressure velocities of each individual.

Jeka JJ & Lackner JR (1994). *Exp Brain Res* **100**, 495-502.

Johannsen L et al. (2009). *J Mot Behav* **41**, 397-399.

Johannsen L et al. (2012). *J Neurophysiol* **107**, 216-225.

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PC257

Interpreting exafferent vestibular information during active head movement

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To maintain balance and orientation the brain must discriminate head motion caused by voluntary movement from that due to external disturbances. Animal data shows that this discrimination process occurs at the earliest stage of vestibular processing where brainstem neurons are uniquely responsive to exafferent information, cancelling out reafference(1). However, indirect evidence from humans suggests that active head movement interferes with the ability to maintain balance(2), raising the possibility that the separation of vestibular exafference from reafference is an imperfect process. To address this we measured the ability of human subjects to transduce exafferent vestibular information into motor responses while performing active head movement.

Ten volunteers (6 male, 19-24yrs) were exposed to Galvanic Vestibular Stimulation (GVS; 1.5mA) for 60s while marching on the spot at 80bpm. Subjects attempted to maintain orientation within the laboratory while visual and acoustic cues were abolished by a blindfold and white-noise headphones. GVS is known to evoke turning responses in such situations, with the rate of turn depending upon the pitch orientation of the head. We therefore asked subjects to adopt various head orientations between ± 45 degrees pitch. In a static condition, head orientation was maintained throughout the trial. Then we examined the effect of voluntary movement upon the GVS response by asking people to produce head movements in time with a sinusoidally-modulated acoustic tone. To determine if the rate of head movement had any influence, three frequencies of modulation were employed: 0.025, 0.05 and 0.1Hz. Head pitch and whole-body turning velocity were measured using motion tracking sensors placed on the head and trunk, respectively(Polhemus Fastrak).

During the static condition GVS evoked turning responses in all subjects. In accordance with previous research(3), the rate of turn was maximal with the head pitched down, minimal with head level, and reversed direction with the head up (Fig 1; $F_{4,36}=10.51$; $p<0.001$). During active head movement turning also occurred. The magnitude and direction of turn velocity was continuously modulated throughout the duration of each trial. However, response gain was systematically reduced with increasing frequency of head movement (Fig 2; $F_{2,18}=10.46$; $p=0.001$).

These results show that humans can transform exafferent vestibular input into motor output even during active head movement. This suggests that the brain successfully discriminates between exafferent and reafferent vestibular input. The reduced response gain with increasing movement frequency may be due to sluggish motor responses. However, it could be due to a breakdown in the discrimination process as reafferent signals become stronger. Future experiments will address this directly by comparing GVS responses during active and passive head pitch movement.

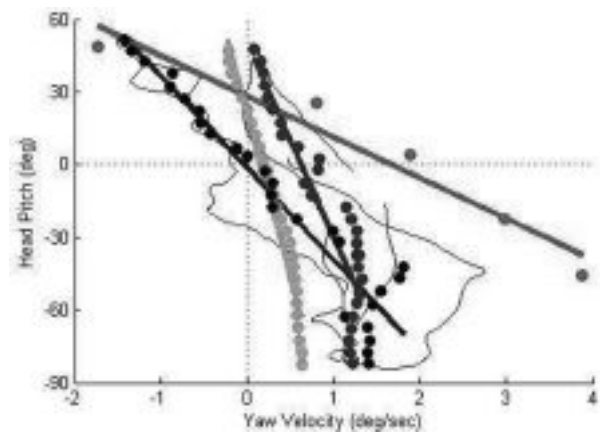


Figure 1. Representative data depicting the relationship between head pitch and turn velocity. Trials where head pitch was static (red) are compared to those where active pitch movements were made (black=0.025; blue=0.05Hz; turquoise=0.1Hz). Positive values of Yaw Velocity represent movement in the anodal direction. Continuous lines show the ongoing time-series trace for movement trials. Circles show time-binned data for these time-series. Linear regression lines were fitted to the data to estimate response gain. It can be seen that the magnitude of turn velocity reduces as movement frequency increases. (Note: a shallower slope signifies greater gain).

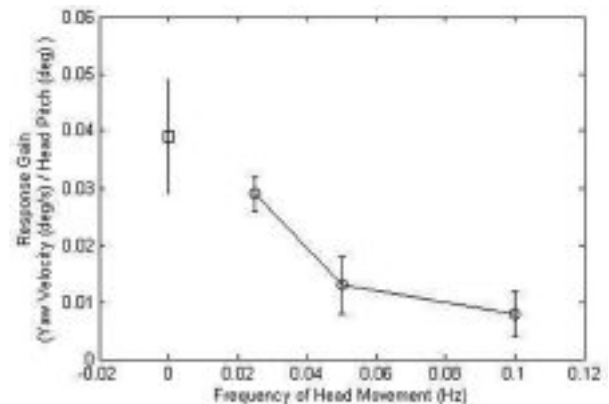


Figure 2. Mean response gain is shown for all frequencies of head movement ($\pm$ SEM). Values were derived from the slope of the regression lines shown in figure 1.

Roy JE, Cullen KE. Dissociating self-generated from passively applied head motion: neural mechanisms in the vestibular nuclei. *J Neurosci.* 2004 Mar 3;24(9):2102-11.

Pozzo T, Berthoz A, Lefort L. Head stabilization during various locomotor tasks in humans. I. Normal subjects. *Exp Brain Res.* 1990;82(1):97-106.

Fitzpatrick RC, Butler JE, Day BL. Resolving head rotation for human bipedalism. *Curr Biol.* 2006 Aug 8;16(15):1509-14.

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PC258

Anodal transcranial direct current stimulation modifies development of a spatial motor skill in healthy adult humans

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Anodal transcranial direct current stimulation (tDCS) is a safe, non-invasive means of increasing the excitability of brain regions, with potential applications in neurorehabilitation of movement (1). Spatial accuracy, though essential in prehension (2) has not been fully integrated in previous skill measures. This study explores the effect of anodal tDCS on task learning and retention in a novel paradigm incorporating temporal and spatial dimensions as a univariate skill measure. 24 healthy right-handed adult volunteers were included in an RCT experimental format. All were trained in a novel sequential motor task requiring rapid, accurate peg transfer to raised targets using the non-dominant upper limb, over two 45 minute sessions spaced 48 hours apart. Adjunctive ACTIVE anodal tDCS ($n=12$, 1.5mA, 43 μ A/cm²) or a SHAM condition ($n=12$) was applied to the scalp overlying contralateral primary motor cortex (M1) during the first 20 minutes of practise. All attended for follow-up measurements after a further 7 days. The behavioural Task Productivity Rate (TPR) skill measure is a function of mean time required to achieve a standard spatial goal. For evaluation of focal M1 corticomotor plasticity related to task learning, measures were evoked using Transcranial Magnetic Stimulation (TMS)(3). Responses were gathered as surface EMG from the medial deltoid (mDelt), a shoulder muscle important in reaching and abductor pollicis brevis (APB), a vital muscle in grasp, analysed as stimulus-response characteristics (SRC). Analysis by ANOVA and student's t-test, values quoted mean $\pm$ SEM.

While task completion times were reduced under ACTIVE anodal tDCS stimulation through to a maximum difference 5.0 $\pm$ 3.5% n.s. at the follow-up session compared to SHAM, skilled performance was significantly better in the SHAM group at the start of the second session by 10.6 $\pm$ 5.0% $p<0.05$, effect size $r=0.416$ (Figure 1), which was not present at follow-up. Group-specific TMS response enhancements compared to baseline were found following training and persisted at follow-up in resting APB SRC $p<0.05$ in favour of the SHAM group but active mDelt SRC $p<0.05$ in favour of the ACTIVE group. In summary, ACTIVE application of anodal tDCS to M1 was associated with lasting plasticity in brain areas projecting to the shoulder muscle. Conversely, more rapid skill learning and retention with superior lasting cortical plasticity in the hand muscle representation was found under the SHAM condition.

In a spatial accuracy task anodal tDCS modified skill learning and retention behaviour in a systematic and lasting fashion. The observed effect may be rooted in action prediction (4) with a secondary impact on task learning. The net clinical benefits of anodal tDCS on practical rehabilitation activities in patient groups may therefore emerge over longer timescales than previously considered.

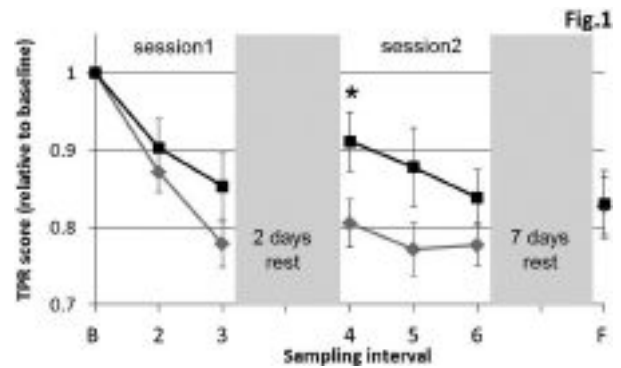


Figure 1: Effect of anodal tDCS intervention group allocation and task practise on spatial motor skill (TPR).

Change in observed motor skill in training, relative to baseline (B) sample mean. Shaded areas: 2 day/48 hour rest between sessions 1 and 2; 7 day/168 hour rest between session 2 and follow-up (F). *Significant difference independent t-test $p\leq 0.05$, the net effect of task experience and retention between ACTIVE (squares) and SHAM (diamond) groups at start of session 2.

◆ SHAM ■ ACTIVE

(1) Reis J, Fritsch B. Modulation of motor performance and motor learning by transcranial direct current stimulation. *Curr Opin Neurol* 2011;24(6):590-6.

(2) Shadmehr R, Smith MA, Krakauer JW. Error correction, sensory prediction, and adaptation in motor control. *Annu Rev Neurosci* 2010;33:89-108.

(3) Reis J, Swayne OB, Vandermeeren Y, Camus M, Dimyan MA, Harris-Love M, et al. Contribution of transcranial magnetic stimulation to the understanding of cortical mechanisms involved in motor control. *J Physiol* 2008;586(2):325-351.

(4) Novick I, Vaadia E. Just do it: action-dependent learning allows sensory prediction. *PLoS One* 2011;6(10):e26020.

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PC259

Modulation of gap junction connectivity can both enhance and disrupt patterned activity within sensory networks of the spinal dorsal horn

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The contribution of gap junctions (GJ's) and connexin (Cx) proteins to pain facilitation may be more prominent than once thought. For example, the non-specific GJ uncoupler carbenoxolone (CBX) reduces mechanical allodynia in inflammatory and neuropathic pain (Spataro et al., 2004). Similarly, in formalin-injected rats CBX prolongs the paw thermal withdrawal latency compared to control (Qin et al., 2006). Despite growing knowledge about GJs in the CNS, there is still relatively little known about GJs in the spinal dorsal horn (DH) although CBX disrupts network-based activity within substantia gelatinosa (SG) in vitro (Chapman et al., 2009). In these studies, pharmacological tools that target GJs and electrophysiological single and multi-electrode array (MEA) recordings were used to further investigate the involvement of GJs in DH network activity. In addition, quantitative PCR (Q-PCR) was used

to determine expression levels of selected Cx proteins in age-matched rat lumbar spinal cord. For single and MEA electrophysiological recordings, transverse spinal slices were obtained from terminally anaesthetized (Urethane, 2g/kg i.p.) Wistar rats aged 10-14 days old. Animal procedures accorded with current UK legislation. Perfusion of 4-Aminopyridine (4-AP, 25 μ M) induced excitation within the DH that was characterized in terms of a) 4-12 Hz rhythmicity, b) population spike frequency and c) spatio-temporal characteristics. The effects of the GJ opener trimethylamine (TMA, 100 μ M), the non-specific GJ uncoupler CBX (100 μ M) and the GAP26 mimetic peptide (100 μ M) which targets Cx43 were assessed. Lumbar spinal cord tissue was removed from terminally anaesthetized (Urethane, 2g/kg i.p.) Wistar rats for Q-PCR analysis of relative expression levels of glial and neuronal Cx subtypes. In single electrode recordings in SG, 4-AP induced population spikes and 4-12 Hz rhythmic activity. During co-application of TMA plus 4-AP, the spike frequency and 4-12 Hz rhythmic activity were significantly increased. Conversely, CBX and GAP26 reduced these quantified parameters. In MEA recordings, 4-AP-induced excitation was observed across the DH and characterized for its spatio-temporal characteristics and rhythmicity in superficial and deep DH laminae. GJ targeting compounds were assessed for their ability to differentially alter characteristics of 4-AP-induced activity across the DH. Q-PCR revealed spinal expression of glial Cx29, Cx32, Cx43 and neuronal Cx36 and Cx45. These data indicate that modulation of GJ connectivity can enhance or diminish excitation across DH, effects which are presumptively mediated via GJs in neurones and/or glia that express Cxs.

Chapman et al., (2009). The Journal of Physiology 587, 2499-2510.

Qin et al., (2006). Neuroscience Research 55, 442-450.

Spataro et al., (2004). The Journal of Pain 5, 392-405.

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PC260

Chemerin attenuation of potentiated C-fibre input to the lamina I pain pathway

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Inflammatory pain treatments frequently lack efficacy and/or exhibit undesirable side effects. Neurokinin 1 receptor expressing (NK1R+) neurons in the lamina I region of the spinal cord are crucial for the manifestation of inflammatory pain [1]. Recent evidence has described an essential role for C-fibre nociceptors in the development of inflammatory pain [2] and it has been established that lamina I NK1R+ neurons predominantly receive monosynaptic C-fibre input [3, 4]. Therefore, identification of drugs which presynaptically modulate C-fibre nociceptive input to these neurons could aid analgesia development. Recent data have shown that chemerin receptor 23 (ChemR23) activation can attenuate inflammatory pain by a mechanism involving inhibition of potentiated spinal cord activity. Interestingly, ChemR23 was found to be expressed by likely C-fibre nociceptors that target lamina I NK1R+ neurons [5]. Therefore, we investigated whether chemerin, the natural

ChemR23 ligand, can modulate potentiated C-fibre input to lamina I NK1R+ neurons. Whole-cell patch clamp recordings were made from presumptive NK1R+ neurons, identified following pre-incubation with tetramethylrhodamine conjugated substance P [3, 4], in rat (~postnatal day 21) spinal cord slices (L4/5), with/without dorsal roots attached. Rats were either untreated (control) or had received an intraplantar injection of complete Freund's adjuvant (CFA: 0.5mg/ml, 1 μ l/g body weight, under brief isoflurane anaesthesia) ~4 days prior. Values are mean $\pm$ SEM, compared with 2-way ANOVA unless stated otherwise. Miniature EPSCs (mEPSCs) were recorded, in control tissue, in the presence of the transient receptor potential subtype vanilloid 1 agonist capsaicin (1 μ M) to pharmacologically presynaptically potentiate likely C-fibre nociceptive input. Capsaicin was applied alone (n=12) or in the presence of chemerin (100ng/ml, n=10). Chemerin significantly reduced capsaicin potentiation of mEPSC frequency (capsaicin vs. capsaicin+chemerin: 20.79 $\pm$ 5.19 vs. 9.67 $\pm$ 2.71Hz, P<0.05, Bonferroni post-test), but was without effect in non-potentiated conditions (baseline vs. chemerin: 1.16 $\pm$ 0.25 vs. 1.46 $\pm$ 0.54Hz, P=0.824, Wilcoxon, n=11). Monosynaptic C-fibre input to lamina I NK1R+ neurons was identified in control and CFA tissue by dorsal root stimulation [3, 4] and C-fibre evoked EPSCs (eEPSCs) were recorded in the absence (no drug control) or presence of chemerin. In control tissue, chemerin exhibited no effect on C-fibre eEPSC peak amplitude. However, in CFA inflammation tissue chemerin significantly attenuated C-fibre eEPSC peak amplitude in a subset of neurons (P<0.01). Thus, we have novel shown functional expression of ChemR23 on presynaptic terminals of likely C-fibre nociceptive synapses with lamina I NK1R+ neurons and suggest that activation of these receptors can attenuate C-fibre nociceptive input to these neurons in inflammatory pain.

Nichols ML, Allen BJ, Rogers SD, Ghilardi JR, Honore P, Luger NM, et al. Transmission of chronic nociception by spinal neurons expressing the substance P receptor. Science. 1999 Nov;286(5444):1558-1561.

Abrahamsen B, Zhao J, Asante CO, Cendan CM, Marsh S, Martinez-Barbera JP, et al. The cell and molecular basis of mechanical, cold, and inflammatory pain. Science. 2008 Aug;321(5889):702-705.

Torsney C, MacDermott AB. Disinhibition opens the gate to pathological pain signaling in superficial neurokinin 1 receptor-expressing neurons in rat spinal cord. The Journal of Neuroscience. 2006 Feb;26(6):1833-1843.

Torsney C. Inflammatory pain unmasks heterosynaptic facilitation in lamina I neurokinin 1 receptor-expressing neurons in rat spinal cord. The Journal of Neuroscience. 2011 Mar;31(13):5158-5168.

Xu ZZ, Zhang L, Liu T, Park JY, Berta T, Yang R, et al. Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. Nature Medicine. 2010 Apr;16(5):592-597.

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PC261

Assessment of myelin thickness and axon circularity in a dorsal root of the mouse

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Previous studies of peripheral nerves and ventral roots have shown that myelin thickness increases non-linearly with axon

size and that axon circularity may be dependent on axon size (e.g. Arbuthnott *et al.* 1980; Biscoe *et al.* 1982; Ceballos *et al.* 1999). In this study, we present preliminary data relating myelin thickness and axon circularity to axon size for the central end of a dorsal root in a mouse cervical segment.

A CD1 strain mouse was anaesthetized with sodium pentobarbitone (50 mg.kg⁻¹ I.P.) and fixed by transcardial perfusion with a Karnovsky fixative. The left C5 dorsal root ganglion, along with the corresponding dorsal and ventral roots, was removed and placed in fresh fixative overnight at 4°C. The tissue was post-fixed with OsO₄ prior to embedding in Araldite. Semithin serial sections (0.5 µm) were cut using an ultramicrotome and stained with 1% toluidine blue in 1% borax. A composite photomicrograph of the central end of the C5 dorsal root was constructed with a resolution of 10 pixels/µm and a magnification of x1000. Areas and perimeters of nerve fibres (axon plus myelin) and of their axons alone were determined using the freeware program Reconstruct (Boston University, Boston, MA, USA; see Fiala, 2005). Fibres were excluded from the analysis if they displayed internal folds, Schwann cell nuclei, Schmidt-Lantermann incisures or paranodal characteristics. From the area measures, axon (D_A) and fibre (D_F) diameters were computed for the equivalent circles. Myelin thickness was calculated as (D_F - D_A)/2. An index of circularity (IC) of the axon was computed by dividing the observed axonal area by the area of a circle with the same perimeter.

Observations on 590 fibres, revealed that for D_A ≤ 6µm, myelin thickness increased linearly with a slope of 0.15. For D_A > 6µm, myelin thickness remained approximately constant (regression slope of -0.03), with a mean value of 1.17µm. Thus, myelin thickness initially increases with axon size prior to reaching a plateau. This conclusion is consistent with data from myelinated axons in peripheral nerves in the mouse (Ceballos *et al.* 1999).

The IC has been reported to depend on axon size (Biscoe *et al.* 1982; Ceballos *et al.* 1999) but the results are conflicting. Biscoe *et al.* (1982) report higher values for smaller axons, whilst Ceballos *et al.* (1999) reported the converse. Our data for IC showed no clear tendency to vary with axon size, with an overall mean value of 0.78. Possible explanations for the differences between these sets of data might be that: (i) the observations of Biscoe *et al.* (1982) were based on lumbar ventral roots which would also include an autonomic component; (ii) our criteria for exclusion of axon profiles appear to be more stringent than those of Ceballos *et al.* (1999).

Arbuthnott ER *et al.* (1980) *J Physiol* **308**, 125-157.

Biscoe TJ *et al.* (1982) *Q J Exp Physiol* **67**, 473-494.

Ceballos D *et al.* (1999) *J Anat* **195**, 563-576.

Fiala CJ (2005) *J Microscopy* **218**, 52-61.

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PC262

Pre- and postsynaptic expression of long term depression at glutamatergic primary afferent synapses on dorsal horn spinal cord neurons in co-culture

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Natural nociceptive stimuli (pinching or heating) produce 5-20 Hz tonic firing in C- and A-delta DRG neurons (1;2). Tran-

scutaneous stimulation at low to medium pain intensities causes hypoalgesic effects, that could be related to the induction of long-term depression (LTD) at the dorsal root ganglion (DRG) synapses onto spinal dorsal horn (DH) neurons (3), but this aspect has not been well studied. In pairs of co-cultured rats DRG and DH neurons we have studied the effect of different durations of DRG neurons 5Hz low-frequency firing (LFF) on LTD induction properties in glutamatergic primary afferent synapses.

Using dual whole-cell patch clamp recording we analyzed the monosynaptic AMPA receptor-mediated eEPSC initiated in the DH neurons by AP of small DRG neurons (10-25 µm diameters). LFF was induced by 5Hz depolarizing impulses (30 ms, 20 - 50 pA) under current clamp mode. LTD was induced by LFF of single DRG neuron with different durations. LFF during 60 sec did not alter the eEPSC amplitudes, but significantly increased the coefficient of variation (CV; 56.8±11.5%, n=5; P<0.002) and the paired-pulse ratio (PPR; 37.8±11.4%, n=5; P<0.05), and decreased the release probability (p; 21.9±5.6%, n=5; P<0.05) calculated using binomial analysis. Data are expressed as percentage of the mean post-LFF value relative to the respective mean pre-LFF controls (± S.E.M.); statistical significance was evaluated using Student's t-test. LFF for 120 sec led to LTD (eEPSCs decreased to 14±3.3%, n=13; P<0.005); no changes in CV, PPR and p were found. LFF for 160, 200, 240 and 360 sec induced robust LTD: eEPSCs decreased to 37.3±4.3%, n=9; 48.1±3.5%, n=7; 58.3±2.5%, n=8, and 57.3±2.8%, n=8, respectively; P<0.001). LTD induced by these LFF durations was not accompanied by significant changing in PPR and p, but showed an increase in CV: 24.4±8.6%, n=6; 35.1±11.2%, n=6; 37.7±12.8%, n=7, and 38.1±9.3, n=8, respectively. LTD magnitude was correlated with the enhancement of the CV at different LFF duration (r²=0.96). Miniature EPSCs did not change in amplitude and variability during LTD induced by LFF with different durations. LTD was unaffected by D-AP5 20 µM and by GABAA- and glycine receptor blockers bicuculline 10 µM and strychnine 1 µM respectively.

Data suggest a possibility of induction 5Hz LFF-induced LTD in the glutamatergic primary afferent synapses in the DH. The magnitude of LTD depends on the duration of DRG neuron LFF. Different mechanisms could be involved in LTD expression according to duration of LFF. Thus, 60 sec LFF induces presynaptic changes, but not change in eEPSC LTD, whereas LTD elicited by 120 and 160 sec LFF affected the postsynaptic site. LTD initiated by longer LFF (200 - 360 sec) probably was caused by a silencing of functional synapses (4;5) without changes in glutamate release probability.

Fitzgerald M (1987). *J Physiol* **383**, 79-92.

Handwerker HO *et al.* (1987). *Exp Brain Res* **65**(3), 493-504.

Sandkuhler J *et al.* (1997). *J Neurosci* **17**(16), 6483-91.

Abrahamsson T *et al.* (2005). *J Physiol* **569**(Pt 3), 737-50.

Meng K *et al.* (2010). *Neuroscience* **165**(3), 684-91.

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PC263

Vascular endothelial growth factor splice variants VEGF165a and VEGF165b modulate spinal nociceptive processing in the rat

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Trauma to the peripheral nervous system can result in the hyperexcitability of peripheral and central neurons, leading to sensitisation and enhanced responses to noxious stimulation. Alternative splicing of vascular endothelial growth factor A (VEGF) mRNA results in the expression of two isoform families, VEGFxxxa and VEGFxxxb. These splice variants are important modulators of nociception, altering the excitability of primary afferent neurons; VEGFxxxa isoforms are pro- and VEGFxxxb anti-nociceptive. In these studies, we determined the contribution of VEGFR2 to spinal nociceptive processing, at the level of spinal cord.

All experiments were carried out in accordance with UK legislation and the Animal (Scientific Procedures) Act 1986. Both VEGF receptor 2 (VEGFR2) and the splice variant isoforms were identified in the rat spinal cord by immunofluorescence. Nociceptive tests of mechanical allodynia (von Frey hair withdrawal threshold) and thermal hyperalgesia (radiant heat withdrawal latency) were performed in male Wistar rats (~300g) or 129OlaHsd mice (~25g) before and one hour after drug administration. Intrathecal injections (10µl, between lumbar vertebra 5/6, 29 gauge needle) of VEGFR2 inhibitor PTK787 (mice), VEGF165a (mice), VEGF165b (mice), or anti-VEGF165b (rat) antibody (56/1) were delivered under isoflurane anaesthesia (2% in O₂). Drug effects were compared to i.t. saline (PTK787, VEGF165a and VEGF165b) or mouse IgG (56/1) injections. Data were compared by two way ANOVA + Bonferroni tests (*p<0.05, **p<0.01, ***p<0.001) and are presented as mean±SEM.

Intrathecal injection of the VEGFR2 full agonist VEGF165a (2.5nM) resulted in significant reductions in withdrawal threshold and latency (thresholds: saline 1.98±0.43g vs. VEGF165a 1.09±0.19g, *, **; latencies: saline 8.84±0.65s, VEGF165a 6.04±0.59s; n=4, *), indicating a pro-nociceptive action. In contrast, intrathecal injection of the partial agonist VEGF165b (2.5nM) or the VEGFR2 inhibitor PTK787 (200nM) increased withdrawal thresholds (saline: 1.17±0.05g, PTK787 2.50±0.29g; VEGF165b 2.42±0.31g, ***) and latencies (saline: 5.99±1.01s; VEGF165b 9.14±0.79s; PTK787 16.31±2.19s; n=3, *, **) indicative of an antinociceptive effect. Intrathecal administration of an antibody raised against VEGF165b (56/1) also led to the reduction in withdrawal thresholds (mouse IgG 32.58±0.64g; 56/1 12.67±2.67g ***) and latencies (mouse IgG 11.06±1.41s; 56/1 7.81±0.49s, *, **). Neutralisation of endogenous VEGF was therefore also antinociceptive in the normal animal.

These data, together with our findings in primary afferents respectively, show that VEGF isoforms have consistent differential effects on nociceptive processing throughout the pain neuroaxis in vivo; VEGF165a and VEGF165b have pronociceptive and antinociceptive actions respectively.

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PC264

An ex vivo Preparation of Mouse Pinna for Recording Afferent Responses from Guard Hair Follicles of Hairy Skin

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In our studies on synaptic-like vesicles (SLVs) in mechanosensory endings we have mainly used isolated nerve-muscle preparations, either of rat lumbrical or mouse soleus, both for recording muscle-spindle responses to stretch and for labelling SLVs with the fluorescent styryl dye FM1-43 (Bewick *et al*, 2005). Recently we extended our observations on FM1-43 labelling to the palisade endings of guard hair follicles of the external ear (pinna) of the mouse (Singh *et al*, 2009). We now report our development of the isolated pinna as a preparation for recording the afferent activity of palisade endings.

Adult mice (C57/Bl6J or Balb/C; 20-35gm) were humanely killed by CO₂ narcosis (ASPA schedule 1 method). Skin was removed near the pinnae, exposing the cartilage of the external auditory meatus (EAM) and the trigeminal (mandibular branch, MDV) and cervical (great auricular) innervation of the concave (anterior) and convex (posterior) aspects of pinna skin respectively. EAM was opened anteriorly and the pinna was flattened and pinned out, anterior skin down, onto a silicone-lined dish (Sylgard) filled with gassed (95% O₂, 5% CO₂) Liley's fluid. Posterior skin and pinna cartilage were removed by blunt dissection, leaving the anterior skin. Branches (usually 2) of the MDV emerge posteriorly between the pinna and EAM cartilages. They were cleaned and inserted into a glass pipette suction electrode. Indifferent suction-electrode impedance was balanced using areolar connective tissue and the bath was earthed to the screen of the recording-electrode cable. Electrical activity was differentially amplified (Neurolog NL104), filtered (Neurolog NL125 band-passed 0.2 - 2 kHz), and recorded using a CED 1401 micro interface and Spike 2 software (Cambridge Electronic Design, UK) that also controlled a ceramic piezoelectric actuator (PZT507, Morgan Electro Ceramics, UK) fitted with a fire-polished 10 cm borosilicate microelectrode glass capillary for mechanical stimulation (3 s at 5 Hz sinusoidal every 30 s) of 1 or 2 hairs. Part of the adipose layer was removed making a window to the base of the skin to access follicles for observation or drug application. To stimulate the hairs of these follicles the edge of the pinna was folded back leaving a small saline-filled gap between the apposed skin layers.

Advantages of the preparation are: there seem to be no down hairs on the anterior skin; guard hairs are sparse compared to most of the pelage; and whereas most possess a palisade ending few, if any, have other types of presumed low-threshold ending. Thus there is little spontaneous activity allowing the evoked responses from the 1 or 2 stimulated follicles to be readily detected. We confirmed that guard-hair palisade endings are rapidly adapting and found that action potentials show a strong tendency to be locked to a specific phase of the sinusoidal movement.

Bewick GS, Reid B, Richardson C & Banks RW (2005). Autogenic modulation of mechanoreceptor excitability by glutamate release from synaptic-like vesicles: Evidence from the rat muscle spindle primary sensory ending. *J Physiol* **562**, 381-394.

Singh P, Simon A, Banks RW & Bewick GS (2009). Glutamatergic modulation of vesicle turnover in primary mechanosensory endings. *Proc Physiol Soc* **17**, C02.

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PC265

A- and C- nociceptor sensitisation in acute and chronic cutaneous inflammation

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This study investigated the sensitisation of different nociceptor types in cutaneous inflammation, using a non-invasive thermal ramping protocol that can selectively activate C- or A-fibre nociceptors (1, 2). As inflammation results in oedema, which can affect the thermal conductivity of skin, we validated the thermal ramping protocol in cutaneous inflammation, and then determined the contributions of C- and A-fibre nociceptors to acute and chronic primary inflammatory hyperalgesia.

In acute experiments, adult male Wistar rats (280-350g) were anaesthetised with halothane (4% in O₂) and maintained on Alfaxalone infusion (25mg.kg.h⁻¹). Cutaneous inflammation was induced with subcutaneous injection of complete Freund's adjuvant (CFA 50µl; 1mg/ml) into the dorsal surface of the hind-paw, either under terminal (acute) or recovery anaesthesia (4% isoflurane in O₂; chronic). Fast (7.5°C.s⁻¹) or slow (2.5°C.s⁻¹) heat ramps were applied at the injection site to preferentially activate A- or C- nociceptors respectively, and surface and subcutaneous heating rates were determined. Withdrawal thresholds to A- and C-nociceptor activation (inter-stimulus interval 8 min) were determined before and for 3 hours after subcutaneous injection of CFA/vehicle. In chronic inflammation, oedema and nociceptive behaviour were assessed for 7 days after CFA injection. Animals were then re-anaesthetised for terminal experiments and A- and C-nociceptor withdrawal thresholds measured. Data were analysed using one way ANOVA + Bonferroni's post tests unless otherwise specified, and are mean±SEM.

Subcutaneous heating rates were equivalent in normal and inflamed skin (slow ramps: normal = 0.6±0.03°C.s⁻¹; inflamed 0.6±0.1 °C.s⁻¹; p>0.05, fast ramps: normal = 2.5±0.07 °C.s⁻¹, inflamed 2.4±0.1 °C.s⁻¹ (n=6)). Hindpaw oedema was evident after 1 hour and maintained for 7 days (paw thickness 4.3±0.1 mm; day 7, 6.3±0.2 mm, p<0.001, n=3). In acute inflammation, thresholds to both C- and A- nociceptor stimulation were lower after 1 hour (C: 53.4±0.3°C vs. 43.6±2.1°C; p<0.001; A: 56.5±0.6 °C vs. 48.5±1.7 °C; p<0.01 n=5). However, thresholds to both C- and A-nociceptor stimulation returned towards baseline values and were not significantly different at hours 2 and 3 after CFA. In chronic inflammation, behavioural withdrawal thresholds were reduced after 7 days (pre-injection: 174.7±33.1 g.mm⁻¹; day 7: 77.6±4.8 g.mm⁻¹. p<0.05, n=4), but in anaesthetised animals, C- and A-heat nociceptor thresholds were raised compared to naïve controls (C-nociceptors, 53.5±0.2°C vs. 56.6±0.7°C (7 day CFA), p<0.001, A-nociceptors, 55.88±0.2°C vs 57.44±0.5 °C (7 day CFA), p<0.01, unpaired t-test, n=4).

Acute cutaneous inflammation results in short-term sensitisation of both A- and C-nociceptors. In contrast, sensitisation was not seen in chronic inflammation.

Leith et al, 2007

Yeoman & Proudfit, 1996

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PC266

Descending tone in the noradrenergic system transiently suppresses ipsilateral and completely masks contralateral neuropathic pain following tibial nerve injury in rat

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Pontospinal noradrenergic neurons form part of the endogenous analgesic system. Plastic changes in this system are thought to be involved in the development of neuropathic pain although previous subtractive approaches to block descending noradrenergic control have been inconclusive (e.g. Howarth et al 2009). Here we use a sequential, repeated catecholaminergic antagonist approach to investigate whether the descending noradrenergic system has a protective role against the development of neuropathic pain using a chronic intrathecal catheter system. Neuropathy was induced in male Wistar rats (300-350g, n=11) using a variation of the spared nerve injury (SNIt) model (tibial nerve ligation and transection (Lee et al., 2000)) under ketamine (50mg/kg) and medetomidine (300µg/kg) anaesthesia i.p. At the same time a chronic intrathecal catheter was implanted at the L5/L6 interspace. Behavioural responses to mechanical (von Frey), cold (acetone) and heat (Hargreaves') stimulation were assayed at baseline and at days 3, 5, 10, 12, 17 and 19 post-surgery. The influence of the descending noradrenergic control over time was tested after single intrathecal dosing with either yohimbine (α2 antagonist, 30µg) or prazosin (α1 antagonist, 30µg). Values are expressed as mean ± SEM, compared by two-way ANOVA. Tibial nerve injury resulted in the development of robust neuropathic signs in the ipsilateral hindlimb by day 10. However, spinal yohimbine unmasked allodynia and hyperalgesia at an earlier stage (e.g. day 3 mechanical threshold: 14.8 ± 2.5g Vs + yohimbine 5.7 ± 1.3g P<0.01 n=6). The SNIt animals showed normal sensation in the contralateral limb, however the same animals treated with yohimbine showed robust cold allodynia from day 3 (day 3: 8 ± 4% paw withdrawal frequency Vs + yohimbine: 64 ± 8% paw withdrawal frequency, P<0.001 n=5) and heat hyperalgesia (day 3: Hargreaves paw withdrawal latency: 13.9 ± 1.0s Vs + yohimbine: 10.4 ± 0.8s P<0.05 n=5). Similarly, yohimbine unmasked robust contralateral mechanical allodynia by day 17 (day 17: 13.9 ± 2.2g Vs + yohimbine: 3.5 ± 1.1g P<0.01 n=6). Yohimbine was without effect in sham animals (n=3). Spinal prazosin did not have an effect at any time point. Thus, endogenous tone in descending noradrenergic systems completely masks the development of contralateral and delays the development of ipsilateral sensitization. Contralateral pain in animal models and in the clinic may therefore be due to a lack of, or reduced activation of these inhibitory systems providing a rationale for the use of noradrenaline re-uptake inhibitors in treating bilateral neuropathic pain. It remains to be determined why the noradrenergic system fails to completely suppress the sensitization of the ipsilateral limb.

Howarth et al (2009) Retrograde viral vector-mediated inhibition of pontospinal noradrenergic neurons causes hyperalgesia in rats. *J. Neurosci* 29, 12855-64

Lee et al (2000) An animal model of neuropathic pain employing injury to the sciatic nerve branches. *Neuroreport* 11, 657-61

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PC267

Vinblastine-induced axoplasmic transport disruption of the rat sciatic nerve causes behavioural changes consistent with neuropathic pain

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In primary sensory neurons, ion channel components necessary for sensory transduction are conveyed by axoplasmic transport from the dorsal root ganglia to the peripheral terminals for insertion into the membrane. We hypothesised that disruption of axoplasmic transport by local nerve inflammation may cause accumulation of these channels at the inflamed site, leading to a hotspot of axonal hyperexcitability. The development of spontaneous firing in sensory axons at the site of hyperexcitability may induce central changes that drive symptoms such as allodynia in patients with neuropathic pain. Consistent with this hypothesis, the blockade of axoplasmic transport by the anti-mitotic agent vinblastine (0.1 mM) causes nociceptors to become mechanically sensitive at the site of application in the absence of axonal degeneration (Dilley & Bove, 2008). Here, we examine the development of cutaneous hypersensitivity (allodynia and hyperalgesia) following the local application of vinblastine to rat sciatic nerves.

The left sciatic nerve was exposed in anaesthetised adult male Sprague Dawley rats (isoflurane, 1.75% in O₂) and treated with 0.1mM vinblastine (n=6) or vehicle (saline; n=6) as previously described (Dilley & Bove, 2008). Untreated animals (n=6) were also tested. Pre-surgery and daily up to 11 days post-surgery, ipsilateral and contralateral hind paws were tested for signs of allodynia using von Frey filaments of increasing stiffness. The lowest filament to produce a rapid foot withdrawal was considered the withdrawal threshold. Heat sensitivity was also tested using a radiant heat source (Hargreaves' method). Latency to foot withdrawal was recorded.

In vehicle and untreated groups there were no signs of mechanical allodynia. In the vinblastine group, mechanical allodynia developed ipsilaterally following surgery, peaking on day 4 (mean withdrawal threshold: pre-surgery = 12.5g (0.7 SEM); day 4 = 5.3g (0.7 SEM); p<0.05 vs. pre-surgery, contralateral and control groups, T test). By day 11, there were signs of recovery (mean = 11.3g (2.3 SEM)). However vinblastine treatment did not induce heat hyperalgesia (mean withdrawal latency: pre-surgery = 16.6s (1.0 SEM); day 4 = 15.2s (1.5 SEM); p>0.5 vs. pre-surgery, contralateral and control groups, T test). In summary, mid-axonal axoplasmic transport disruption using vinblastine causes development of mechanical allodynia, as seen in other models of neuropathic pain. Thus, the disruption of axoplasmic transport may contribute to symptoms in patients with neuropathic pain. Consistent with this hypothesis, electrophysiological recordings from isolated C-fibre neurons in the vinblastine model have revealed evidence of spontaneous activity. These changes may also be relevant to neuropathies induced by chemotherapy.

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PC267a

TRPV1 is modulated by VEGF in an isoform and PKC dependent manner

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TRPV1 is known to be important in the sensitisation of sensory neurones in inflammation and nerve injury. VEGF has two counteracting splice isoform families, VEGF_{xxx}a and VEGF_{xxx}b (1). VEGF is involved in nociception, possibly via TRPV1, as VEGF165a causes mechanical allodynia, which is not seen in TRPV1 KO mice and is blocked by TRPV1 antagonism (Hulse et al unpublished). VEGF165a activates PKC through VEGFR2 ligand dependent activation (2), and TRPV1 is sensitised by PKC-dependent phosphorylation (3). We therefore investigated whether VEGF isoforms might modulate sensory neuronal activity by controlling TRPV1 phosphorylation by PKC.

Rats were killed by anaesthetic overdose, and primary cultured DRG neurons were treated either with vehicle (HBSS) or 2.5nM VEGF165b. Varying concentrations of capsaicin (0, 0.01, 0.05, 0.1, 0.5, 1, 5, 10μM) were used to stimulate TRPV1. The resulting calcium influx response was measured on a plate reader using Fluo-4 dye as a Ca²⁺ indicator, and the response quantified by measuring the area under the curve relative to before capsaicin. The cultures were also incubated with or without the PKC inhibitor bisindolylmaleimide-1 (BIM1, 10μM). In this instance sub-threshold 0.2μM capsaicin was used and the calcium response measured as above. Immortalised embryonic DRG neurons (50B11) were also cultured and treated with either vehicle, VEGF165a, VEGF165b or NGF. Protein was extracted and immunoprecipitation used to isolate TRPV1. Once isolated phosphorylated TRPV1 levels were examined by western blotting with a phosphoserine-specific antibody (n=3). VEGF165b appeared to reduce sensitisation of TRPV1. A significant suppression of TRPV1 response was seen at 0.1-5μM capsaicin in the presence of VEGF165b (p<0.001, 1-way ANOVA). BIM1 blocked the sensitisation of TRPV1 by VEGF165a, indicating a PKC-dependent mechanism. 0.2μM capsaicin with 2.5nM VEGF165a caused an increase in calcium response, which was prevented with BIM1 (p<0.01, 1-way ANOVA). VEGF165a and NGF showed an increase in phosphorylated TRPV1 by Western blotting, which VEGF165b did not.

VEGF165a therefore appears to sensitise TRPV1 by PKC-dependent phosphorylation, while VEGF165b acts to suppress this sensitisation. The finding that VEGF165b did not increase phosphorylated TRPV1 levels also supports the idea that it may be involved in preventing sensitisation of TRPV1. Thus TRPV1 activation by VEGF isoforms in DRG neurons is isoform and PKC dependent.

S. J. Harper, D. O. Bates, VEGF-A splicing: the key to anti-angiogenic therapeutics? Nature reviews (2008) 8, 880.

M. Nilsson, J.V. Heymach, Vascular Endothelial Growth Factor (VEGF) Pathway. Journal of Thoracic Oncology (2006) 1, 768.

J-K Cheng, R-R Ji, Intracellular signalling in primary sensory neurons and persistent pain. Neurochem Res. (2008) 33, 1970.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

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PC273

Teaching baroreflex physiology to medical students: A comparison of quiz-based and conventional teaching strategies in a laboratory exercise

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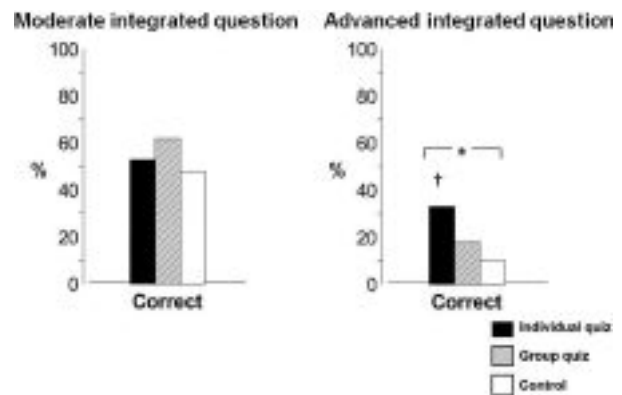
Background. Quiz-based and collaborative teaching strategies have previously been found to be efficient for improving meaningful learning of physiology during lectures (1). These approaches have, however, not been investigated during laboratory exercises. In the present study, we compared the impact of solving quizzes individually and in groups to conventional teaching on the immediate learning during a laboratory exercise.

Methods. We implemented two quizzes in a mandatory 4-hour laboratory exercise on baroreflex physiology. A total of 155 second-year medical students were randomized to solve quizzes individually (intervention group I, n=57), in groups of

3-4 (intervention group II, n=56), or not to perform any quizzes (intervention group III, control, n=42). After the laboratory exercise, all students completed an individual test, which encompassed two recall questions, two intermediate questions, and two integrated questions. The integrated questions were of moderate and advanced difficulty, respectively. Finally, the students completed an evaluation form.

Results. Intervention group I reached the highest total test scores, and proved best at answering the integrated question of advanced difficulty ($P < 0.05$, Table 1). Moreover, there was an overall difference between groups for the student evaluations of the quality of the teaching, which was highest for intervention group II.

Conclusion. Solving quizzes individually during a laboratory exercise may enhance learning, whereas solving quizzes in groups is associated with higher student satisfaction.



Test scores. Specific scores on integrated questions of moderate and advanced difficulty. The ordinate indicates the percentage of students in each group that responded correctly. * Overall difference between groups, $P < 0.05$ (Kruskal-Wallis test). † Different from control, Holm-Bonferroni-corrected $P < 0.05$ (Fisher's exact test).

Michael J. Where's the evidence that active learning works? Adv Physiol Educ 30: 159-167, 2006.

None.

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PC274

Formative Assessment and Active Learning: a tool to improve learning of physiology

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Students learn best when they are focused and thinking about the subject at hand. To teach physiology, we must offer opportunities for students to actively participate in class. This approach aids in focusing their attention on the topic and thus generating genuine interest in the mechanisms involved. This study was conducted to determine if offering voluntary active learning activities would improve student understanding and application of the material covered. To compare performance, an anonymous cardiorespiratory evaluation was distributed to two groups of students during the fall (control, n = 168) and spring (treatment, n = 176) semesters. Students in both groups were taught by traditional methods, and students in the treatment group had the option to voluntarily participate in two additional active learning exercises: 1) a small group discus-

sion, where students would discuss a physiology topic with their Teaching Assistant before running BIOPAC software for the laboratory exercise and 2) a free response question, where students anonymously responded to one short essay question after the laboratory exercise. In these formative assessments, students received feedback about their present state of learning from the discussion with their peers and also from the instructor comments regarding perceived misconceptions. As a result of the participation in these activities, students in the treatment group had a better overall performance [X^2 (degree of freedom = 1) = 31.2, $P < 0.001$] on the evaluation (treatment group: 62% of responses correct and control group: 49%) with an observed difference of 13% (95% confidence interval: 8, 17). In conclusion, this study presents sufficient evidence that when the opportunity presents itself, students become active participants in the learning process, which translates into an improvement in their understanding and application of physiological concepts.

Carvalho H. Active teaching and learning for a deeper understanding of physiology. *Adv Physiol Educ* 33: 132–133, 2009.

Carvalho H., Crystal West. Voluntary Participation leads to a better understanding in physiology. *Advances in Physiology Education*, 35: 53–58, 2011.

Carvalho H. A group dynamic activity for learning the cardiac cycle and action potential. *Advances in Physiology Education*, 35: 312–313, 2011.

Crystal West

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PC275

The use of an Audience Response System (ARS) in the assessment of practical classes in Human Physiology (Biomedical Sciences)

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The use of interactive ARS, or “clickers”, has been proposed as an innovative methodology to capture the attention of students in practical classes. Its success has been previously demonstrated in disciplines as varied as Nursing (1), Biochemistry (2) and Chemistry (3).

The aim of the study was to promote a student-centered learning process through active participation in lab tasks in a Human Physiology course on the Biomedical Sciences degree. For each lab activity, a batch of 10 multiple choice questions were prepared to measure the level of knowledge acquisition and the connections between theoretical classes and students' practical skills.

Immediately after the assessment, clickers informed students of their results. The total of correct and incorrect answers was displayed on a screen (in pie or bar charts). A rate of correct answers of between 70–100% among all the students answering the questionnaire indicate that the topic had been well understood, but results below 60% showed that more time was needed to explain or work on the subject in question. This system of immediate feed-back allowed us to resolve doubts and also stimulated discussion, promoted self-criticism, peer collaboration and the degree of student satisfaction.

Item analysis of the answers to the questions was then applied to improve the formative aspects of the questionnaires. The results of the item analysis helped students and teachers to identify problems and overcome difficulties. Teachers can adjust the instructions (a) to redefine questions in order to promote peer discussion, (b) to identify poorly learned concepts (misconceptions), (c) to formulate questions that can be answered in the time stipulated, and (d) to identify ineffective methods.

ARS appears to be well suited for the assessment of student performance

Jennie C. de Gagne. 2011. *Nurse Education Today*. 31, 8, 34–40

Stephen Addison, Adrienne Wright and Rachel Milner. 2009. *Biochemistry and Molecular Biology Education* 37, 2, 84–91

Michael Shaver. 2010. *Journal of Chemical Education* 87, 12, 1320–1323

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PC276

On-line discussion groups as a tool for enhancing student understanding following practical-based teaching

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Teaching on the BSc Animal Science course at the University of Nottingham has traditionally sought to maintain a significant level of practical-based classes at the heart of its modules. Data collection, analysis and interpretation are primary learning objectives of practical-based classes in the biosciences however time limitations often mean the focus during classes is on data collection with analysis being performed by the student retrospectively. This can be challenging for a student as inexperience often means they have difficulty dealing with the individuality and variability of biological data. The current study therefore looked at using a computer-mediated communication facility, WebCT discussion groups, as a means of providing support and advice beyond the practical. Eighty-two undergraduate students on Year 2 module D224Z5 Neurophysiology & Pharmacology were involved in the study, none of whom had previously used discussion groups. Use of the discussion boards was demonstrated at the start of the module and 2h on-line discussion sessions, after each of 3 practical classes on the module, were initially timetabled for 1 week after the practical and 1 before the associated coursework submission deadline; during the session the lecturer would be available on-line to answer questions and to encourage discussion between students. However on reflection and in response to requested feedback following each session, boards were subsequently run throughout the two week period with the lecturer contributing when available. Student opinion on the use of discussion groups was surveyed at the end of the module using an anonymous questionnaire. Forty completed questionnaires were returned giving a response rate of 48.8%. The data indicate that students engaged with the practice of using discussion boards to enhance their understanding. Only 5% of respondents did not use them, whilst 74% of everyone else used the discussion boards after each practical; 84.2% indicated they got useful information from the discussion boards. However the lack of anonymity when using the boards meant that some

students (13.8%) were reluctant to participate. Interestingly, the vast majority of students (82.5%) did not consider course-work until at least a week after the practical therefore many (57.5%) thought initial timetabling of sessions was too early to be effective. Therefore overall on-line discussion groups appear to be a valuable tool to provide support that is available and of benefit to all students on a module. It would appear that to satisfy different work strategies of individual students a discussion board without time limitations (asynchronous computer-mediated communication) is the most suitable format. When questioned, most students preferred the use of discussion groups over alternative forms of providing feedback such as e-mails and specific seminars.

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PC277

A role for proteasome subunits in cholecystokinin 2 receptor-mediated gene transcription

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The hormone gastrin helps maintain gastric mucosal integrity by regulating expression of genes such as *plasminogen activator inhibitor 2* (PAI-2; 1) and *regenerating protein 1* (Reg1; 2), but the mechanisms involved are incompletely understood. We recently identified a gastrin response element in the *vesicular monoamine transporter 2* promoter and showed its activity depended on the proteasome beta subunit PSMB1 (3). We have used the gastric cancer cell line AGS-GR, which expresses the cholecystokinin 2 receptor (CCK2R), to determine if PSMB1 is involved in CCK2R-mediated expression of other gastrin sensitive genes, and to visualize its cellular distribution during gastrin stimulation. AGS-GR cells were transfected with siRNA against PSMB1 (30nM), or scrambled control, and 48h later with firefly luciferase reporter constructs containing 1.6kb of the PAI-2 promoter or 2.1kb of the Reg1 promoter, together with a control *renilla* reporter vector. 18h later cells were stimulated with gastrin (G17, 2nM) or vehicle for 6h then extracted for dual luciferase assay. Western blots showed that 72h after transfection with PSMB1 siRNA, PSMB1 protein was reduced by 69.5±5.5% (mean±SEM, n=3) compared to the level in cells transfected with scrambled siRNA. In cells transfected with scrambled siRNA, gastrin increased transcription of the PAI-2 promoter 9.2±1.5 fold (n=6) and the Reg1 promoter 2.4±0.9 fold (n=9), compared to unstimulated cells. In cells with PSMB1 knocked down, gastrin-stimulated PAI-2 promoter activity was significantly reduced to 2.1±0.2 fold (p<0.001, ANOVA) and Reg1 activity to 1.2±0.1 fold (p<0.03). In unstimulated AGS-GR cells, immunocytochemistry using a rabbit antiserum against PSMB1 revealed distribution throughout the cytoplasm and nucleus. After 2h stimulation with 2nM G17, cytoplasmic PSMB1 was barely detectable and nuclear staining was intensified; after 6h stimulation, PSMB1 was again visible in cytoplasm. Western blotting of nuclear and cytoplasmic extracts showed that the nuclear:cytoplasmic ratio of PSMB1 changed from 1.7±0.4 in untreated cells to 10.6±2.6 (n=7, p<0.01, ANOVA) after 2h stimulation with G17. This subcellular redistribution was not seen with alpha (PSMA5) or regulatory (PSMC1) proteasome subunits. PSMB1 distribution was unaffected by activation of receptors for histamine, epidermal growth factor, PGE2, or interleukin-8. The response to G17 was

however mimicked by phorbol 12-myristate 13-acetate (10⁻⁷M), and prevented by the protein kinase C (PKC) antagonist Ro-32-0432 (10⁻⁶M). We conclude that proteasome beta subunits are involved in gastrin-stimulated expression of some genes important for gastric mucosal integrity and function. CCK2R activation induces a PKC-dependent subcellular redistribution of proteasome subunits that may be linked to their transcriptional functions.

Ashcroft FJ, Varro A, Dimaline R, Dockray GJ (2004). Control of expression of the lectin-like protein Reg-1 by gastrin: role of the Rho family GTPase RhoA and a C-rich promoter element. *Biochem J*. 381:397-403.

Almeida-Vega S, Catlow K, Kenny S, Dimaline R, Varro A (2009). Gastrin activates paracrine networks leading to induction of PAI-2 via MAZ and ASC-1. *Am J Physiol Gastrointest Liver Physiol*. 296:G414-23.

Catlow K, Ashurst HL, Varro A, Dimaline R (2007). Identification of a gastrin response element in the vesicular monoamine transporter type 2 promoter and requirement of 20 S proteasome subunits for transcriptional activity. *J Biol Chem*. 282:17069-77

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PC278

The effect of aspirin and smoking on excretion of lactulose and mannitol in fit young women: Towards an aspirin augmented test of gut permeability

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Gut health maybe envisaged as the ability to continue to function i.e. withstand, large fluctuations in lumen conditions. We developed a standardized test to quantitatively assess the response of the gut to a standardized noxious stimulus. Twenty healthy carefully screened female volunteers (9 smokers and 11 non smokers) were recruited. They each received 600mg of aspirin or placebo in random sequence and were subsequently dosed with 10g lactulose and 5g mannitol. Half hourly urine samples were collected over six hours. Urinary sugar concentrations were determined by HPLC using a method similar to Trehan et al. Lactulose mannitol ratios (LMR) and rates of excretion of lactulose and mannitol were found to be normally distributed and were compared by doubly repeated measures ANOVA. The magnitude of the LMR depended significantly on the period over which the urine was collected, the six hour period being significantly higher than the three hour collection period (d.f. 1, 19; F = 48.74; P < 0.001). Dosage with 600mg aspirin caused a significant increase in LMR (d.f. 1, 19; F = 23.95; P < 0.001) over the six hour period of collection. Variation in LMR over time was driven by differences in the patterns with which the two probes were absorbed and excreted. Greater quantities of mannitol were excreted during the first three hours than the subsequent three hours (d.f. 1, 19; F = 48.88; P < 0.001) of collection, with the excretion rate peaking at 1.5 to 2 hours post dosage. The pattern of excretion of mannitol was unchanged after dosage with aspirin. Lactulose excretion over the six hour period was maintained at a similar rate but the overall rate was significantly increased after dosage with aspirin (d.f. 1, 18; F = 16.83; P = 0.001). There were no differences in the patterns of excretion of the two probes between smokers and non smokers. The LMR test can repro-

ducibly detect increases in permeability resulting from a 600mg dose of aspirin, particularly when the patterns of absorption of the component sugars are considered in relation to the duration of the sampling period. It remains to examine whether the test is able to identify agents that promote the recovery of permeability from, or reduce the response to, the dose of aspirin.

Trehan I, Shulman RJ, Ou C-N, Maleta K, Manary MJ. A Randomized, Double-Blind, Placebo-Controlled Trial of Rifaximin, a Nonabsorbable Antibiotic, in the Treatment of Tropical Enteropathy. *Am J Gastroenterol* 2009;104:2326-2333.

This work was funded by the Ministry of Science and Innovation, New Zealand, contract number C06X0807, awarded to The New Zealand Institute for Plant & Food Research Limited. We would like to thank Chris Booth and Michelle McGrath for their assistance during the trial.

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PC279

UT-A and UT-B urea transporter expression in the human gastrointestinal tract

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The symbiotic relationship that exists between humans and their intestinal bacteria is crucial in maintaining our gastrointestinal health. Growth of bacterial populations in the human colon is enhanced by the provision of nitrogen, in the form of urea, via colonic urea transporters (1). Our previous studies have identified two distinct urea transporters within the human colon, namely UT-B1 (2) and UT-A6 (3). The aim of the present study was to investigate the expression of these two transporters along the length of the human gastrointestinal tract. Using purchased cDNA samples (AMS Biotechnology, UK), initial PCR experiments surprisingly showed that both UT-B1 (colon > small intestine > stomach) and UT-A6 (stomach = small intestine = colon) were expressed at the RNA level throughout the gastrointestinal tract. Next, western analysis was performed using protein samples produced from gastrointestinal tissues obtained with patient consent at surgical resection procedures. Using the characterised BUTB-PAN antibody (2), experiments confirmed the now expected abundance pattern for the 35 kDa UT-B1 protein signal (i.e. colon > small intestine > stomach). Finally, western analysis was also performed using the hUT-A6#C antibody, raised to the unique C-terminal of UT-A6. This novel antibody successfully detected a 23 kDa signal, corresponding to the predicted size for UT-A6 protein, within colonic plasma membrane-enriched protein samples. This study shows that urea transporters are expressed all along the human gastrointestinal tract, and not just in the colon as predicted. It is also the first study to detect UT-A6 at the protein level. These findings suggest that urea transport mechanisms within the human gastrointestinal tract are more extensive than previously proposed.

Stewart GS (2011). *Br J Pharm* 164, 1780-1792

Collins D et al. (2010). *Am J Physiol Gastro Physiol* 298, G345-G351

Smith CP et al. (2004). *Am J Physiol Cell Physiol* 287, C1087-C1093

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PC280

Salivary acinar cells: functional expression of different cannabinoid receptor subtypes

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Cannabinoid receptors (CBRs) belong to the G protein-coupled receptor superfamily and are widely expressed within the body. Although the expression of CBRs was predicted in nonexitable acinar cells, the molecular identification of different receptor subtypes in acinar cells has not been performed and the physiological consequences of their activation in salivary glands remain to be fully established. To elucidate these issues in the acinar cells of submandibular salivary glands, we used different molecular biological and fluorescent imaging techniques. Experiments were performed on acinar cells freshly isolated from the submandibular salivary glands of deeply isoflurane-anesthetized Wistar rats deeply anesthetized with isoflurane (2%). The animals were used in accordance with protocols that were approved by the Animal Care and Use Committee at Bogomoletz Institute of Physiology, Ivan Franko National University, and The University of Dundee.

By utilizing a set of molecular biology approaches we have demonstrated the prominent expression of classical CB1Rs and CB2Rs in acinar cells of the rat submandibular gland. In addition, we also identified expression of the putative novel cannabinoid receptor GPR55 in these cells. Using Ca^{2+} imaging technique, we found that the activation of different CBRs subtypes triggers $[Ca^{2+}]_{cyt}$ signalling in acinar cells by distinct pathways, involving Ca^{2+} release from the endoplasmic reticulum (ER) and store-operated Ca^{2+} entry. Altogether, our findings directly demonstrate a functional expression of different CBRs subtypes, especially CB1Rs, CB2Rs, and GPR55, in acinar cells from the submandibular salivary glands. The presence of CBR subtypes in acinar cells suggests that they may play a novel role in regulating the activity of the salivary gland.

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PC281

Bile acids regulate autophagy in colonic epithelial cells

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Background: Colonic epithelial cells function as a physical barrier to luminal toxins and antigens and regulate the transport

of fluid, nutrients and electrolytes to and from the gut lumen. Autophagy is an evolutionarily-conserved mechanism by which self-digestion of cellular proteins and organelles during periods of cellular stress can occur. The importance of autophagy in colonic epithelial cells in development of intestinal disorders, such as inflammatory bowel disease (IBD) and cancer, is becoming increasingly apparent. Bile acids are classically known for their roles in facilitating digestion and absorption of fats. However, they have more recently become appreciated as a family of intestinal hormones and are known to be important in the pathogenesis of colonic inflammation and cancer. However, the mechanisms underlying bile acid actions are still poorly understood, and their roles in regulating colonic epithelial autophagy are not yet known.

Aim: The aim of this study was to investigate the effects of the common colonic bile acids, deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA), on autophagy in colonic epithelial cells.

Methods: T₈₄ colonic adenocarcinoma cells were grown on 30 mm Millicell-HA inserts until they formed a polarized monolayer, mimicking the phenotype of native epithelial cells. Cells were then treated bilaterally with DCA (200 μ M) or UDCA (500 μ M) for 24h. Expression levels of LC3 protein, a reliable indicator of ongoing autophagy, were then investigated by western blotting.

Results: In cells treated with DCA for 24 h, expression levels of LC3 protein were increased $444 \pm 145\%$ ($n = 4$; $p < 0.05$) compared to untreated control cells. In contrast, UDCA exerted a relatively weak effect on autophagy that was not significantly different from untreated controls ($203 \pm 62\%$; $n = 4$).

Conclusions: Our studies suggest that the composition of the colonic bile acid pool is likely to be important in regulating the extent of epithelial autophagy in the colon. Given the important roles that autophagy plays in regulating inflammatory responses and cell survival, these data are important for developing our understanding of the role that bile acids play in the pathogenesis such diseases. Future studies will focus on characterising the effects of bile acids on epithelial autophagy, with the hope that this will ultimately lead to the development of new approaches to treat intestinal diseases.

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PC282

Role of acid-sensitive two-pore domain potassium (K_{2p}) channels in cancer progression

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Potassium channel activity is required for cell cycle progression and altered expression and activity has been implicated in a number of carcinomas. One family of channels the two-pore domain potassium (K_{2p}) channels are of particular interest since when expressed on the cell surface these channels are constitutively active. Altered expression of acid-sensitive K_{2p} channels has been identified in a number of carcinomas and their expression is thought to be beneficial to cancers under conditions such as hypoxia or low glucose.

To examine the expression of acid-sensitive K_{2p} channels ($K_{2p3.1}$, $K_{2p9.1}$, $K_{2p15.1}$) in cancer cell lines a combined approach using reverse transcriptase PCR, immunofluorescence and Western blotting was used. Expression of these channels was also assessed in 10 oesophageal carcinoma samples by immunohistochemistry. Proliferation (MTS) assays were performed to assess proliferation in K_{2p} positive cancer cell lines when exposed to specific K_{2p} channel inhibitors (genistein, methanandamide and ruthenium red). In addition, whole cell currents of cancer cell lines were analysed and K_{2p} channels were found to contribute to these currents.

Expression of $K_{2p3.1}$ and $K_{2p9.1}$ mRNA and protein expression was identified in several human cancer cell lines including breast MCF7, colorectal HCT116, SW480, SW620, lung A549 and oesophageal OE19. Also $K_{2p9.1}$ positivity was found in a significant proportion, $>30\%$ of oesophageal carcinoma tissue. Proliferation was significantly decreased in response to K_{2p} inhibitors for colorectal but not for breast or lung cancer cell lines. SW480 proliferation was reduced by $46 \pm 4\%$ $p \leq 0.05$ (100 μ M genistein), $53 \pm 6\%$ $p \leq 0.05$ (50 μ M methanandamide) and $72 \pm 10\%$ $p \leq 0.05$ (100 μ M ruthenium red). The other colorectal cell lines showed similar reductions HCT116 $57 \pm 4\%$ $p \leq 0.01$ (50 μ M methanandamide) and SW620 $59 \pm 5\%$ $p \leq 0.01$ (50 μ M methanandamide), $76 \pm 4\%$ $p \leq 0.05$ (100 μ M ruthenium red). Cell proliferation was also reduced $>40\%$ by a broad spectrum potassium channel blocker 4-AP (5mM).

K_{2p} channels alter cell resting membrane potential therefore increased expression in cancer cells would change this potential and could lead to activation of other potassium channels causing progression in the cell cycle. Acid-sensitive K_{2p} channels are modulated by cell stress conditions (pH, hypoxia) and the physiological relevance of this in a cancer environment needs to be investigated. These findings provide evidence for a role of acid-sensitive K_{2p} channels in cancer cell proliferation and identify K_{2p} channels as a potential therapeutic target in cancer.

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Human Ether à-gogo K^+ channel 1 regulate MDA-MB-231 breast cancer cell migration by modulating resting membrane potential and Orai1-dependent calcium entry

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Breast cancer has a poor prognosis due to its strong metastatic ability. Accumulating data present ether à go-go (hEag1) K^+ channels as relevant players in controlling cell cycle and proliferation of non-invasive breast cancer cells. However, the role of hEag1 in invasive breast cancer cells migration is still unknown. In this study, we studied both the functional expression and the involvement in cell migration of hEag1 in the highly metastatic MDA-MB-231 human epithelial breast cancer cells. We showed that hEag1 mRNA and proteins were expressed in human invasive ductal carcinoma tissues and breast cancer cell lines. Functional activity of hEag1 channels in MDA-MB-231 cells was confirmed using astemizole, a hEag1

blocker, and a specific siRNA targeting this channel (sihEag1). Using patch-clamp technique, calcium imaging and Boyden chambers assays, we demonstrated that blocking or silencing hEag1 depolarized the membrane potential and reduced both Ca^{2+} entry and MDA-MB-231 cell migration without affecting cell proliferation. Recent studies have reported that Ca^{2+} entry through Orai1 channels is required for MDA-MB-231 cell migration. Down-regulation of hEag1 or Orai1 expression reduced Ca^{2+} influx and cell migration with similar efficiency. Interestingly, no additive effects on Ca^{2+} influx or cell migration were observed in cells co-transfected with sihEag1 and siOrai1. Finally, we found, by immunohistochemistry, that both Orai1 and hEag1 are expressed in invasive breast adenocarcinoma tissues and invaded metastatic lymph node samples (LNM⁺). Furthermore, in LNM⁺, high hEag1 expression level was associated with high Orai1 expression level, and inversely. These data supported the possibility of a link between hEag1 and Orai1 in pathological conditions. In conclusion, this study is the first to demonstrate that hEag1 channels are involved in the serum-induced migration of breast cancer cell by controlling Ca^{2+} entry through Orai1 channels. hEag1 may therefore represent a potential target for the suppression of breast cancer cell migration, and thus prevention of metastasis development.

(* These authors contributed equally to this work)

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Transient receptor potential melastatin 7 channel is involved in migration of metastatic breast cancer cells through its kinase domain

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Estrogen receptors absence (ER-) has been shown to direct human Invasive Ductal Carcinoma (IDC) toward a more aggressive and malignant state. In this study, we examined the expression and function of TRPM7 in the metastatic ER- breast cancer cells. Clinical relevance was established by immunohistochemistry in ER- IDC tissues samples. We found that TRPM7 expression was higher in the invasive tumoral areas (staining score 2.8 ± 0.2) and invaded lymph nodes (staining score 2.3 ± 0.1) than in the non-invasive tumoral areas (staining score 0.8 ± 0.4). In the highly metastatic MDA-MB-231 cell line, a Magnesium Inhibited Cation (MIC) current was recorded in whole cell patch-clamp and was reduced using siRNA directed against TRPM7. TRPM7 silencing neither affected Ca^{2+} , Mg^{2+} -homeostasis nor cell proliferation but significantly reduced cell migration (by 68 ± 3 %). Moreover, the heterolo-

gous overexpression of zebrafish wild type TRPM7 strongly increased cell migration in the weakly migratory MCF-7 cells and in the MDA-MB-231 cells (2.4 fold increase). This migration increase was not observed when we overexpressed a kinase domain truncated TRPM7 form (TRPM7 Δ kinase) in both cell lines, demonstrating the role of the kinase domain in breast cancer cell migration. Furthermore, the use of low and high external calcium concentrations did not affect the migration decrease induced by TRPM7 silencing, suggesting that TRPM7 regulates cell migration independently of calcium entry. We finally observed that TRPM7 silencing had no effect on the focal adhesion kinase (FAK) phosphorylation but reduced the myosin IIA heavy chain phosphorylation (by 41 ± 14). Taken together, our findings suggest that the channel TRPM7 regulates breast cancer cell migration via phosphorylation of myosin IIA, and independently of the calcium influx. Our data support also the consideration of TRPM7 channel as a novel biomarker and/or possibly a pharmaceutical target of the most aggressive human ER- IDC.

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PC285

Type 3 IP3 receptors are linked to BK channels via lipid rafts and promote human breast cancer cells proliferation

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Breast cancer is the leading cancer in terms of incidence and mortality in the French female population. Concerning the process of carcinogenesis, including deregulation of proliferation, migration and invasion of cancer cells, the role of ion channels is increasingly studied.

The implication of Ca^{2+} signalling, particularly the one involving inositol (1,4,5)-trisphosphate receptors (IP3R) which includes 3 subtypes (IP3R1, IP3R2 and IP3R3), has been shown in several cancers. Furthermore, Ca^{2+} -dependent K^{+} channels (KCa) are known for their roles in the development of many neoplastic diseases. A recent study from our laboratory has shown that IP3R3-mediated Ca^{2+} signalling is involved in the proliferation of human breast cancer MCF-7 cells. Likewise, our laboratory showed that KCa channels (BK and hK1) are also involved in human breast cancer cells proliferation. In this context, we investigated the role of IP3-dependent Ca^{2+} signalling and its probable interaction with the BK channels in the pathophysiology of breast cancer cells.

Our results show that IP3R3 and BK channels are expressed in normal and cancerous breast cell lines and that both proteins are overexpressed in breast cancer tissues. Moreover, our results are in favor of a functional coupling between IP3R3 and BK channels in MCF-7 cancerous cells. Indeed, application of ATP causes an elevation of internal Ca^{2+} triggering a TEA-sensitive membrane hyperpolarization that is very strongly reduced in cells in which IP3R3 or BK channels expression was silenced by the use of specific siRNAs. Furthermore, we show that ATP increases the proliferation of MCF-7 cells and that this effect is impaired in cells in which the expression of BK channels, IP3R3, or both has been reduced following transfection by specific siRNAs. Our results show also that BK channel and IP3R3 co-immunoprecipitate and that the interaction seems to occur via lipid rafts. However, in the normal breast cell line MCF10A, neither ATP application nor reduction of BK channel

expression level by a specific siRNA affect the proliferation of these cells. Furthermore, IP3R3 and BK channels are not co-immunoprecipitated, suggesting the absence of a molecular coupling between BK channels and IP3R3.

Taken together, our results suggest a molecular and functional interaction between BK channel and IP3R3 via lipid rafts in cancer cells. Moreover, while this interaction was highlighted in the tumor cell line MCF-7, it is, however, absent in the normal cell line MCF-10A. Our findings permit us to propose this coupling between BK and IP3R3 as a necessary mechanism for tumor cells proliferation.

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PC286

The role of cytosolic domains in the trafficking of human Nav1.7

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Chronic pain is a highly unmet clinical need. However, the voltage-gated sodium (Nav) channel Nav1.7 has emerged as an attractive target in the treatment of pain [1]. Expressed preferentially in nociceptive dorsal root ganglion neurons, Nav1.7 functions at the cell membrane to transmit peripherally generated pain signals towards the central nervous system [2]. Manipulation of Nav1.7 trafficking mechanisms to decrease the number of these channels expressed at the cell membrane is a promising approach to reducing the sensation of pain. However, very little is currently known about the mechanisms regulating Nav1.7 cell surface expression.

Nav channels are composed of four 6-transmembrane domains (I to IV), and have cytosolic N- and C-terminal domains [3]. To examine trafficking by conventional imaging approaches, we have introduced haemagglutinin A (HA) and bungarotoxin binding site (BBS) epitopes into three extracellular loops of the channel. Introduction of these epitopes reduced the surface expression of the channel, preventing the use of this approach to study trafficking. We have thus employed an indirect approach where we have expressed the cytosolic domains of Nav1.7 (from an IRES- EYFP vector) in HEK293 cells stably expressing the channel, and measured channel cell surface expression using the whole-cell patch-clamp method and cell surface biotinylation. The rationale was that the presence of active trafficking motifs within these domains would exert a dominant negative effect on Nav1.7 trafficking, resulting in a change in the cell surface expression of the channel. We found that neither the N-terminus nor inter-domain loop 3 (IDL3) significantly altered Nav1.7 surface expression. It was unclear whether this result was due to inadequate expression of the domains, or because of a lack of effect of the domains. Taken together, applying conventional approaches to investigate Nav1.7 trafficking has proven unsuccessful.

In a parallel approach, we have substituted the cytosolic domain of human CD4 with the cytosolic domains of Nav1.7, and examined the effect of the substituted domains on the trafficking of CD4 using immunocytochemistry. Our results show that while human CD4 does not undergo endocytosis when expressed in HEK293 cells, addition of the C-terminus of Nav1.7 results in robust endocytosis, indicating the presence of endocytic signals in the C-terminus of the channel. Consistent with this observed effect, the C-terminus has two consensus endocytosis motifs- a tyrosine-based motif and a

dileucine motif. Our results therefore demonstrate that by using reporter proteins such as CD4, it is possible to circumvent the difficulties associated with using full-length Nav1.7, to investigate the trafficking of this channel.

Cox, J.J., et al., An SCN9A channelopathy causes congenital inability to experience pain. *Nature*, 2006. 444(7121): p. 894-8.

Djouhri, L., et al., Sensory and electrophysiological properties of guinea-pig sensory neurones expressing Na(v)1.7 (PN1) Na⁺ channel alpha subunit protein. *Journal of Physiology-London*, 2003. 546(2): p. 565-576.

Catterall, W.A., From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron*, 2000. 26(1): p. 13-25.

Professor Asipu Sivaprasadarao, Dr Jonathan Lippiat, Dr Christopher Cockcroft

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PC287

Comparative analysis of the molecular mechanisms mediating the Ca²⁺-, Cd²⁺- and Zn²⁺-induced mitochondrial permeability transition

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It is known that several divalent cations induce the mitochondrial permeability transition (MPT) that accompanied with opening the MPT pore, Ca²⁺ release from the mitochondria and swelling of these organelles. Such processes frequently lead to the cell damage and death. Nevertheless the characteristics of the metal-induced MPT remain elusive. The aim of this research was to compare the molecular mechanisms of the Ca²⁺ (5.0-100.0 μM)-, Cd²⁺ (0.1-50.0 μM)- and Zn²⁺ (0.1-7.0 μM)-induced MPT.

The experiments were performed on the isolated mitochondria obtained from outbred adult rat liver by differential centrifugation (1). The animals were anesthetized by inhalation with diethyl ether and decapitated. Mitochondrial swelling was measured using spectrophotometer and analyzed by: i) maximal rate; ii) lag time; iii) peak amplitude (changes of the mitochondrial volume). Ca²⁺ release from the mitochondria was investigated using Ca²⁺-selective electrode and analyzed by: i) maximal rate; ii) duration; iii) peak amplitude (concentration of released Ca²⁺). Cyclosporin A (10 μM), dithiothreitol (1 mM), 2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (1 mM) and 7-Chloro-5-(2-chlorophenyl)-1,5-dihydro-4,1-benzothiazepin-2(3H)-one (1 μM) were used as modulators of the metal-induced MPT. The concentration of mitochondrial protein was measured by Lowry method (2).

We showed, that the metal-induced MPT is mediated by megachannel activation and involves cyclophilin D. Carboxyl groups of the potential-dependent anion channel contribute to the metal-induced MPT. Sulfhydryl groups of the ADP/ATP-antiporter are involved in the Cd²⁺- and Zn²⁺-induced MPT. Binding of Ca²⁺ and Zn²⁺ with cyclophilin D and Cd²⁺ – with ADP/ATP-antiporter represents key events for the activation of the MPT pore. Mitochondrial Na⁺/Ca²⁺-exchanger stimulates the metal-induced MPT. In general, it was discovered different mechanisms of the Ca²⁺-, Cd²⁺- and Zn²⁺-induced MPT, swelling of mitochondria and Ca²⁺ release from them under the influ-

ence of these cations and also the MPT initiated by low and high concentrations of Ca^{2+} . We believe that the obtained differences depend on the ability of cations to interact with constituents of the MPT pore and on the functional state of other transporting systems of these organelles.

Thus, as a result of our investigations the molecular mechanisms of the interaction of Ca^{2+} , Cd^{2+} and Zn^{2+} with the MPT pore were determined and compared.

1. Schneider WC, Hogeboom GH (1950). *J Biol Chem* **183**(1), 123–128

2. Lowry OH, Rosebrough NJ, Farr AL, and Randall RJ (1951). *J Biol Chem* **193**(1), 265–275

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PC288

β 4-subunit palmitoylation controls cell surface expression of large conductance calcium- and voltage- dependent potassium (BK) channels

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BK channels control diverse physiological functions including neuronal excitability and thus mechanisms that control cell surface expression of these channels are important determinates of function in health and disease. We have recently identified that the cell surface expression of BK pore-forming α subunit is potently regulated by s-acylation, the only reversible lipid modification of proteins. In many neurons, α subunits assemble with accessory β -subunits to modify channel properties, however, whether these β -subunits may also be regulated by s-acylation to control trafficking or function is not known.

Using the CSS-Palm 2.0 algorithm, we predicted the neuronal β 4-subunit is a palmitoylated membrane protein and confirmed this biochemically using 3H-palmitate incorporation and acyl-RAC of β 4-subunits expressed in HEK293 cells and neuronal N2a cells. Using quantitative immunofluorescent imaging assays, wild type β 4 expressed in HEK293 or N2a cells alone displayed robust cell-surface expression. However, mutation of a single predicted palmitoylated cysteine to alanine (β 4-mut) resulted in enhanced trapping in the endoplasmic reticulum (ER colocalisation using Pearson's R value was increased from 0.36 to 0.83) associated with a significant decrease (> 50%) in cell surface expression without significant effects on total β 4-subunit expression. To investigate whether palmitoylation of the β 4-subunit controlled trafficking of the α subunit, we co-expressed β 4 with an N-terminal Flag tagged α subunit construct (Flag-ZERO) in both HEK293 and N2a cells, and then monitored α subunit surface expression and ER co-localisation. Cell surface expression of Flag-ZERO was significantly enhanced in both HEK293 (by $50 \pm 5.7\%$) and N2a (by $90 \pm 8.1\%$) cells upon co-expression of Flag-ZERO with β 4. This was associated with a decreased ER co-localization of Flag-ZERO upon β 4 expression (R value shifted from 0.80 to 0.48 in both HEK293 and N2a cells). However, this enhanced cell surface expression of Flag-ZERO promoted by β 4 was dependent upon the palmitoylation of β 4. Co-expression of Flag-ZERO with β 4-mut resulted in no significant enhancement of Flag-ZERO surface expression in either HEK293 or N2a cells.

Therefore, the accessory β 4-subunit is a palmitoylated membrane protein that regulates the trafficking of BK channel to

the cell surface in a palmitoylation dependent mechanism. Dynamic regulation of accessory β 4-subunit palmitoylation may have important functional consequences for BK channel function in the control of neuronal excitability. β 4-subunit palmitoylation may have important functional consequences for BK channel function in the control of neuronal excitability. β 4-subunit palmitoylation may have important functional consequences for BK channel function in the control of neuronal excitability.

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PC289

A maternal high fat diet dysregulates expression of clock related and amino acid transporter genes in the mouse placenta

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In mice, a maternal high fat diet (mHFD) has been shown to predispose the offspring to develop a cluster of cardio-metabolic risk factors (1, 2). The mHFD could potentially induce its long-term effects by impairing placental function and subsequently fetal development. This study investigates the effect of mHFD on the expression of genes, whose encoded proteins play key roles in placental metabolism such as the endogenous molecular clock network, nutrient transporter proteins, and Sirtuins. Female C57/BL6J mice were maintained under controlled conditions and fed either a HF diet (HF; 45% kcal fat) or standard chow diet (C; 21% kcal fat) 4 weeks before conception and during gestation until E16 when pregnant dams were killed by cervical dislocation and placentas dissected, weighed and stored at -80°C . In the HF and C litters ($n=5$ and 4, respectively), RNA was extracted from 4 placentas per litter. Quantitative real-time PCR was performed to determine the transcript levels for the circadian clock genes *Clock*, *Bmal1*, *Cry2* and *Per2*, the apoptosis associated gene *Foxo3A*, the deacetylases *Sirt1* and *Sirt3*, the gene marker for lipid transport lipoprotein lipase (*Lpl*) and the amino acid transporter genes *Snat2* and *Tat1*. Data were normalised using the endogenous control genes *Actb* and *Ywhaz*. In the HF group, there were significant reductions in mRNA levels of *Snat2*, *Tat1*, *Lpl*, *Bmal1*, *Sirt1* and *Sirt3*, compared to C group. In the C group mRNA levels were significantly correlated between all but one gene pair (*Cry2* and *Bmal1*, $P=0.06$), whereas in the HF group the overall correlation between genes was lost and only specific sets of genes remained correlated including *Sirt1*, *Tat1* and *Snat1* ($R > 0.6$, $P < 0.01$ for all pairs). This study demonstrates that a mHFD affects placental mRNA levels for key nutrient transport and regulatory genes. This supports the hypothesis that mHFDs alter placental metabolism to effect fetal development. Metabolic effects of a mHFD on the placenta may be sensed by *Sirt1* which is known to interact with the circadian clock system and may also influence regulation of nutrient transporters.

1. Bruce et al. (2009) *Hepatology* 50:1796-808

2. Elahi et al. (2009) *Br J Nutri* 10:1-6

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PC290

An acute duodenal phosphate load does not downregulate renal sodium phosphate co-transporters

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Regulation of dietary phosphate (Pi) in patients with renal failure is important to prevent secondary hyperparathyroidism and to reduce cardiovascular morbidity and mortality. Expression of sodium phosphate co-transporters (NaPis) at the brush border membrane (BBM) of the small intestine and kidney (NaPi2b, 2a and 2c respectively) is critical to Pi homeostasis. A newly characterized group of regulators, known as phosphatonins, respond to elevated serum Pi levels and down-regulate NaPi expression in the kidney to increase Pi excretion. A phosphatonin-like factor was reported by Berndt et al (2007)[1] to cause rapid Pi excretion (<30 min) following an acute duodenal Pi load, a response that was independent of known regulators of Pi such as PTH, vitamin D, and FGF23. However, this group did not examine potential altered expression of NaPi2a and 2c, the predominant NaPi isoforms in the kidney, and the present work serves to address this. Male Sprague Dawley rats (200-250g) were anaesthetized by injection of pentobarbitone sodium into the intraperitoneal cavity (60mg/kg). The duodenum was cannulated ~ 2cm from the pylorus and 1mL of a solution containing 10mM (simulated post-prandial) or 1.3M Pi (as used by Berndt et al) and matched saline controls was instilled and allowed to flow through the digestive tract in an open loop. After 30 min, the animal was killed via cardiac exsanguination, both kidneys removed, and the cortex isolated and snap frozen for subsequent BBM vesicle preparation[2]. Alkaline phosphatase and protein assays were performed to determine the purity of the BBMV[3, 4]. For western blotting, 20 µg BBMV was combined with 2x Laemmli buffer at a 1:1 ratio, denatured at 90°C for 2 min, run on a 10% SDS-PAGE gel, transferred to a PVDF membrane, blocked with skimmed milk, probed with an antibody against NaPi2a overnight[5] and an HRP-conjugated anti-rabbit secondary antibody. Blots were visualized using a "homemade" enzyme chemiluminescent reagent and results expressed relative to b-actin (n=6 for all conditions). Statistical analysis used a Student's unpaired t-test with P ≤ 0.05 considered significant. No significant difference was seen in the protein expression of NaPi2a at the kidney brush border membrane between animals instilled with 10mM or 1.3M Pi and their respective saline controls (NaPi2a: 10mM Pi vs 154mM saline P=0.56; 1.3M Pi vs. 1.15M saline (osmolality equal to 1.3 Pi solution) P=0.92). Thus, we conclude that the intestinal phosphaturic factor observed by Berndt et al, if present, did not affect the renal expression of NaPi2a - it is possible that the factor might influence the expression of NaPi2c, Pit1 and Pit2, though these transporters handle a much smaller percentage of Pi. A duodenal Pi load may also affect the undefined and uncharacterized phosphate transport mechanisms at the renal basolateral membrane.

Berndt, T. and R. Kumar, Phosphatonins and the regulation of phosphate homeostasis. *Annu Rev Physiol*, 2007. 69: p. 341-59.

Biber, J., et al., Isolation of renal proximal tubular brush-border membranes. *Nat Protoc*, 2007. 2(6): p. 1356-9.

Forstner, G.G., S.M. Sabesin, and K.J. Isselbacher, Rat intestinal microvillus membranes. Purification and biochemical characterization. *Biochem J*, 1968. 106(2): p. 381-90.

Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*, 1976. 72: p. 248-54.

Custer, M., et al., Expression of Na-P(i) cotransport in rat kidney: localization by RT-PCR and immunohistochemistry. *Am J Physiol*, 1994. 266(5 Pt 2): p. F767-74.

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PC291

Functional characterization of CLC-Kb mutations found in patients with Bartter syndrome

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Mutations in the CLCNKB gene encoding the CLC-Kb, kidney chloride channel, are the cause of classic Bartter syndrome (BS) type III. This is an autosomal recessive disease characterized by salt-wasting tubulopathy and associated with secondary hyperaldosteronism, hypokalaemic metabolic alkalosis and normal blood pressure (1). To understand better the implication of CLC-Kb channels in the development of BS is necessary to deepen the knowledge on mutated forms of CLC-Kb.

In this study we investigate the functional consequences of 2 novel (L81P and G246R) and 3 previously reported CLC-Kb homozygous missense mutations (R92W, A204T and R438H) (2, 3, and 4).

By using *X. laevis* oocytes as heterologous expression system we functionally characterize the CLC-Kb mutants. Surface expression and Cl⁻ currents data were collected for each mutant. Results are shown as mean ± SEM. Experiments included at least 8 measurements with 3 different batches of oocytes. Significance was analyzed with one-way ANOVA followed by Holm-Sidak test. P < 0.05 was considered significant. The mutants fell into 2 categories, those carrying no current: G246R (0.8±0.1 µA), R438H (0.6±0.1 µA) and those showing a residual conductance, with reduction by 30%, R92W (2.9 ±0.2 µA) or 60 %: L81P (1.6±0.2 µA) and A204T (1.3±0.3 µA) as compared to CLC-Kb WT (5.4±0.5 µA). Overall, the reduction in surface expression, estimated by chemiluminescence assay (oocytes), corresponded to a decrease in current amplitude for the different mutants.

Functionally we investigated the sensitivities to external H⁺ and Ca²⁺, two characteristic properties of CLC-Kb, in mutants showing residual activity. We observed no difference in half-maximal pH inhibition (pKa) for L81P (7.74±0.67), R92W (7.77±0.13) and R351P (7.78±0.40) compared to WT (7.82±0.51). In contrast, there was a dramatic alteration in inhibition by H⁺ for A204T (6.72±0.01). No change in the slope factor was shown for any mutants respect to the WT. The stimulating effect of external Ca²⁺ was largely blunted.

Altogether, our results show that reduced targeting to the membrane and/or lower stability of the membrane protein is the main functional consequence of these mutations. Additionally, the altered regulation of A204T would tend to alleviate the phenotypic consequences of the impairment of mem-

brane expression by maintaining higher activity of the channel under physiological conditions. From a mechanistic point of view, the dramatic functional effects of this last mutation underscores a major role in CLC-Kb gating for this region which have never been described before.

Unwin, Robert J, and Giovambattista Capasso. "Bartter's and Gitelman's Syndromes: Their Relationship to the Actions of Loop and Thiazide Diuretics." *Current Opinion in Pharmacology* 6, no. 2 (April 2006): 208–213.

Konrad M, Vollmer M, Lemmink HH, van den Heuvel LP, et al. Mutations in the chloride channel gene CLCNKB as a cause of classic Bartter syndrome. *J Am Soc Nephrol* 2000; 11: 1449-1459.

Zelikovic I, Szargel R, Hawash A, Labay V, et al. A novel mutation in the chloride channel gene, CLCNKB, as a cause of Gitelman and Bartter syndromes. *Kidney Int* 2003; 63: 24-32.

Vargas-Poussou R, Dahan K, Kahila D, Venisse A, et al. Spectrum of mutations in Gitelman syndrome. *J Am Soc Nephrol* 2011; 22: 693-703.

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PC292

An investigation of the effects of Caffeic acid against oxidative and endoplasmic reticulum stress in renal proximal tubular cells

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INTRODUCTION: The aminoglycoside antibiotics gentamicin and geneticin (G418) can both cause nephrotoxicity at high concentrations. Specifically, gentamicin causes renal cell death via oxidative stress and endoplasmic reticulum (ER) stress (Quiros *et al.*, 2011) whereas geneticin primarily causes ER stress via modification of intracellular proteins (Jin *et al.*, 2004). Caffeic acid is a polyphenol antioxidant which can be derived from the diet and has recently been shown to reduce gentamicin nephrotoxicity in rats (Aygün *et al.*, 2012).

AIMS: The aims of this study were (i) to compare the toxicity of both gentamicin and geneticin on renal epithelial proximal tubular cells and (ii) to investigate the effects of the hydroxycinnamate caffeic acid on aminoglycoside toxicity. The effects of caffeic acid on these aminoglycosides were compared with its effects on paraquat – a recognised pro-oxidant and inducer of ER stress (Samai *et al.*, 2007, Chinta *et al.*, 2008).

METHODS: Confluent cultures of a rat proximal tubular cell-line (NRK-52E cells) were dosed once daily for 72 hours with sub-lethal concentrations of gentamicin (10 mg/mL) or geneticin (1 mg/mL) in the absence or presence of caffeic acid (0.5 mM). NRK-52E cells were also incubated with paraquat (5 mM) for 24 hours with or without caffeic acid (0.5 mM). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay was used to measure cell viability. Data are presented as mean % cell viability $\pm$ S.D. analysed using one-way ANOVA followed by Bonferroni's post-test.

RESULTS: Gentamicin (GENT) produced a significant reduction in the viability of NRK-52E cells at a concentration of 10 mg/mL (untreated cells: 100.0 $\pm$ 3.8% vs. GENT only: 38.9 $\pm$ 14.3%, $p < 0.05$, $n = 6$). Geneticin (G418) also produced a significant reduction in cell viability at a concentration of 1 mg/mL (untreated cells: 100.0 $\pm$ 3.8% vs. GENT only: 24.8 $\pm$ 5.1%, $p < 0.05$, $n = 6$). Caffeic acid (CA) did not affect gentamicin toxicity (GENT

only: 38.9 $\pm$ 14.3% vs. GENT+CA: 33.5 $\pm$ 17.0%, $p > 0.05$, $n = 6$) however, it was able to reduce geneticin toxicity significantly (G418 only: 24.8 $\pm$ 5.1% vs. G418+CA: 35.8 $\pm$ 7.6%, $p < 0.05$, $n = 6$). Paraquat (PQ) also produced a significant reduction in NRK-52E cell viability at a concentration of 5 mM (untreated cells: 100.0 $\pm$ 3.4% vs. PQ only: 12.6 $\pm$ 6.3%, $p < 0.05$, $n = 6$). Caffeic acid reduced paraquat toxicity significantly (PQ only: 12.6 $\pm$ 6.3% vs. PA+CA: 24.2 $\pm$ 4.4%, $p < 0.05$, $n = 6$). Caffeic acid only (0.5 mM) did not have any effect on the viability of NRK-52E cells (data not shown).

CONCLUSIONS: These results suggest that caffeic acid has a differential effect on aminoglycoside toxicity which requires further study. As it was able to reduce renal cell toxicity produced by geneticin and paraquat significantly, the possible effect of caffeic acid on ER stress pathways within these cells warrants further investigation.

Quiros Y *et al.* (2011). *Toxicol Sci* **119**, 245-256.

Jin QH *et al.* (2004). *Cell Mol Life Sci* **61**, 1816-1825.

Aygün FO *et al.* (2012). *Biol Trace Elem Res* **145**, 211-216.

Samai M *et al.* (2007). *Free Radic Biol Med* **43**, 528-534.

Chinta SJ *et al.* (2008). *Neuromolecular Med* **10**, 333-342.

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PC293

An investigation of the effects of fetuin-A on paraquat-induced oxidative injury in renal proximal tubular epithelial cells

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INTRODUCTION: Fetuin-A or $\alpha 2$ -Heremans-Schmid glycoprotein (AHSG) is a hepatically synthesised negative acute phase plasma protein belonging to the cystatin superfamily of cysteine protease inhibitors. It acts as a potent inhibitor of vascular calcification (Schafer *et al.*, 2003). Sub-physiological levels of serum fetuin-A are found in patients with chronic kidney disease (CKD) and is associated consequent ectopic vascular calcification (Ketteler *et al.*, 2003). Patients with CKD also have a pro-oxidant milieu which contributes significantly to associated morbidity and mortality.

AIM: The aim of this study was to investigate the effects of fetuin-A at a range of physiological and sub-physiological concentrations on oxidant injury caused to renal epithelial cells by the pro-oxidant paraquat, which generates superoxide anions and thereby causes oxidant injury (Samai *et al.*, 2007).

METHODS: Confluent cultures of NRK-52E cells, a rat proximal tubular cell-line, were incubated with increasing concentrations of paraquat (0-5 mM) in Dulbecco's Modified Eagle's Medium (DMEM) for 24 hours. Cultures were also incubated with fetuin-A (0.01, 0.1 and 1.0 mg/mL) for either 24 hours prior to (pre-incubations) or at the same time as paraquat (co-incubations). Cell viability was assessed via spectrophotometric measurement of the mitochondrial-dependent conversion of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) into formazan. Data are presented as mean % cell viability $\pm$ S.D., analysed using one-way ANOVA and Bonferroni's post-testing.

RESULTS: Paraquat (PQ) produced a significant reduction in the viability of NRK-52E cells at a concentration of 3 mM (untreated cells: $100.0 \pm 3.5\%$ vs. PQ only: $15.9 \pm 7.7\%$, $p < 0.05$, $n = 6$). Co-incubation with fetuin-A at a physiological concentration (1 mg/mL) and paraquat (3 mM) for 24 hours produced a significant reduction in paraquat toxicity (PQ only: $15.9 \pm 7.7\%$ vs. PQ+fetuin-A: $33.8 \pm 6.2\%$, $p < 0.05$, $n = 6$). Pre-incubation with fetuin-A (1 mg/mL) for 24 hours also produced a reduction in paraquat toxicity (PQ only: $15.9 \pm 7.7\%$ vs. fetuin-A then PQ: $67.5 \pm 7.1\%$, $p < 0.05$, $n = 6$). Pre-incubation with fetuin-A produced a greater effect than co-incubation. A sub-physiological concentration of fetuin-A (0.1 mg/mL) was also able to protect against paraquat toxicity following pre-incubation (PQ only: $15.9 \pm 7.7\%$ vs. fetuin-A then PQ: $46.44 \pm 7\%$, $p < 0.05$, $n = 6$).

CONCLUSIONS: These results suggest that physiological and sub-physiological concentrations of fetuin-A can provide significant protection against oxidant injury. Our results demonstrate that pre-incubation is significantly more protective than co-incubation. The cellular mechanisms underlying this renoprotection warrant further investigation.

Schafer C *et al.* (2003). *J Clin Invest* **112**, 357-366.

Ketteler M *et al.* (2003). *Lancet* **361**, 827-833.

Samai M *et al.* (2007). *Free Radic Biol Med* **43**, 528-534.

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PC294

A comparison of the effects of minocycline and 5-aminoisoquinolinone on paraquat-induced oxidant injury in renal epithelial cells

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INTRODUCTION: Oxidant injury is implicated in the development of acute kidney injury (AKI). During severe oxidative stress, the generation of reactive oxygen species (ROS) leads to the over-activation of the DNA repair enzyme poly(ADP-ribose) polymerase-1 (PARP-1) resulting in ATP depletion and cell death. The tetracycline antibiotic minocycline has been reported to inhibit PARP-1 activation (Alano *et al.*, 2006) and is able to provide protection against oxidant injury to the kidney (Xia *et al.*, 2011).

AIM: The aim of this study was to investigate and compare the effect of minocycline and 5-aminoisoquinolinone (5-AIQ), the latter being an established PARP-1 inhibitor which has been shown to protect the kidney *in vitro* and *in vivo* (Chatterjee *et al.*, 2004), on oxidant injury caused by paraquat, a potent pro-oxidant which leads to ROS-mediated oxidant injury and renal cell death (Samai *et al.*, 2007).

METHODS: Confluent cultures of NRK-52E cells, a rat proximal tubular cell-line, were incubated with increasing concentrations of paraquat (0-5 mM) in Dulbecco's Modified Eagle's Medium for 24 hours. Cultures were also incubated with paraquat in the presence of high and low concentrations of minocycline (10 μ M and 100 nM) and 5-AIQ (100 μ M) for 24 hours. Cell viability was then assessed via spectrophotometric measurement of the mitochondrial-dependent conversion

of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) into formazan. Data are presented as mean % cell viability $\pm$ S.D. analysed using one-way ANOVA followed by Bonferroni's post-testing.

RESULTS: Paraquat (PQ) produced a significant reduction in the viability of NRK-52E cells at a concentration of 3 mM (untreated cells: $100.0 \pm 1.7\%$ vs. PQ only: $38.0 \pm 7.4\%$, $p < 0.05$, $n = 12$). Minocycline (MC) produced a significant reduction in paraquat toxicity both at a high concentration of 10 μ M (PQ only: $38.0 \pm 7.4\%$ vs. PQ+MC: $49.0 \pm 6.0\%$, $p < 0.05$, $n = 12$) and at a lower concentration of 100 nM (PQ only: $38.0 \pm 7.4\%$ vs. PQ+MC: $50.2 \pm 11.5\%$, $p < 0.05$, $n = 8-12$). 5-AIQ was also able to produce a significant reduction of paraquat toxicity at a concentration of 100 μ M (PQ only: $38.0 \pm 7.4\%$ vs. PQ+5-AIQ: $73.9 \pm 8.9\%$, $p < 0.05$, $n = 12$). Minocycline or 5-AIQ alone did not have any effect on NRK-52E cell viability at the concentrations tested against paraquat (data not shown).

CONCLUSIONS: These results suggest that minocycline is able to reduce paraquat toxicity significantly at nM concentrations. The PARP-1 inhibitor 5-AIQ was also able to protect against paraquat toxicity but at a much higher (μ M) concentration. Minocycline may also be able to provide protection via its ability to inhibit endoplasmic reticulum stress which has recently been proposed as a mechanism of paraquat-induced cell death (Huang *et al.*, 2012). This potential mechanism of protection warrants further investigation in renal cells.

Alano CC *et al.* (2006). *Proc Natl Acad Sci* **103**, 9685-9690.

Xia D *et al.* (2011). *Clin Invest Med* **34**, E55-E63.

Chatterjee PK *et al.* (2004). *Kidney Int* **65**, 499-509.

Samai M *et al.* (2007). *Free Radic Biol Med* **43**, 528-534.

Huang CL *et al.* (2012). *Toxicol Lett* **209**, 203-210.

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PC295

Acute kinematic and neuromuscular responses to multiple-joint accentuated eccentric load resistance exercise

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Acute upper body resistance exercise whereby the eccentric muscle action is overloaded (AEL) has demonstrated enhanced kinematic variables in the subsequent concentric phase compared to equivalent constant load (CL) practices (1,2). Repeated application of the AEL model is believed to promote superior chronic strength adaptations (1). Whether such enhancements of kinematic and physiological variables occur in the lower body with AEL protocols remains unclear (3,4). Therefore, the aim of this study was to investigate differences in concentric kinematic and neuromuscular variables in acute lower limb AEL and CL models. Multiple-joint resistance exercise was employed in the current study given the greater neuromuscular and coordination demands believed to lead to better transference of chronic strength gains to competition and mobility in athletic and clinical populations, respectively. Strength trained males ($n = 10$) completed experimental test day sessions (AEL and CL) in a randomised, cross over study after baseline strength (3RM) and familiarisation sessions. Test day sessions required 4 sets of 3 back squat repetitions to be

completed with either an AEL (105% of 3RM) and a lighter subsequent concentric phase (65%, 75%, 85% and 95% of 3RM) or CL where both phases were the same absolute load (65%, 75%, 85% and 95% of 3RM). Concentric phase kinematic variables and lower limb surface EMG were assessed. EMG was normalised to a submaximal back squat warm-up set as previously described (5). Repeated measures ANOVAs were conducted to assess differences between conditions ($P < 0.05$). No statistically significant differences in concentric kinematic variables were detected (peak power [$P = 0.273$, CL: 2700.7 ± 155.1 W; AEL: 2881.5 ± 321.5 W], peak force [$P = 0.974$, CL: 2606.8 ± 286.6 N; AEL: 2589.5 ± 275.6 N] or peak velocity [$P = 0.588$, CL: 1.2 ± 0.2 m.s⁻¹; AEL: 1.2 ± 0.2 m.s⁻¹], mean $\pm$ SD across the four sets for each condition). Vastus lateralis ($P = 0.560$, CL: $133.1 \pm 7.5\%$ of normalised EMG [%]; AEL: $127.9 \pm 1.4\%$), Bicep femoris ($P = 0.126$, CL: $142.3 \pm 25.0\%$; AEL: $157.2 \pm 24.2\%$) and Vastus medialis ($P = 0.887$, CL: $140.1 \pm 9.0\%$; AEL: $134.9 \pm 5.5\%$) EMG was not significantly different between conditions. Gluteus maximus ($P = 0.090$, CL: $136.8 \pm 16.4\%$; AEL: $143.0 \pm 12.0\%$) demonstrated tendencies towards greater activation during the concentric phase for the AEL condition. In conclusion, an acute AEL protocol did not enhance concentric phase kinematic variables above those in the CL condition despite a tendency for increased Gluteus maximus neuromuscular activation. This suggests that there would be no advantages over CL resistance exercise in achieving chronic strength adaptations. Future research employing well-controlled training programme interventions adopting both a battery of kinematic and physiological measures are required to substantiate this thesis.

1. Doan BK, Newton RU, Marsit JL, Triplett-McBride NT, Koziris LP et al (2002) Effects of increased eccentric loading on bench press 1RM. JSCR 16: 9-13.

2. Sheppard JM, Young K (2010) Using additional eccentric loads to increase concentric performance in the bench throw. JSCR 24: 2853-2856.

3. Moore CA, Weiss LW, Schilling BK, Fry AC, Li Y (2007) Acute effects of augmented eccentric loading on jump squat performance. JSCR 21: 372-377.

4. Sheppard JM, Young K (2010) Using additional eccentric loads to increase concentric performance in the bench throw. JSCR 24: 2853-2856.

5. Balshaw TG, Hunter AM (2012) Evaluation of electromyography normalisation methods for the back squat. J Electromyogr Kinesiol 22: 308-319.

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PC296

Measuring in vivo body fluid compartments by bioimpedance – a validation study

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Body water and body fluid compartments evaluation is most important in human physiology and pathophysiology. Several sophisticated methods, from hydrostatic weighting to air displacement plethysmography, have been developed, although not so easy to apply.

Bioimpedance based technologies have been extensively developed and are currently used from diagnostic (e.g. ECG) to various therapeutical procedures (e.g. iontophoresis). Recently, a bioimpedance system was proposed to provide quantitative

information, non invasively, about Total Body Water (TBW) Extracellular Water (ECW) and Intracellular Water (ICW) (among other variables). As known, these methods are very practical and easy to use, being based in the electrical properties of human tissues through the skin. By using different frequency ranges, appropriate correlations with biological variables are explored by specific mathematical algorithms. According with the manufacturer, TBW is calculated at 200kHz, ECW at 5kHz and ICW is obtained from these two measurements. However, a clear demonstration of those relationships is not available. So, the authors tested, in the present paper, validity of bioimpedance data gathered from human volunteers, and relationship with reference plasma electrolytes known to determine or influence body fluid distribution.

32 healthy volunteers, both gender (6 male and 26 female) aged 21 to 82 (mean $36,16 \pm 17,46$ years old) giving previous informed written consent, were selected during routine lab check up. All procedures fully respected Helsinki principles and respective amendments. This varied sample regarding age, diet habits and physical condition, is essential to validate the method. After inclusion, a venous blood sample was collected in order to determine Na⁺, K⁺ and Cl⁻ concentrations (selective ions potenciometry). Body fluid compartments were than obtained by the Bodystat QuadScan 4000 (Bodystat UK) measurements involving the application of 2 electrodes according with the manufacturer specification. Results were compared by Pearson's correlation test and by the qui-square test, and a 95% level of confidence adopted.

Results confirmed the sample's heterogeneous profile. TBW was highly correlated with weight and with the body mass index, and higher in males. Plasma ion concentration was within regular ranges in all volunteers. K⁺ correlated well with ICW while Na⁺ and Cl⁻ correlated well with ECW. Nevertheless correlation between Na⁺ and ECW is reportedly more consistent than the correlation between Cl⁻ and ECW.

In conclusion this work demonstrated this bioimpedance method is valid to measure the body water compartments, confirming that observed specificity, linearity, measurement range, and precision fully are consistent with the device application purposes.

Segal KR, Burastero S, Chun A, Coronel P, Pierson RN Jr, Wang J. Estimation of extracellular and total body water by multiple-frequency bioelectrical-impedance measurement. Am J Clin Nutr. 1991;54:26-9

Amstrong LE, Asssing hydration status - the elusive Gold Standard, J Am Coll Nutr., 2007; 26 (5) : 575-84

Bodystat. Bodystat® QuadScan 4000 Hardware User's Guide. Isle of Man:2007.

Hannan WJ, Cowen SJ, Plester CE, Fearon K, deBeau A., Comparison of bioimpedance spectroscopy and multifrequency bioimpedance analysis for the assessment of extracellular and total body water in surgical patients. Clin Sc, 1995: 89: 851-8

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PC297

The central pressor response and the perception of force during human standing

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With the forces the leg muscles apply when we stand, it is surprising how easy it feels.

We had 10 subjects aged 19-55 stand and consider the force applied to the ground. A body brace was then quickly positioned to hold them in the same standing posture so that the muscles were relaxed. They then made a plantarflexion contraction to reproduce the force perceived during standing. This was a 71.8% CI [64.4, 79.2] underestimate of exerted active torque when standing ($P < 0.001$ ANOVA). When the experiment was performed with subjects balancing an inverted pendulum matched to their own bodies, they overestimated 5.3% [-4.4, 15.0] the force exerted ($P = 0.75$).

We interpret this as indicating that cortical perceptual centres cannot access the signal of force exerted during standing, whether central or reafferent [1], because the cortical system does not issue the motor command. This implicates a sub-cortical system in balance drive to the muscles. Reciprocal changes in corticomuscular coherence and galvanic-evoked vestibular reflexes corroborated this.

When a muscle is contracted voluntarily through cortical drive, a pressor signal generated centrally is sent to medullary cardiovascular centres to raise blood pressure (BP). We therefore asked whether this response is also generated by the sub-cortical balance drive.

Subjects were supported upright in the posture of standing with the muscles relaxed for 5 minutes. BP remained stable. At that time, the brace was removed so that subjects stood by contracting the leg muscles (18.2% [17.8, 18.6] of measured maximal contraction force). BP did not change. Thus, at this workload, the muscle did not generate a significant metaboreflex response. When repeated by producing a matched contraction voluntarily to balance the inverted pendulum, BP increased by 6.2 mmHg [6.1, 6.3] or 7.2% ($P < 0.001$ by ANOVA) after 3 minutes.

In 8 subjects, we stimulated over the motor point of tibialis anterior with supramaximal tetanic trains (5 @ 40ms) repeated at 1Hz; a workload similar to the calf muscles during standing). When force output was stable, muscle perfusion pressure was changed by raising or lowering the leg. This produced a rapid decline of 4.8% [3.6 6.0] in force output for the 6.2mmHg loss of pressor response, equivalent to that reported for a hand muscle [2]. In the hand muscle, loss of this augmented contractility through the pressor response meant that muscle fatigued twice as fast [3].

Thus, without this pressor response, the leg muscles during standing rely crucially on the hydrostatic head of pressure provided by orthostatic posture. Put another way, you can only stand because you are standing.

Luu, BL. et al. The fusimotor and reafferent origin of the sense of force and weight. *JPhysiol* 2011 589 3135-47.

Wright JR et al. Effects of arterial perfusion pressure on force production in working human hand muscles. *JPhysiol* 1996 495 885-91.

Wright JR et al. Effects of systemic arterial blood pressure on the contractile force of a human hand muscle. *JApplPhysiol* 2000 88 1390-6.

National Health and Medical Research Council of Australia

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PC298

Altering oxygen tension during exercise does not affect flow mediated dilation in healthy young males

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Hypoxia increases systemic vasodilation during exercise whereas breathing hyperoxia is known to increase systemic vascular resistance. Little is known about the effect of altered oxygen tensions on endothelial function, particularly following an acute bout of exercise. The purpose of this study was to investigate the effects of constant load cycling and different levels of inspired oxygen (F_{iO_2}) on endothelial function in healthy, recreationally active men. It was hypothesized that exercising while breathing normoxic or hypoxic gas would improve FMD, whereas hyperoxic gas would not alter FMD. Thirteen healthy, recreationally active males (22 ± 3 ; mean $\pm$ SD yrs) volunteered to participate. Subjects randomly completed three graded exercise tests breathing either 16% O_2 (HYPO), 21% O_2 (AIR) or 100% O_2 (HYPER) to determine gas-specific maximal workload (W_{max}) on separate days. Subjects then returned on three separate days and performed, 40-min, constant-load cycling trials at 50% of their gas specific W_{max} . Baseline flow-mediated dilation (FMD), performed using standardized techniques, was measured at rest, after 30 min of gas exposure and 30 min following each exercise trial. Subjects remained on the test gas for the entire protocol. No differences in absolute FMD were observed for AIR (0.1 ± 2.2 mm), HYPO (-0.7 ± 1.2 mm) and HYPER (0.3 ± 1.3 mm), relative FMD (1.2 ± 4.5 ; -1.0 ± 4.6 ; $1.1 \pm 3.3\%$, respectively), baseline diameter (-0.4 ± 4.0 ; -0.2 ± 2.3 ; 0.1 ± 1.2 mm, respectively), shear rate (19 ± 133 ; -34 ± 138 ; 26 ± 120 $L \cdot s^{-1}$, respectively) or time-to-peak dilation (16.9 ± 31.2 ; -0.52 ± 51 ; 7.6 ± 24.7 sec, respectively) were observed at rest or after exercise with any gas. These novel data show that in recreationally active males, neither F_{iO_2} nor exercise have a significant effect on endothelial function at rest or following an acute bout of exercise. Vascular remodeling and alterations in antioxidant defense associated with training status in these individuals could account for the lack of change observed with FMD after exercise.

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Is haemostasis in humans subject to redox regulation?

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Introduction: Patients with occlusive arterial disease undergo hypoxia (hyp) in affected limbs & exercise training is used to improve perfusion in the ischaemic tissues.

Hyp & exercise increase oxidative stress (Bailey et al., 2009). Oxidative stress has been implicated as a modulator of haemostasis (Görlach, 2005), & as a catalyst for thrombin generation (Wang et al., 2009).

We designed a proof-of-concept study in healthy individuals to assess the impact of high-dose antioxidant prophylaxis on the haemostatic system.

We hypothesised that since oxidative stress increases global thrombogenicity, the intervention would attenuate changes previously shown by our laboratory (Fall et al., 2011).

Methods: Following pre-intervention blood sampling, 40 male subjects were randomly assigned to either a placebo or intervention group. The intervention group were administered 1000mg per day of vitamin C & 900IU per day of vitamin E, & the placebo group were given tablets of identical appearance but no nutritional value for 8 weeks & returned to the laboratory after an overnight fast. Bloods were taken after 10min supine rest in normoxia, after 6 hours of passive exposure to hyp (12% inspired oxygen) in an environmental chamber & after a cycling challenge to volitional exhaustion in hyp. Bloods were batch-analysed for plasma levels of thrombin-antithrombin complex (T-AT), prothrombin fragments 1&2 (PF1+2), activated partial thromboplastin time (aPTT), prothrombin time (PT), thrombin time (TT), fibrinogen (FB) & d-dimer (DD).

Results: Eight weeks intervention significantly increased levels of T-AT and PF1+2 compared to controls ($P < 0.05$) but this difference was abrogated after hyp & remained so after exercise. There were no differences between groups in aPTT, PT, TT, FB or DD in any time point.

There were group effects in several markers; aPTT shortened with exercise, as did PT. TT elongated with hyp & remained so post-exercise; there was a reduction in FB with hyp which returned to baseline following exercise. D-dimer remained unchanged.

Discussion & conclusions: Increases in T-AT & PF1+2 with intervention suggest an increase in thrombin generation & factor Xa activity (Mannucci, 1994) in the active group. This does not translate into the more routine analysis that would be performed in the patient population. The group data suggest that hyp alone does not affect haemostasis; contrary to existing research (Wang et al., 2009). The addition of exercise hastens both the intrinsic and extrinsic pathways of coagulation in line with previous research (Fall et al., 2011), but this is not translated into down-stream markers, most likely due to activation of antithrombin III.

The T-AT & PF1+2 data suggest that free radicals in fact limit thrombin generation at rest. Hyp reverses this, normalising thrombin generation. We are currently focusing on understanding whether the haemostatic:fibrinolytic balance is disturbed.

Bailey DM, Evans KA, James PE, McEneny J, Young IS, Fall L, Gutowski M, Kewley E, McCord JM, Möller K & Ainslie PN. (2009). Altered free radical metabolism in acute mountain sickness: implications for dynamic cerebral autoregulation and blood-brain barrier function. *The Journal of Physiology* 587, 73-85.

Fall L, Evans KA, Lewis MH & Bailey DM. (2011). Haemostatic response to hypoxaemic/exercise stress: the dilemma of plasma volume correction. *Journal of Clinical Pathology* 64, 269-271.

Görlach A. (2005). Redox Regulation of the Coagulation Cascade. *Antioxidants & Redox Signaling* 7, 1398-1404.

Mannucci PM. (1994). Mechanisms, markers and management of coagulation activation. *Br Med Bull* 50, 851-870.

Wang JS, Cheng ML, Yen HC, Lou BS & Liu HC. (2009). Vitamin E suppresses enhancement of factor VIII-dependent thrombin generation by systemic hypoxia. *Stroke* 40, 656-659.

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PC300

Disassociation between exhaled NO and venous NO₂⁻; implications for quantification

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Nitric oxide (NO) is critical in the regulation of vascular function. Vascular NO quantification is complex due to its short half-life and high reactivity. Exhaled NO has been being extensively as a surrogate measure of vascular NO bioavailability due to its ease of measurement. We have previously shown a lack of relationship between changes in exhaled and venous nitrite (NO₂⁻) before and after an acute exercise challenge in normoxia (Evans & Bailey, 2011). We therefore wanted to extend this work by examining the relationship between exhaled NO and venous NO₂⁻ after a passive hypoxic exposure, since hypoxia can stimulate the generation of vascular NO. Exhaled NO and venous NO₂⁻ was quantified in sixteen apparently healthy males in normoxia and following a 6hr passive exposure hypoxia to (12% O₂). Venous blood was collected from an indwelling catheter placed in an antecubital vein and was immediately centrifuged (2500rpm; 4°C). Plasma was flash frozen and stored at -80°C before later analysis. Both exhaled NO and plasma NO₂⁻ concentrations were measured using established ozone-chemiluminescence techniques (Sievers NOA 280i).

Passive hypoxia elicited no change in either exhaled NO (Norm: 10 ± 7 vs. Hyp: 10 ± 8 ppb $P > 0.05$) or plasma NO₂⁻ (Norm: 201 ± 87 vs. Hyp: 209 ± 105 nmol; $P > 0.05$) (Paired sample T-test). No relationship was observed between delta changes (hypoxia minus normoxia) in exhaled NO and plasma NO₂⁻ ($r = 0.167$, $P > 0.05$) (Pearson's correlation).

Current data suggests there is no relationship between changes in exhaled NO and NO₂⁻. This could be due to the fact that there were not any observable differences between absolute exhaled NO and NO₂⁻ concentrations. The use of exhaled NO should still therefore be used with caution when evaluating NO bioavailability and its physiological action. Exhaled NO could still over or under estimate vascular NO bioavailability. K.A. Evans, D. M. Bailey, *Proc Physiol Soc* 23. (2011)

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PC301

Effect of dual tasking on anticipatory postural responses to rapid lower limb movement while seated on an exercise ball

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A key component of motor rehabilitation is the improvement of anticipatory postural adjustments (APAs) using tools such as the exercise ball. Concurrent cognitive tasks are often added during this rehabilitation, but their impact on APAs is unknown. This study examined the effect of concurrent cognitive tasks

on anticipatory postural adjustments to voluntary movements performed while sitting on an exercise ball.

Bilateral EMG activity was recorded from the external oblique, internal oblique, rectus abdominis and erector spinae muscles, as well as the right rectus femoris muscle of 20 healthy subjects (mean (SD) age of 22 (0.8) years), during rapid hip flexion movement performed in response to an auditory cue under 3 conditions: no concurrent task (QF), counting out loud up from one serially (S1s) and counting down in sevens; serial sevens task (S7s). The onset of the rise and magnitude of EMG activity in the prime mover and postural trunk muscles were measured and compared across the three conditions.

The cognitive task conditions (S1s and S7s) showed a delayed response in the prime mover (40ms) after the cue to move and a reduced EMG activity ($p=0.019$) compared to condition with no concurrent activity, which occurred 20ms after the cue. Only the ES muscle, on the opposite side to prime mover, showed a tendency for earlier onset and higher EMG activity in the S7s task than in the S1s in the APA time window. The EO muscle on the opposite side to RF was less active in the S7s than either the S1s or the no concurrent conditions in the CPA time windows. The ES and EO muscles on the side of RF were more active during S7s than the QF condition during the CPA time windows. The RA and IO muscles showed no differences between the conditions in any APA or CPA window. The result of this study indicates that the effect of dual tasking is related to the attention demands of the cognitive task. A more demanding cognitive task results to a delayed and reduced magnitude of voluntary motor response and earlier anticipatory postural adjustments. It may be possible that dual tasking facilitates earlier postural adjustments in patients with deficient postural control, but this would need to be tested in older individuals and patients with deficient postural control.

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PC302

The role of central catecholamines in the development of fatigue during prolonged exercise in women

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Central catecholamines influence attention, arousal, motivation and motor control. Bupropion, a dual noradrenaline/dopamine reuptake inhibitor, improves time trial performance in male subjects in the heat (Watson et al. 2005). Gender differences in neuropsychopharmacology have been shown in animal and human studies (Young & Becker 2009), but it is not known whether these differences may alter the effect of Bupropion on exercise performance in women. With local ethics committee approval, 9 habitually physically active women (Mean $\pm$ SD age 21 ± 2 y; height 1.68 ± 0.08 m; body mass 64.1 ± 6.0 kg; VO_{2max} 50.9 ± 7.2 ml/kg/min) were recruited to examine the effect of pre-exercise administration of Bupropion (4x150 mg) on prolonged exercise performance in a warm environment ($30.2 \pm 0.2^{\circ}C$, $50\% \pm 1\%$ rh). Subjects completed a VO_{2max} test, a familiarisation trial, and a single-blinded placebo control trial before a randomised, double-blind, placebo-controlled crossover design was employed.

Experimental trials took place during the first 10 days of the follicular phase of the menstrual cycle. Subjects cycled for 1 h at 60% VO_{2max} followed by a 30 min workload challenge, during which they completed as much work as possible. Heart rate, skin and core temperature, and ratings of perceived exertion and thermal comfort were recorded throughout exercise. Data were analysed with repeated measures ANOVA. Pairwise comparisons were made with LSD where appropriate and Bonferroni's test for multiple comparisons. Total work done was higher on the Bupropion trial (291 ± 48 kJ) than on the single-blind (267 ± 48 kJ, $P=0.021$) and double-blind trials (269 ± 46 kJ, $P=0.042$). No differences were found between all trials for core temperature throughout rest, the first hour or the workload challenge. However, at the end of the workload challenge core temperature was higher on the Bupropion trial ($39.5 \pm 0.4^{\circ}C$) than the single-blind ($39.2 \pm 0.6^{\circ}C$, $P=0.028$) and double-blind trials ($39.2 \pm 0.6^{\circ}C$, $P=0.021$). Heart rate was also higher at the end of the workload challenge on the Bupropion trial (185 ± 9 bpm) than the single-blind (180 ± 13 bpm, $P=0.048$) and double-blind trials (179 ± 13 bpm, $P=0.043$). The results indicate that during the follicular phase of the menstrual cycle an acute dosing protocol of Bupropion can improve self-regulated work rate in warm conditions.

Watson, P. et al., 2005. Acute dopamine/noradrenaline reuptake inhibition enhances human exercise performance in warm, but not temperate conditions. *The Journal of Physiology*, 565(Pt 3), pp.873-83.

Young, E.A. & Becker, J.B., 2009. Perspective: sex matters: gonadal steroids and the brain. *Neuropsychopharmacology*, 34(3), pp.537-8.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC303

The effect of acute normobaric hypoxia and exercise on cognitive performance

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The effects of chronic exposure to high altitudes on physiology have been extensively investigated and are well understood (1). However, less research has focussed on the affect of altitude on psychological and cognitive performance and studies that have report conflicting findings (2, 3). The purpose of this study was to determine the effect of acute normobaric hypoxia in isolation and combined with light sub-maximal exercise on cognitive performance. Twelve volunteers (8 women; age 20 ± 1 years; height 175 ± 10 cm; body mass 69 ± 10 kg) completed the study. Following familiarisation and practice sessions participants completed experimental sessions in normoxia (ambient temperature $19^{\circ}C$; relative humidity 50%) and hypoxia (5500 m equivalent altitude; O_2 10.5%; CO_2 0.04%; N_2 balance; ambient temperature $19^{\circ}C$; relative humidity 50%). Sessions were completed following a crossover design and separate by 10 days. Each session comprised a 20 min stepping exercise divided into four 5 min bouts (4 min stepping at 50 steps/min, 1 min seated rest) preceded and followed by a Stroop test. Arterial oxygen saturation (SpO_2), heart rate and ratings of perceived exertion (RPE) were recorded before and after each exercise session. Data were analysed with paired t-tests or a Wilcoxon signed-rank tests, as appropriate, $p < 0.05$ was regarded as statistically significant. There

were no differences between conditions for heart rate or RPE pre-exercise, but following the last bout both were greater in hypoxia (normoxia vs. hypoxia: HR: 95 vs. 126 beats/min; RPE: 8 vs. 13; $p < 0.01$). In hypoxia SpO_2 was lower both pre- and post-exercise (97 vs. 74%, $p < 0.01$). There was no effect of exercise or hypoxia on Stroop test errors. There were marginal changes in RT after exercise with normoxia decreasing by 3% and hypoxia increasing by 7%, but neither significantly. Results suggest that complex reaction time is not affected by an acute exposure to hypoxia in isolation or when combined with light exercise.

Milledge JS, West JB, Schoene RB. High Altitude Medicine and Physiology (4th edition), 4 edition. 2007 London, Hodder Arnold.

Cudaback, DD. Four-Km altitude effects of performance and health. Publications of the Astronomical Society of the Pacific. 1984;96:463-77.

Bahrke MS, Shukitt-Hale B. Effects of altitude on mood, behaviour and cognitive functioning. A review. Sports Medicine, 1993;16:97-125.

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PC304

Heart rate variability in trained and untrained subjects after two changes in body position

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Heart rate variability (HRV) is a noninvasive tool to study cardiac autonomic regulation. In recent years there is growing evidence that training affects HRV indices (1). The active orthostatic task has been used to evaluate HRV in trained and untrained subjects, but the results are contradictory. Studies using second change of body position (lying down after been in upright posture) are scarce. Aim of this investigation was to assess the differences in HRV between endurance trained athletes and sedentary controls at rest and during active orthoclinostatic test.

Sixteen endurance trained athletes (age=22.0±2.42 years) and sixteen (age=21.5±1.03 years) untrained controls participated in the study. In the supine position after 20 min of rest RR intervals of each subject were recorded for 5 min with Polar S810i heart rate monitor. After that subjects actively stood up and remained in upright position for 8 min. The subjects then returned in the supine position for 8 min (orthoclinostatic test). RR intervals were collected for 5 min from 3th to 8th minutes during upright position and second supine position. The power spectrum indices included: LF (low frequency, 0.04 Hz-0.15 Hz) and HF (high frequency, 0.15-0.40 Hz), both expressed in absolute (ms^2) and in normalized units (nu). LF/HF ratio was calculated. Mean HR (bpm) and Mean RR (ms) were also determined.

VO_{2max} in athletes was significantly higher in athletes than in untrained. During supine rest Mean HR was lower ($p < 0.001$) in athletes than in untrained (58.3±6.29 vs. 68.78±8.66 bpm). Mean RR was higher ($p < 0.001$) in athletes (1046.47±113.42 vs. 890.17±116.98 ms). HF ms^2 was higher ($p < 0.05$) in athletes than in untrained (2063.22±1598.77 vs. 1102.60±849.04). HF nu was higher ($p < 0.05$) in athletes than in untrained (57.86±16.75 vs. 43.45±6.77). LF nu was lower ($p < 0.05$) in athletes than in untrained (42.13±16.75 vs. 56.55±6.77). LF/HF ratio was lower ($p < 0.05$) in athletes than in untrained

(0.88±0.57 vs. 1.37±0.47). There was no difference between the two groups in regard to spectral HRV indices after the two changes in body position.

The differences in HRV indices between the two groups at rest (first supine position) suggest more pronounced parasympathetic influence on cardiac regulation in athletes in comparison with untrained. There is tendency showing less marked sympathetic cardiac influences in athletes. Active standing eliminates the differences in HRV between trained and untrained subjects. The lack of differences between the groups remains after assuming second supine position which may be caused by the saturation of HRV (2). These results suggest that the more complex autonomic heart regulation elicited by two changes in body position suppress the differences in HRV related to endurance training. This effect during orthoclinostatic test requires further study.

Aubert AE et al. (2003). Sports medicine, 33, 889-919

Goldberger JJ et al. (1996). J Cardiovasc Electrophysiol, 7, 594-602.

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PC305

Improvement in neurocognitive function: time to re-assess evaluation techniques?

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Background and aims: Neurocognitive function assessment (NFA) consists of a battery of psychometric tests designed to challenge memory, mental agility and co-ordination and is traditionally used to assess the effect of a vascular surgical intervention (e.g day before surgery and 24 hours, 5-7 days and/or 6 weeks post-operatively) such as coronary artery graft by-pass (1) and carotid endarterectomy (2). However, changes typically recorded post-operatively may be misinterpreted as a consequence of a 24% improvement in score, which was identified when NFA was repeated over 4 days (3). As a result, post-operative NFA scores, may in fact underestimate the degree of neurocognitive decline or alternatively, overestimate neurocognitive improvement observed post-operatively. In the present study we aimed to identify if i) the learning process continues over 8 days and ii) a learning effect exists despite a 6 week gap between tests.

Methods: 30 healthy volunteers performed 4 consecutive days of testing, while 10 were tested for a further 4 days. A separate cohort of 15 subjects was tested on 2 separate occasions, 6 weeks apart. All participants completed a battery of psychometric tests, randomly administered and performed at a similar time of day. The tests included the core tests according to the recommendations of the statement of consensus on the assessment of neurobehavioral outcomes after cardiac surgery (4). The battery of tests were further grouped into 3 specific domains; i) *Memory* ii) *Mental Agility* iii) *Visual-motor Co-ordination*.

Results: Detailed results are provided in Table 1. We confirmed our previous findings (3) showing an improvement over 4 days ($P < 0.05$). Thereafter, NFA score began to plateau. In addition, significant improvements in all memory and mental agility tests ($P < 0.05$) were recorded in those who repeated the tests 6 weeks apart.

Conclusion: These findings are the first to identify that NFA scores stabilise after 4 consecutive days of testing. Furthermore, the learning effect is apparent despite a 6 week break between tests. Further research is required with a greater sample size to confirm these preliminary observations.

Table 1: Changes in neurocognitive function performance over 8 days and when repeated twice 6 weeks apart

	Test	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Baseline - 6 Weeks
Memory	Rey Auditory Verbal Learning Test (score)	49 ± 61 ± 65 ± 68 ± 72 ± 73 ± 75 ± 55 ± 8 - 61								
	Repetition Digits Forwards (score)	8 ± 10 ^a	8 ^a	7 ^a	5	5	5	4	4	8 ^a
	Repetition Digits Backwards (score)	5 ± 2.8 ± 2.8 ± 2.9 ± 2.9 ± 3	10 ± 5	10 ± 5	10 ± 5	10 ± 5	10 ± 5	10 ± 5	10 ± 5	8 ± 2 - 9 ± 2 ^a
Mental Agility	Trail Making Test A (seconds)	28 ± 22 ± 22 ± 20 ± 20 ± 19 ± 17 ± 17 ± 29 ± 8 - 22								
	Trail Making Test B (seconds)	58 ± 49 ± 39 ± 42 ± 34 ± 35 ± 30 ± 30 ± 57 ± 17 - 48								
	Digit Symbol Substitution Test (score)	62 ± 70 ± 74 ± 78 ± 82 ± 78 ± 82 ± 83 ± 82 ± 5 - 68								
Visual-motor Co-ordination	Grooved Pegboard Dominant Hand (seconds)	62 ± 58 ± 57 ± 55 ± 53 ± 56 ± 54 ± 54 ± 61 ± 5 - 57								
	Grooved Pegboard Non-Dominant Hand (seconds)	70 ± 67 ± 64 ± 62 ± 58 ± 61 ± 62 ± 58 ± 65 ± 6 - 63								

mean ± SD; * = $P < 0.05$ vs. previous day

Newman *et al.* (2001) *NEJM*; **344**, 395-402

Mazul-Sunko *et al.* (2010) *J Neurosurg Anesth*; **22**, 195-201

Marley *et al.* (2011) *Proc Physiol Soc*

Murkin *et al.* (1995) *Ann Thorac Surg*; **59**, 1289-95

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PC306

Low-dose aspirin does not affect carotid-cardiac or carotid-vasomotor baroreflex sensitivity during muscle metaboreflex activation in humans

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Human cardiovascular control during exercise is regulated by the arterial baroreflex and muscle afferent feedback (Raven *et al.*, 2006). Muscle metaboreflex activation does not affect carotid-cardiac (CBR-HR) or carotid-vasomotor (CBR-MAP) baroreflex maximal gain in humans (Drew *et al.*, 2008). Low-dose aspirin inhibits production of the metabolite thromboxane (TX) A_2 . However, it is unknown if inhibiting TX A_2 affects CBR-HR and CBR-MAP maximal gain during muscle metaboreflex activation. Therefore, we examined the effect of inhibiting TX A_2 production via low-dose aspirin on CBR-HR and CBR-MAP maximal gain during muscle metaboreflex activation in humans. 9 subjects performed two trials twice, each after 7-days of placebo or low-dose aspirin. A 5min baseline (rest) was followed by either 1.5min of continued rest (0% maximal voluntary contraction (MVC)) or isometric right calf exercise at 70% MVC, then 3.5min of circulatory occlusion (post-exercise circulatory occlusion (PECO) if exercise was performed). Paired-samples *t*-test and repeated measures ANOVA were used for statistical analysis. Aspirin reduced baseline TX B_2 by ~90% ($p < 0.05$). Maximal gain during rest was similar in all trials for CBR-HR and CBR-MAP ($p > 0.05$) (Table 1). Both

CBR-HR and CBR-MAP maximal gain during circulatory occlusion were similar in all trials, including during PECO ($p > 0.05$) (Table 1). These results suggest that TX A_2 inhibition via low-dose aspirin does not affect CBR-HR or CBR-MAP maximal gain during muscle metaboreflex activation in humans. This contrasts with previous preliminary findings showing that low-dose aspirin increases CBR-HR maximal gain during concurrent muscle mechanoreflex and metaboreflex activation in humans, without affecting CBR-MAP maximal gain (Drew *et al.*, 2012). When viewed together, this may reveal a specific link between TX and metabolite-sensitised muscle mechanoreceptors that influences cardiac vagal activity. Supported by P01 HL096570 (LIS); UL1 RR033184 (LIS).

Table 1 – Maximal gain for carotid-cardiac (CBR-HR) and carotid-vasomotor (CBR-MAP) baroreflex control during rest and circulatory occlusion after placebo or low-dose aspirin.

Treatment	Trial (%MVC)	CBR-HR (beats $^{-1}$ mmHg $^{-1}$)		CBR-MAP (mmHg mmHg $^{-1}$)	
		Rest	Circulatory occlusion	Rest	Circulatory occlusion
Placebo	0%	-0.41 ± 0.06	-0.33 ± 0.08	-0.17 ± 0.02	-0.11 ± 0.02
	70%	-0.33 ± 0.06	-0.48 ± 0.11	-0.25 ± 0.05	-0.21 ± 0.04
Aspirin	0%	-0.43 ± 0.10	-0.44 ± 0.10	-0.22 ± 0.05	-0.21 ± 0.04
	70%	-0.42 ± 0.10	-0.50 ± 0.11	-0.24 ± 0.05	-0.24 ± 0.04

MVC, maximal voluntary contraction. Data are mean ± standard error of the mean.

Drew RC *et al.* (2008). *Exp Physiol* **93**, 1044-1057.

Drew RC *et al.* (2012). *FASEB J* **26**, 893.13.

Raven PB *et al.* (2006). *Exp Physiol* **91**, 37-49.

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PC307

Variation of ingested water may affect *in vivo* skin's physiology

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The importance of diet water on skin's physiology has been explored as a marketing argument specially for cosmetic and nutritional claims, while many studies have shown that the amount of water present in food and produced by metabolic pathways, is not sufficient to meet regular daily needs. The normal water intake is determined by a complex set of highly sensitive neurophysiological mechanisms ruling osmolarity and water balance. It varies between individuals but, unlike other fundamental nutrients, daily water requirements are not clearly identified. And the relative impact of this nutrient over normal *in vivo* human skin is far from being established. So, the present project intends to respond to these specific questions.

Twelve healthy volunteers, female, participated in the study (mean age 23.2 ± 1.85), after informed written consent. All procedures respected Helsinki principles and respective amendments. In order to assess the total amount of water consumed, daily, volunteers were asked about their regular dietary habits. Based on a survey of food frequency previously validated, it was possible to identify 2 groups of patients regarding their water consumption through regular diet (a) those consuming less than 500ml of water/day (b) those consuming more than 500ml of water/day. A stress test was designed involv-

ing the additional daily consumption of 2liters of water, during 4 weeks. This amount corresponds to the referenced water consumption recommended by the Dietary Guidelines for Americans 2010 Report, and to the European Food Safety Authority (EFSA). Representative variables were transepidermal water loss (TEWL, measured by Tewameter TM300, CK electronics), epidermal hydration (measured by MMSC, Delphin UK) and skin's biomechanics (Cutometer CM575, CK electronics). Measurements took place at T0 (inclusion) T2 (2 weeks after beginning the test) and T4 (by the end of the test, 4 weeks after beginning) and involved several anatomical regions (face, ventral forearm, breast, abdomen and, external leg). Statistical analyses were performed using SPSS 17 and a 95% level of confidence adopted.

Data seems to suggest that variation of the dietary water intake may alter normal skin physiology in all anatomical sites. Significant changes on epidermal hydration are already detected at T2 and biomechanical descriptors seem to improve at T4. No significant changes were detected regarding the barrier function. The proposed methodology may be optimized to further explore the importance of water in our regular diet and normal physiology

Jequier E, Constant F, Warer as an essential nutrient: the physiological basis of hydration, *Europ J Clin Nutrition*, 2010; 64, 115-123

European Food Safety Authority; Outcome of the Public Consultation on the Draft Opinion of the Scientific Panel on Dietetic Products, Nutrition, and Allergies (NDA) on establishing Food-Based Dietary Guidelines. *EFSA Journal* 2010; 8(5):1506.

Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans, 2010. http://www.cnpp.usda.gov/Publications/DietaryGuidelines/2010_08/12/2012

Palma ML, Monteiro C., Bujan MJ, Rodrigues LM, Water intake and relationship with the water content of the diet in a healthy population, *Biom Biopharm Res.*, 2011; (8) 2: 261-271

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PC308

A minimally invasive *in vivo* model to assess skin repair physiology

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Many animal models were specifically developed for safety prediction of numerous (human) consumer products, including medicines. These models are often regarded with skepticism due the well known distance between human and animal skin. Also, restriction to the use of animal for scientific purposes prudently limited developments in these directions, rather pointing to alternative methods. However, refinement is still admissible, eg using rodents instead of nonrodents, specially if data relevance and animal distress reduction are objective outcomes.

Skin healing is a complex issue where animal models are an additional resource often used in basic studies (eg in trauma pathophysiology) involving, highly aggressive procedures that limit its application.

Authors developed a micromodel in rats to study recovery of cutaneous "barrier" following controlled impairment. The model uses a contact challenger - sodium lauryl sulphate (SLS)

whose toxicology in human skin is well known, especially when applied at low concentrations. It does not cause any relevant histological changes, but evokes inflammation, edema and barrier impairment.

This study was developed in Wistar rats (n=10, mean weigh - 250g) and aimed to establish the minimal concentration of topically applied SLS able to evoke barrier impairment. Four contralateral areas (2cm²) were marked in the animal dorsum, 2 in each side of the median line, after mechanical removal of the hair (24hours before testing, with a razor). Rats were previously sedated with inhaled ether for 15-30min to avoid additional stressing stimulus. Patches with different SLS solution (0,5; 1; 2 % v/v) were randomly applied for 24h, 1 site serving as control. After 24hours, patches were removed and variables measured.

For this purpose, rats were previously sedated with inhaled ethyl ether using a 2,5g cotton wool impregnated with 7ml of the anesthetic, in a glass goblet set for inhalation. Animals were kept in stages II and III for 15-30min, time needed to perform measurements without additional stressing stimulus. No pain or other distress are associated to the procedure which included clinical (visual scoring scale) and biometrical assessments by non-invasive technologies such as transepidermal water loss (TEWL) (Tewameter TM300 CK electronics, Germany), expressed in g/h.m²; erythema (Chromameter® CR 300, Minolta, Japan), expressed in arbitrary units, and local microcirculation by laser doppler flowmetry (LDF - Periflux® PF5010, Perimed, Sweden), expressed in arbitrary units of perfusion. Measurements took place under controlled room conditions as recommended (22 ± 2°C at 40–50% humidity), before patching (T0) 1hour (T1a) 24hours (T1b) and daily (Tx) after patch removal until full recovery of TEWL used as the statistical end-point. Statistical comparisons between groups were performed using SPSS 17.0 and a confidence level of 95% adopted.

Results suggest a very good correlation with previously published data from human skin, justifying to further explore the application of this model to other domains such as comparative patch and dressings efficacy.

Aronrerk and Kamkaen, *J. Health Research*, 2009, 23: 17-22

Flavell et al, *Br J Pharmacol*. 2008 March; 153(S1): S241–S246.

Rodrigues LM, Roberto MA. *Burns*. 2006; 32 (7): 797-801.

Pereira MM, Saraiva J, Rodrigues LM, *Biom Biopharm Res*, 2011 (8) 2: 313-320

Pharmaceutical Sciences Postgraduate Program, Federal University of Paraná (UFPR), Curitiba, PR, Brazil. CAPES Grantee - Proc. n° 9878/11-4"

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PC309

Doxazosin causes cell death by autophagy (mitophagy) in hormone resistant prostate cancer cells

N.M. Pavithran and C.S. Thompson

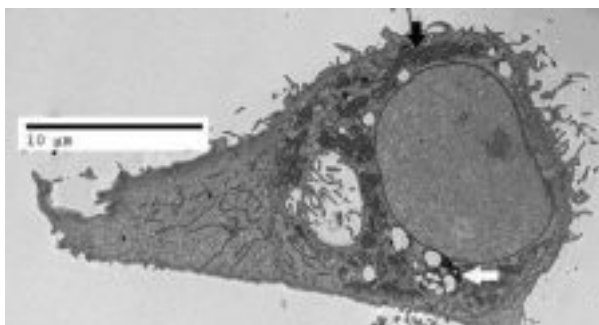
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Prostate cancer (PCa) is the most common cancer in men in the UK. Initially PCa is androgen sensitive; it then progresses to hormone resistant PCa (HRPC), which eventually becomes resistant to chemotherapy as well. Doxazosin, an α1A adren-

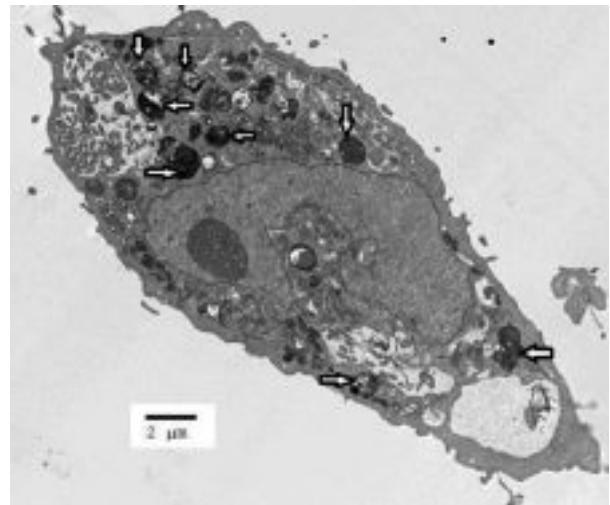
ergic antagonist used in the management of benign prostatic hyperplasia, decreases the incidence of PCa (Harris et al, 2007). Furthermore, in vitro studies have shown that doxazosin reverses drug resistance to chemotherapy (Takara et al, 2009), with an additive anti-neoplastic effect when combined with chemotherapy (Cal et al, 2000) or radiotherapy (Ceullar et al, 2002). These cytotoxic actions of doxazosin against PCa are by an adrenergic-independent mechanism (Kyprianou et al, 2000). However, the molecular target and type of cell death induced by doxazosin is unclear. Our objective was to identify, using chemical inhibitors of autophagy and transmission electron microscopy (TEM), whether autophagy is the mode of cell death induced by doxazosin.

PC-3 (p53 null, HRPC) cells were grown in F-12 HAM media and skin fibroblasts were grown in DMEM, respectively, at 37 °C in humidified 5% CO₂. Both media were supplemented with 1 % antibiotic solution containing penicillin/streptomycin and 8% foetal bovine serum. (a) PC-3 cells were seeded (5000 cell/well) in 96-well plates. After 24 h incubation they were pre-treated for 4 h with/without autophagy inhibitors, 3-methyl adenine (2 mM) and dansylcadaverine (75 µM), and then exposed to doxazosin (37 µM dissolved in H₂O) for a further 48 h. Subsequently, cell viability was assessed by CellTiter 96® assay. Results are expressed as means ± S.E.M. and statistical differences are determined using Student's two-tail paired t test. (b) Each cell line was treated for 48 h with doxazosin (37 µM) or vehicle (control) and morphology assessed by TEM. Pre-treatment with 3-methyl adenine or dansylcadaverine significantly ($p=0.02$ and $p<0.0001$, respectively) reduced cytotoxic action of doxazosin. TEM appearances of untreated PC-3 cells (Fig.1) remarkably differed from those treated with doxazosin (Fig 2). The latter showed distinct morphological characteristics of autophagy with autophagosomes containing mitochondria (mitophagy) accompanied by extensive lipofuscin-like accumulation. Skin fibroblasts also demonstrated identical findings on TEM following exposure to doxazosin. Thus, p53-null status of PC-3 cells could not be accounted for autophagy as the skin fibroblasts have p53 gene. Also, notable features of apoptosis and necrosis such as karyorrhexis and pyknosis were absent in both treated groups.

We have shown, for the first time doxazosin-induced PCa cell death by autophagy (mitophagy) that results in lipofuscin like accumulation. This novel finding may be exploited as a potential therapeutic target for the treatment of HRPC.



TEM image of control PC-3 cells showing numerous mitochondria (black arrow) and scant lipofuscin like granule (white arrow)



TEM image of PC-3 cell exposed to doxazosin showing accumulation of lipofuscin like material

Harris AM, Warner BW, Wilson JM, Becker A, Rowland RG, Conner W, Lane M, Kimbler K, Durbin EB, Baron AT, Kyprianou N. Effect of alpha1-adrenoceptor antagonist exposure on prostate cancer incidence: an observational cohort study. *J Urol*. 2007 Nov;178(5):2176-80.

Takara K, Sakaeda T, Kakumoto M, Tanigawara Y, Kobayashi H, Okumura K, Ohnishi N, Yokoyama T. Effects of alpha-adrenoceptor antagonist doxazosin on MDR1-mediated multidrug resistance and transcellular transport. *Oncol Res*. 2009;17(11-12):527-33.

Cal C, Uslu R, Gunaydin G, Ozyurt C, Omay SB. Doxazosin: a new cytotoxic agent for prostate cancer? *BJU Int*. 2000 Apr;85(6):672-5.

Cuellar DC, Rhee J, Kyprianou N. Alpha1-adrenoceptor antagonists radiosensitize prostate cancer cells via apoptosis induction. *Anticancer Res*. 2002 May-Jun;22(3):1673-9.

Kyprianou N, Benning CM. Suppression of human prostate cancer cell growth by alpha1-adrenoceptor antagonists doxazosin and terazosin via induction of apoptosis. *Cancer Res*. 2000 Aug 15;60(16):4550-5.

None

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC310

Potential Systemic Shear-Stress Inducing Effect of Whole Body Vibration

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There is growing interest in the use of whole body vibration (WBV) for physical training and rehabilitation applications. Subjecting the body to prolonged periods of high-frequency vibration have long been known to be potential injurious, whereas lower frequencies and durations of exposure may have potentially beneficial effects (Rittweger, 2010). Neuromuscular activation appears to be highest in the frequency range 15-20 Hz (Wakeling *et al.*, 2002). WBV has been reported to increase local muscular blood flow (Kersch-Schindl *et al.*, 2001) but there are no data reporting any systemic effects of WBV on blood flow. Vibration induced shear stress is of interest for its angiogenesis inducing potential. The aim of this study was to investigate the potential shear stress effects of WBV, its effect on skeletal muscle blood flow distal (forearm) to the main site (lower limbs) of muscle activation was investigated. Ten (8 male) healthy young adults aged (mean±SD)

20±1 years, height 1.76±0.08 m, body mass (BM) 76.1±6.8 kg undertook bouts of squatting exercise under four separate conditions, completing 60 squats in two minutes at a constant rate. The conditions were unloaded, loaded with a mass added to a back pack equivalent to 15 %BM and with and without WBV (30 Hz, 3 mm amplitude). A latin square design was used and subjects undertook two exercise conditions separated by 30 minutes in each of two separate sessions on different days. Immediately after completing each exercise bout, subjects moved to a supine position on an examination couch. Forearm blood flow (FBF) was measured in triplicate using venous occlusion plethysmography pre-exercise commencing 1½ minutes post-exercise and at 3 minutes intervals thereafter until 23 minutes had elapsed. Blood pressure was measured using an automated monitor on the opposite arm. Data were analysed using repeated measures ANOVA. Overall there were no significant differences in the post-exercise FBF (Fig 1) or mean arterial pressure (MAP) (Fig 2) between the various conditions. However, there was a trend towards differences in FBF at the first post-exercise time point (2 minutes) where FBF appears to increase in the unloaded condition. In contrast, with 15 %BM loading this increase in FBF was absent, most likely due to a selective reduction in lower limb peripheral resistance in response to the increased metabolic load which was associated with a trend towards a lower MAP (Fig 2). However, with the addition of WBV, it appears that this partially restored the observed increase in FBF. One tentative explanation consistent with this observation is that WBV induced shear stress resulted in a general systemic vasodilatory effect that partially counteracted the increased lower limb vasodilatation. These results should be considered preliminary, but indicate that the potential systemic effect of WBV induced shear stress warrant further investigation.

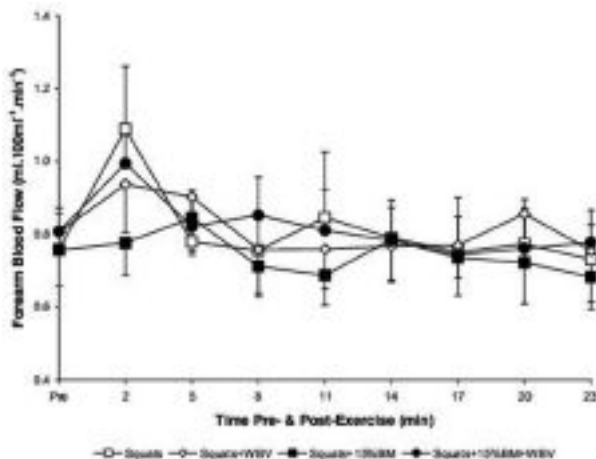


Fig 1 Forearm Blood Flow pre- and post-exercise at various time points. Data show as mean (n=10) and error bars indicate SEM.

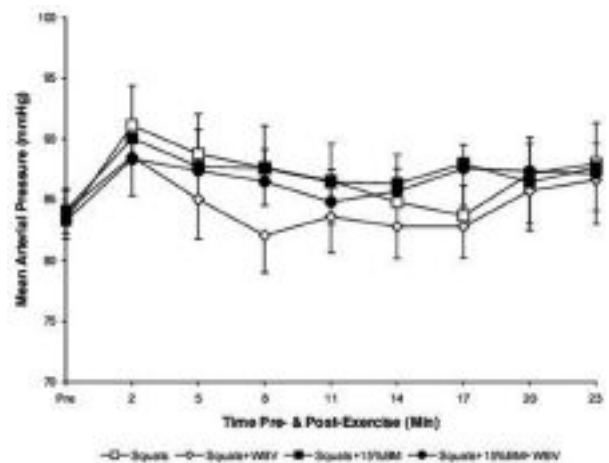


Fig 2 Mean Arterial Blood Pressure pre- and post-exercise at various time points. Data show as mean (n=10) and error bars indicate SEM.

Kerschman-Schindl K, Grampp S, Henk C, Resch H, Preisinger E, Fialka-Moser V & Imhof H (2001). Whole-body vibration exercise leads to alterations in muscle blood volume. *Clin Physiol* **21**, 377–382.

Rittweger J (2010). Vibration as an exercise modality: how it may work, and what its potential might be. *Eur J Appl Physiol* **108**, 877–904.

Wakeling JM, Nigg BM & Rozitis AI (2002). Muscle activity damps the soft tissue resonance that occurs in response to pulsed and continuous vibrations. *J Appl Physiol* **93**, 1093–1103.

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PC311

Effect of cryotherapy on blood lactate concentration and blood pH and performance in two swimming races in one session

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BACKGROUND: there have been substantial researches to show that an increase concentration of hydrogen ions and a decrease in pH (increase in acidity) within muscle or plasma causes fatigue.

Perform Cryotherapy, Ice massage, is related the fact that intense exercise actually causes microtrauma. This muscle damage not only stimulates muscle cell activity and helps repair the damage and strengthen the muscle but it is also delayed onset muscle pain and soreness (DOMS).

AIM: to investigate the impact of swimming two events (200 meter IM event and 200 meters butterfly) in one session regarding the National swimming competition schedule of Egypt on blood lactate concentration and pH. Also, the impact of Ice massage after each event.

METHODS: 20 competitive swimmers (National level) aged 17 ±1.56, weight 75±2.3 and height 177.21) divided into two groups (experimental and control) experimental group perform 200 meter Individual medley. Five minutes following this maximal effort, an ice massage has performed to the swimmers to their shoulder and legs (3 minutes, 2minutes and 1 minute soft massage and one minute rest interval between each massage) then completed by another 200 meters but butterfly after 30 minutes of the first race. The same ice massage has been performed. Blood sample has been collected to measure both blood lactate concentration and pH pre-race,

immediately after and after 30 minutes of each race. Control group performed the same races but without ice massage in between the two races.

RESULTS: revealed significant increase ($P < 0.01$) in blood lactate levels and decreased pH immediately after exercise in both groups, decreased significantly after 30 minutes of the first race compared to the control group.

DISCUSSION AND CONCLUSION: It is concluded that ice massage may help recovery from short maximal efforts or after perform one race followed by another race in one swimming session within 30 minutes.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC312

Different responses to exercise in glabrous and nonglabrous skin

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The cutaneous circulation is the main effector for human thermoregulation. As exercise leads to enhanced production of heat, skin microcirculation is under competing thermo- and nonthermoregulatory demands. The exact mechanism governing the response of skin blood flow (SkBF) after exercise remains to be elucidated. Furthermore, it has been shown that nonglabrous and glabrous skin sites differ in structural and microvascular characteristics as well as in the type of innervation, especially with regard to the abundance of arteriovenous anastomoses but the exact mechanism has not been established yet. Thus, the aim of our study was to evaluate the response of SkBF to an acute exercise in glabrous and nonglabrous skin sites, respectively.

Nine healthy nonsmoking subjects (6 males, 3 females, 20 years old, BMI 21.91 ± 0.44 , $VO_{2\max}$ 53.22 ± 2.68 ml/kg min) participated in the study. SkBF was assessed using laser Doppler flowmetry (LDF) on two measuring skin sites: the finger pulp and the ventral forearm. Simultaneously, skin temperature (T) was recorded on both measuring sites. Throughout the experiment, a standard ECG as well as blood pressure was recorded. After 5 minutes rest in supine position subjects mounted the cycloergometer and rested for another 5 minutes. The right arm was fixed on an armrest and SkBF probes were positioned at the heart level. Afterwards subjects started a graded exercise until 85% of the estimated maximal heart rate was reached. After ceasing exercising, subject remained in a sitting position and the parameters were measured for subsequent 25 minutes. Values of SkBF, T, RR interval and RR interval difference (RMSSD) were averaged over a 3-min interval and are shown in table 1.

After exercise, SkBF at the volar forearm started to diminish and returned to the resting value by the end of the experiment whereas the SkBF in the finger pulp started to rise abruptly and did not return to the baseline values by the end of the experiment. SkBF in the finger pulp raised for 269.6 ± 60.1 AU in 128.3 ± 18.5 s after cessation of exercise and T for 4.6 ± 0.6 °C in 160 ± 19.6 s. The data of a typical response to exercise are presented in fig. 1, where the upper two signals represent the SkBF of finger pulp and volar forearm respectively and the lower two the corresponding skin temperatures.

Our results have shown that glabrous and nonglabrous skin sites respond differently to exercise. We might speculate that vasoconstrictor tone in glabrous parts is withdrawn after exer-

cise due to an increase in core temperature. Further experiments are needed to elucidate the exact mechanisms involved in the regulation of SkBF in response to exercise.

Table 1

	Finger pulp SkBF (AU)	Finger pulp T (°C)	ventral forearm SkBF (AU)	ventral forearm T (°C)	RR (ms)	RMSSD (ms)
before exercise	91.8 $\pm$ 17.3	29.4 $\pm$ 0.8	11.5 $\pm$ 3.6	32.7 $\pm$ 0.3	996.7 $\pm$ 53.1	64.5 $\pm$ 8.8
24 min after exercise	247.9 $\pm$ 58.9*	31.8 $\pm$ 0.2*	11.9 $\pm$ 3.9	31.7 $\pm$ 0.5	863.4 $\pm$ 34.0*	19.8 $\pm$ 3.7*

* - significantly different (paired t-test, $p < 0.01$)

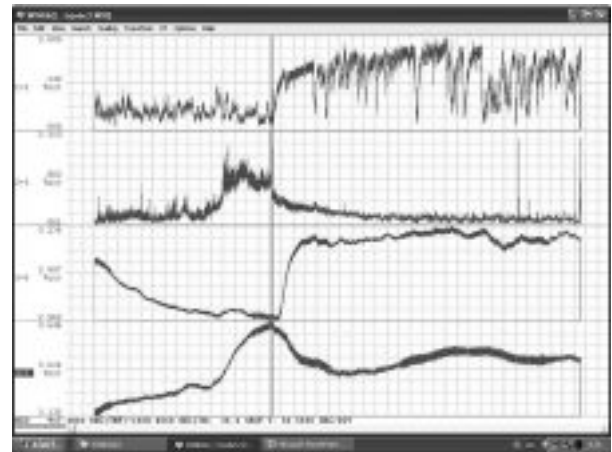


Figure 1

Yamazaki F, Kinoshita K, Sone R (2009). J Physiol Sci 59, 137-142

Hodges GJ, Johnson JM (2009). Appl. Physiol. Nutr. Metab. 34, 829-839

Lenasi H, Struelens M (2010). Clin Hemorheol Microcirc 45(2-4), 365-74

Johnson JM (1992). Exerc Sport Sci Rev 20, 59-97

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PC313

Assessment of the recovery mechanisms after a motor control intervention in young adults with shoulder impingement

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Motivation

Shoulder impingement (SI) is a common problem for which management approaches lack evidence [1], it is commonly associated with altered motor control of scapulothoracic muscles [2]. This study aimed to elucidate the effects of a motor control (MC) based intervention on pain and muscle function. Method

Sixteen SI participants (mean age 24.6 ± 1.6 , 11 males) and 16 healthy participants (22 ± 3.1 , 11 males) were studied. The Shoulder Pain and Arm Disability Index (SPADI) assessed pain/function. Surface electromyography (EMG) recorded muscle activity of serratus anterior (SA) and three regions of trapezius (upper, middle, and lower (LT)) during arm elevation to 90°

and lowering in three planes. Elevation angles were measured by 3-D motion analysis (Vicon, Oxford). Patients were assessed pre and post a 10-week MC intervention, which involved scapular orientation retraining. EMG data were band pass filtered and raw signals were analysed visually for onset and termination of activation, with respect to arm elevation. Analysis between groups was performed by independent and paired t-tests.

Results

Pre-intervention, patients reported pain and reduced function compared to the healthy participants (pre-intervention 20 ± 9.2 ; healthy 0 ± 0), these scores improved significantly post-intervention ($p < 0.001$) by a mean of 10 ± 4 . Pre-intervention, EMG onset was delayed and length of contraction reduced significantly in SA (sagittal and frontal arm elevation) and LT (sagittal and scapular arm elevation) compared to the healthy participants (Table 1). Onset and duration of muscle activity improved significantly post-intervention ($p < 0.05$ - 0.01) in both SA and LT, reaching similar values to the healthy group (Fig. 1).

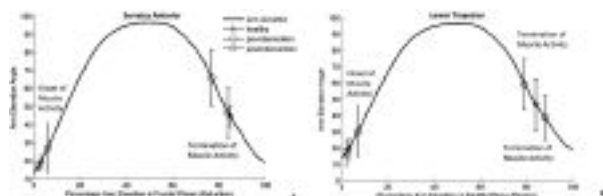
Conclusions

Recovery mechanisms of the clinical effects of the 10 week MC intervention involve improvements in muscle recruitment patterns. It is proposed that MC exercises targeting scapular orientation may be required to improve muscle activation and influence biomechanics, to improve function in SI patients.

Mean and standard deviation muscle activation timing for serratus anterior and lower trapezius.

Group		Serratus Anterior						Lower Trapezius					
		Healthy			Pre-intervention			Healthy			Pre-intervention		
		Onset	Termination	Duration	Onset	Termination	Duration	Onset	Termination	Duration	Onset	Termination	Duration
Arm Movement Plane	Sagittal	14 ± 3	47 ± 13	33 ± 17	48 ± 18	23 ± 14	15 ± 7	46 ± 11	38 ± 17	29 ± 16	17 ± 4*	42 ± 14	35 ± 11
	Frontal	30 ± 5	44 ± 5	14 ± 5	47 ± 10	38 ± 5	11 ± 3	57 ± 30	36 ± 16	67 ± 16	34 ± 21	59 ± 23	25 ± 13
	Scapular	17 ± 3	46 ± 11	29 ± 14	49 ± 14	21 ± 15	13 ± 4	38 ± 8	39 ± 12	31 ± 17	44 ± 14	29 ± 13	51 ± 30*

*Significant difference pre-post intervention. # Significant difference between healthy and pain participants. Significance levels: */# $p < 0.05$, **/## $p < 0.001$, ***/### $p < 0.0001$.



Muscle activation timing in relation to arm elevation: (a) serratus anterior during frontal plane movement. (b) lower trapezius during sagittal plane movement. Mean and standard deviation of muscle onset and termination of activity.

Dorrestijn O, et al. (2009). J Shoulder Elbow Surg.18(4):652-60.

Ludewig PM & Cook TM. (2000). Phys Ther.80(3):276-91.

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The determinants of t-system volume in resting skeletal muscle

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The transverse tubular (t-)system of skeletal muscle couples sarcolemmal electrical excitation with contraction deep within the fibre. Despite continuity of the t-system lumen with the extracellular fluid (ECF), t-system volume (t-vol) is labile, ranging from $<0.5\%$ of fibre volume in resting muscle to as much as 10-15% in glycerol-induced vacuolation in both fast and slow twitch fibres of amphibians and mammals (Krotenko & Lucy, 2001). T-vol changes occur in exercise, pathologies including muscular dystrophy, and following experimental manipulation of extracellular ion concentrations or osmolality. This may contribute to fatigue, rhabdomyolysis, and disruption of excitation-contraction coupling. Yet, the mechanisms that underlie t-vol changes are poorly understood.

To address this, a multi-compartment computer model of rat skeletal muscle was developed (Fraser et al., 2011) and shown to reach a unique steady state independent of intracellular or intra-t-system ion concentrations. It was used to define the minimum conditions for t-system stability at rest, and to determine the mechanism of t-vol changes observed in previously published experimental work.

Simulations were conducted to define the influence of positive and negative hydrostatic pressures, fixed charges, ionic permeabilities and Na^+/K^+ -ATPase density (N). A general tendency was found for the t-system to swell due to net ionic fluxes from the ECF across the access resistance. A stable t-vol is possible when this ionic influx is offset by a net ionic efflux from the t-system to the cell and thence to the ECF, thereby forming a net ion cycle $\text{ECF} \rightarrow \text{t-system} \rightarrow \text{sarcoplasm} \rightarrow \text{ECF}$ that is ultimately dependent on Na^+/K^+ -ATPase activity (Fig 1). Surface and tubular membrane properties that maximise this circuit flux were shown to decrease t-vol; such properties include $P_{\text{Na}(t)} > P_{\text{Na}(s)}$, $P_{\text{K}(t)} < P_{\text{K}(s)}$ and $N_t < N_s$ (where P denotes permeability and subscripts t and s denote t-system membrane and sarcolemma respectively). Hydrostatic pressure influences the magnitude of volume changes that result from alterations in this circuit flux, whereas fixed charge in the t-system influences both the magnitude and direction of the circuit flux.

Using a parameter set derived, where possible, from literature values, the circuit flux theory of t-vol determination was tested against all available experimental studies (including Rapoport, 1969; Dulhunty, 1982; Usher-Smith et al., 2007), as summarized in Table 2. Although these studies generally measured t-system diameter in several amphibian and mammalian systems, the present model predicted t-vol changes that correlate satisfactorily. This work therefore provides a unifying and robust theoretical framework for understanding the determination of t-system volume.

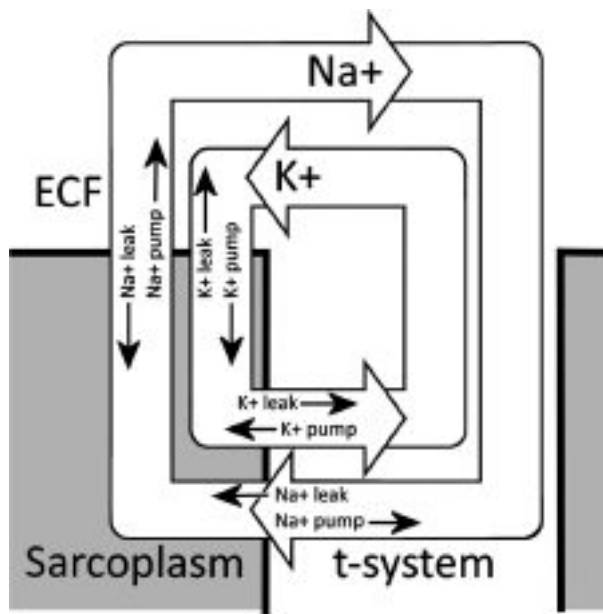


Fig. 1. A diagram of circuit fluxes at steady state. Net Na⁺ and K⁺ fluxes are shown by the large open arrows; their magnitudes are equal. The larger transmembrane components of these smaller net fluxes are shown by the black arrows. The model t-system is divided into 20 sub-compartments not shown here.

Protocol	Range studied (mM)	Result [experiment]	Result [model]
1. Hypertonic NaCl addition	[Na ⁺], 80→230	-16% diameter	-50% volume
2. Addition and withdrawal of glycerol (gly)	[Gly], 0→300→0	Vacuolation on gly withdrawal	+3000% volume on gly withdrawal
3. Isotonic NaCl increase	[Na ⁺], 150→50	+100% diameter	+200% volume
4. Isotonic Cl ⁻ →sulphate	[Cl ⁻], 115→0	Transient 250% volume increase	Transient 400% volume increase
5. Isotonic Na ⁺ →K ⁺	[K ⁺], 2.5→40	+100% diameter	+500% volume

Table 1. Comparison of model results with experimental findings in amphibian and mammalian muscle: 1) Freygang et al. 1967; 2) Rapoport et al. 1967; 3) Dulhunty et al. 1982; 4) Fraser et al. 1998. J muscle res cell motil 19, 613–629; 5) Usher-Smith et al. 2007. J muscle res cell motil 28, 19–28.

Dulhunty AF (1982). J Memb Biol 67, 81–90.

Fraser JA, Huang CL-H & Pedersen TH (2011). J Gen Physiol 138, 95–116.

Krotenko SA & Lucy JA (2001). Int Rev Cytol 202, 243–298.

Rapoport SI (1969). J Gen Physiol 54, 178–187.

Usher-Smith JA, Fraser JA, Huang CL-H & Skepper JN (2007). J Muscle Res cell Motil 28, 19–28.

JAF hold a BBSRC David Phillips Fellowship

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PC315

Mechano-sensitivity of the vascular network and its modulation by Gd₃⁺ in the rabbit masseter muscle

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A number of studies have recently reinvestigated the rapid hyperaemia that occurs in response to a brief muscle contraction and to external compression of the muscle (Clifford & Tschakovsky, 2008). Both stimuli have been suggested to produce a rapid dilatation, induced by the transmural pressure changes in the vascular network. Several types of mechano-sensitive ion channels (MSC) in smooth muscle and/or endothelium are potentially involved. Gadolinium (Gd₃⁺) is considered

to be one among the most effective MSC blockers (Schubert & Mulvany, 1999). It was reported to effectively reduce the myogenic response to sustained transmural pressure changes in isolated vessels but its effectiveness in vivo has been poorly investigated.

Aim of the present study is to investigate in vivo the effect of local administration of Gd₃⁺ on basal blood flow and on the rapid hyperaemia produced by external muscle compression. The adopted model allows for continuous monitoring of blood flow in the purely muscular masseteric artery. The study complies with the national guidelines for animal care. Eight rabbits were anesthetized with i.v. administration of urethane (400 mg/kg), ketamine and xylazine (initial dose of 5 and 1.5 mg/kg, respectively, then continuously infused as needed).

Hyperemic responses were induced by 1-s lasting compression of the masseter muscle by means of a cylindrical head (diameter: 2 cm) moved by a computer-driven motor. Hyperemic responses were repeatedly evoked before and 15, 30 and 45 min after close arterial injection of Gd₃⁺, administered at progressive concentrations of 0.1, 1 and 10 mM. Each dose was infused in 2 min and after 1-h interval from previous administration. Injection rate was set to 50% of measured blood flow in the masseteric artery resulting in approximate plasma concentrations of 0.045, 0.45 and 4.5 mM.

Both basal blood flow and the amplitude of the hyperaemic response were significantly dependent on Gd₃⁺ concentration ($p < 0.05$) but not on time (2-ways ANOVA). With respect to the control (pre-Gd₃⁺) condition the following changes were observed in basal blood flow at the different plasma concentrations: +11 ± 25% at 0.045 mM ($p = 0.90$), +69 ± 71% at 0.45 mM ($p < 0.05$) and -54 ± 35% at 4.5 mM ($p < 0.05$) and in the amplitude of the hyperaemic response: -12 ± 4% at 0.045 mM ($p = 0.24$), -26 ± 26% at 0.45 mM ($p < 0.05$) and -94 ± 4% at 4.5 mM ($p < 0.05$).

While at the highest concentration the observed effects could be secondary to the low solubility of Gd₃⁺, at lower concentrations Gd₃⁺ significantly increased basal muscle blood flow and decreased the rapid hyperaemic response to muscle compression. This supports the notion that MSCs are implicated in the maintenance of basal myogenic tone and that they have a role in the rapid dilatation induced by muscle compression. Clifford PS & Tschakovsky ME (2008) *Exerc Sport Sci Rev*, 36, 25-29. Schubert R & Mulvany MJ (1999) *Clin Sci* 96, 313-326.

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PC316

Attenuation of the rapid hyperaemic response during repetitive muscle compression

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The rapid hyperaemia at the onset of exercise is considered to partly rely on the mechano-sensitivity of the vascular network (Clifford & Tschakovsky, 2008). This is conveniently investigated by means of short lasting muscle compressions (MC), which mimic the hyperaemic response of brief muscle contractions. How the hyperaemia develops upon repetitive mechanical stimulation is currently unknown, both potentiated and attenuated responses having been observed following repetition of the stimulus (Clifford et al., 2006; Kirby et al

2007). The issue is however of interest, given that motor activity is frequently characterized by repetitive muscle contractions.

The present study aims at investigating the hyperaemic response to repetitive MC.

The study complies with the national guidelines for animal care and has been approved by the local ethical committee.

Muscle blood flow is continuously measured from the masseteric artery of anesthetized rabbits. Data are collected bilaterally from 5 rabbits (n=10 arteries) anesthetized with urethane (1.2 g/kg, i.v.). Mechanical compression of the masseter muscle is exerted by a cylindrical head (diameter=2cm) moved by a PC-driven servo-controlled motor (local pressure = about 120 mmHg).

Repetitive MC (1 s ON, 1 s OFF, for 25 s) transiently increased blood flow (measured during OFF periods) up to 580 ± 145 % (mean + STD), time to peak= 4.1 ± 1.4 s. Blood flow then decreased towards baseline in spite of continuing stimulation (half return time= 8 ± 5.5 s).

Repetitive 1-s MC at constant rate (stimulation period: 2 s to 4 min) produced instead stable hyperaemic responses whose peak amplitude decreased with decreasing stimulation period (1-way ANOVA, $p < 0.01$): from 457 ± 208 % (4 min) to 163 ± 60 % (20 s) to 25 ± 15 % (2 s).

The observation that the amplitude of the hyperaemic response decreases by more than 50% when the stimulation period decreases from 4 min in to 20 s suggests that the mechano-sensitivity of the vascular network undergoes some transient "inactivation" after stimulation.

The results highlight the transient nature of the hyperaemia produced by repetitive muscle compression and may explain the contradictory data in the literature as well as the similar hyperaemic pattern observed in response to the mechanical stimulation produced by repetitive passive limb movement (Trinity et al 2011). In addition, this characteristic fits well with the hypothesis that the mechano-sensitivity of the vascular network mediates a feed-forward control of muscle blood flow at the beginning of exercise, leaving to other mechanisms the responsibility to match blood flow to the actual metabolic demand during prolonged muscle activity.

Clifford PS et al (2006) *J Physiol* **572**, 561-7

Clifford PS & Tschakovsky ME (2008) *Exerc Sport Sci Rev*, **36**, 25-29

Kirby BS et al (2007) *J Physiol* **583**, 861-74

Trinity jd et al (2011) *Am J Physiol* **300**, H1885-91

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PC317

Inflammatory responses to high-intensity intermittent training in diet-induced obese rats

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Adipose tissue is an endocrine organ and the main source of inflammatory cytokines, such as TNF- α and IL-6, characterizing a chronic low-grade inflammatory state (1). Lifestyle behavioral interventions, as physical activity, may have clinically significant benefits for improving inflammation (2). This study aimed to investigate the inflammation responses after intermittent training of high intensity squat jump type in Wistar obese rats. Forty adults male Wistar rats of 2 months-old were

divided into: sedentary control (SC), exercise control (EC), sedentary obese (SO), exercise obese (EO) groups. Control groups were fed with standard chow and obese received a cafeteria diet+standard chow ad libitum. The training protocol was performed following the adapted model of force from Tamaki et al. (3). It consisted on 3x a week, interposed by 24 h among sessions, 3 series of 12 repetitions of 60s of interval among series with a strength load equivalent to 50% of the body weight (BW) during 6 weeks. An insulin tolerance test (ITT) was performed with venous blood collected from tail distal end for glycemia measurement at 0, 4, 8, 12 and 16 min after ip injection of regular human insulin (0.5 IU/kg BW). The sacrifice was performed for removing of soleus muscle under anesthesia (Ketamine 80mg/kg, Xylazine 5mg/kg, ip). RT-PCR was performed for evaluation of TNF- α and IL-10 mRNA levels. ANOVA one-way was used for comparison among means with post-hoc (Tukey) if necessary, considering $P < 0.05$ for significance. The experimental protocol was approved by the Ethics Committee for Animal Research (#15/2009). The obese groups (SO=538 $\pm$ 19.6, EO=494.6 $\pm$ 14; $P < 0.01$ vs SC, EC) presented a final weight significantly higher than the control groups (SC=415 $\pm$ 18, EC=393 $\pm$ 14), and significant increase in fat mass (around 49% in OS, $P < 0.001$ vs SC, 42% in EO $P < 0.01$ vs EC). ITT showed a reduction in insulin sensitivity in obese, however reversed by training (kITT expressed as %/min: SC=5.76 $\pm$ 0.36; EC=6.63 $\pm$ 0.45; SO=3.60 $\pm$ 0.62*#; EO=7.07 $\pm$ 0.47#; * $P < 0.05$ vs SC; # $P < 0.01$ vs. EC, & $P < 0.001$ vs. SO). The diet caused increase of TNF- α RNAm (SC=100.5 $\pm$ 5.3, OS=133 $\pm$ 7.9 AU, $P < 0.001$). However, the training caused a 27%-reduction ($P < 0.01$ vs SO) in fat mass, and 39%-reduction in TNF- α level (EO=80.7 $\pm$ 6.2, $P < 0.001$ vs SO). The cafeteria diet positively modulated while the exercise reduced IL-10 levels mRNA (SC=100.6 $\pm$ 4.2, SO=210.4 $\pm$ 10, EC=42.8 $\pm$ 9.58#, EO=153.9 $\pm$ 13.7*, * $P < 0.05$ vs OS, & $P < 0.001$ vs OS, # $P < 0.01$ vs OE). We can conclude that the cafeteria diet was detrimental to insulin sensitivity in rats, and effective in body weight gain and increased inflammatory cytokine TNF- α levels. On the other hand, high-intensity intermittent training was effective in reversing this situation. Surprisingly the levels of anti-inflammatory cytokine IL-10 followed the levels of TNF- α in this model of training.

Galic S. et al. (2010) *Mol Cell Endocrinol* 316: 129–139.
Nicklas, B.J. et al. (2005) *CMAJ* 172:1199–209.
Tamaki, T. et al. *Med. Sci. Sports Exerc.* (1992) 24:881-6.

Rafael J Moreira is recipient of Fapesp fellowship (# 2010/17078-4).

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PC318

The capillary pumps regulate muscle blood flow by the myocytes' CO₂ and heat production and the mechanical impact of their contraction

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One of the greatest fallacies in the physiology is the paradigm that the immediate exercise hyperemia could be governed by the arteriolar dilation. Striated muscle cells represent thermal engines with internal combustion, which must be cooled and thermo-regulated, like every man-made one, individually,

not in bulk. These cells are extremely vulnerable to overheating, since they can increase their energy production enormously in far less than a second anaerobically (humming bird makes 80 wing-beats/sec). They have not intracellular convective heat transfer, since about 80% of their water is entrapped in the myofibrils. For that, more than other cells, they need an immediate response of their external cooling, in order not to raise their surface temperature, which would hamper the rapid elevation of the intracellular temperature gradient (2) determining their internal conductive heat transfer. This is especially valid for the white muscle cells, with their great anaerobic loading, which would first expand and die from overheating (1). That's why we tested our hypothesis that CO₂ plays decisive role in the homeotherms' thermo-baric homeostasis, allowing coupling the capillary blood flow with the positive temperature gradient between their adherent cells and the capillary blood, which proves the latter's primary cooling role. For that we measured the thermo-baric relationship of CO₂ dissolved in saline for different concentrations and plotted pressure against temperature. We discovered that in each diagram (Fig. 1) there are several inflex points and that the first derivatives of all of them (Fig. 2) have one maximum always near the 37 deg C, independently from the CO₂ content. Thus, we discovered a physical constant, explaining the human rest body core temperature, auto-regulated by the increased curve slope at this point. For that we created the following theory: a) The capillary blood flow is promoted using the Malone heat engine-cycle; b) The working medium is the containing dissolved CO₂ blood plasma, expanding by all factors reducing the CO₂ solubility in it or producing additional amounts of CO₂, like cell metabolism (anaerobic-5-6 times greater) or acids; c) The capillary glycocalyx molecular-chains (bended toward the venous limb by the erythrocyte passage) and the parachute-formed erythrocytes act as ratchet and pawl, determining their one-way motion e. g.. a capillary pump; d) The plasma expands due to the nanobubbles formed at the liquid/solid interfaces at the glycocalyx molecular-chains, their density reaching maximum at 37 deg C and decreasing at a higher temperature (4, 5). This pumping explains the inflammation, as well (3). Since muscle contractions cause mechanically capillary pressure elevation with a net positive effect by the "ratchet" system, we suggest an additional term "capillary muscle pump".

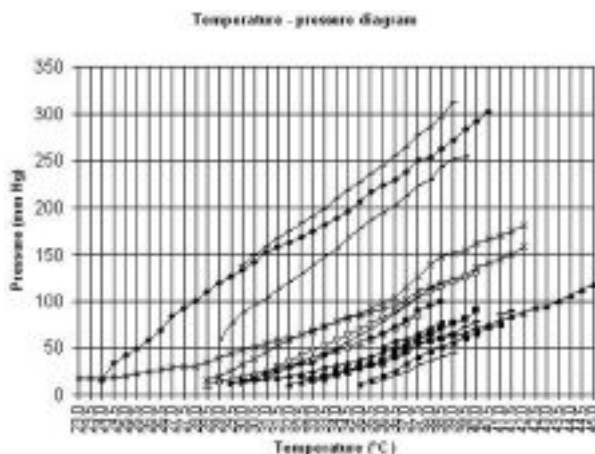


Fig.. 1 Pressure-temperature relationship for carbon dioxide in saline solution. The curves are for different CO₂ - concentrations.

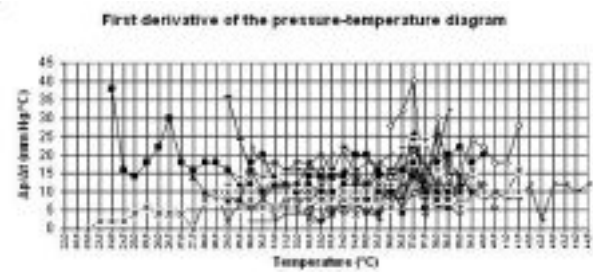


Fig. 2 First derivatives of the diagrams from Fig. 1, obtained as a moving derivative between adjacent points ($\Delta P/\Delta T$). The symbols in the both figures do not conform to each other, because the aim was only to show the overlapping in the 37 deg C area.

1. Gervais P et al. (2003). Jour Biotech/102, 267-279.
2. Okabe K et al. (2012). Nature Commun 3 Article Numb 705.
3. Panchev V et al. (2011) 10-th world Congr inflamm, Paris, poster p-323.
4. Zhang XH et al. (2005). Chinese phys/14, 1774.
5. Zhang, XH et al. (2004). Langmuir/20, 3813.

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PC319

Physical performance does not differ between right and left footed soccer young players. Field and isokinetic tests analysis

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Objective

To compare physical performance using field tests and isokinetic muscle strength between right and elite young soccer players.

Material and methods

Forty-nine young, healthy, male soccer players (mean age of 13.6 years) who were scholarship holders of the Moroccan academy Mohammed VI of soccer served as subjects in this study. Players were divided into 2 groups comparable for age, weight and height : right dominant limb (group 1) and left dominant limb (group 2). All participants received the same the entrainment schedule.

Soccer players underwent a clinical examination (age, weight, height and body mass index), field tests (30 M sprint, Tube II and vertical jump) and evaluation of knee flexor and extensor muscle strength of their dominant limb on an isokinetic dynamometer. To ensure consistent and accurate measurements, each parameter was evaluated by the same examiner utilizing the same methods.

Results

Field tests results show no differences between players of the 2 groups (table 1). Muscular strength did not differ between right and left footed as well at low as at speed velocities.

Discussion

This study highlights that physical performance tests, including field tests and isokinetic, did not differ between right and left footed young soccer players. During the 90-minute of Soccer game, numerous explosive bursts of activity are required, including jumping, kicking, tackling, turning, sprinting, changing pace, and sustaining forceful contractions to maintain balance and control of the ball against defensive pressure. Briefly, soccer performance improves need not only physical but also technical and tactical development. Further studies on large samples should be conducted. It's extremely important that those studies integrate a complete analysis of soccer performance including physical, technical/biomechanical, tactical and mental properties.

table 1: Field tests comparison between right and left footed soccer players

Field tests	Right footed N=30	Left footed N=29	P
Vertical jump	38.4±5.2	37.2±4.8	0.5
Sprint 30 M	4.9±0.4	4.9±0.5	0.9
Table II	17±1.8	17.2±1.3	0.4

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PC320

Continuous and intermittent training protocols improve expression of proteins involved in muscle metabolism of diet-induced obese rats

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Obesity is associated to reduced glucose transporter 4 (GLUT4) expression and/or impaired insulin signaling pathway in skeletal muscle (1). Muscle contraction provokes reduction on ATP rate, culminating in 5-Adenosine Monophosphate activated protein kinase (AMPK) activation, provoking GLUT4 translocation (2). Training stimulates peroxisome proliferator-activated receptor- γ coactivator (PGC1 α) transcription in muscle which stimulates GLUT4 expression (3). This study aimed to evaluate the effects of continuous or intermittent training protocols on AMPK, PGC1 α and GLUT4 expression in muscle of diet-induced obese rats. Sixty male Wistar rats aged 90 days were divided into: sedentary control (SC), control continuous exercise (CCE), control intermittent exercise (CIE) obese sedentary (OS), obese continuous exercise (OCE), obese intermittent exercise (OIE). All animals were fed with standard chow and obese group received hiperlipidic diet. Insulin tolerance test and the double effort test were performed in two moments: after 8 weeks (wk) of diet and at the end of 8 wk of training using blood collected from the tail distal end. using blood collected from the tail distal end. The CCE and OCE groups trained for 30 min at 90% of anaerobic threshold, 3x/wk, 8 wk. The groups CIE and OIE performed 11 efforts lasting 2 min with 1 min of passive interval, 3x/wk, 8 wk, over the delta zero to 120% of the critical load. The sacrifice was performed for removing of gastrocnemius muscle under anesthesia (Ketamine 80mg/kg, Xylazine 5mg/kg, ip). RT-PCR and Western blotting were performed for gene expression and protein content analysis. The experimental protocol was approved by the Ethics Committee for Animal Research (#74/2009).

ANOVA one-way was used for comparison among means with post-hoc (Tukey) if necessary, considering $P < 0.05$ for significance. Obese animals presented higher fat mass and reduced insulin sensitivity ($SC = 2.3 \pm 0.23$; $SO = 1.8 \pm 0.2$, $P < 0.05$), but both protocols reverted this condition ($OCE = 2.8 \pm 0.7$; $OIE = 2.4 \pm 0.4$, $P < 0.05$ vs OS). AMPK $\alpha 2$ mRNA was increased in all exercised groups ($CEC = 70\%$, $CEI = 72\%$, $P < 0.01$ vs SC; $OEC = 75\%$, $OEI = 59\%$, $P < 0.05$ vs OS), AMPK $\alpha 2$ protein content was increased only in control ($CCE = 47\%$, $CIE = 51\%$, $P < 0.05$ vs SC). pAMPK $\alpha 2$ was very reduced in SO (39% , $P < 0.05$ vs SC), and increased with training ($OCE = 264\%$, $OIE = 299\%$, $P < 0.01$ vs OS). Both protocols were efficient to increase PGC1 α expression ($CCE = 62\%$, $CIE = 126\%$, $P < 0.05$ vs SC; $OCE = 104\%$, $OIE = 152\%$, $P < 0.05$ vs OS). Continuous training increased in 53% the GLUT4 expression only in obese ($P < 0.05$) and Intermittent training improved only in control (55% , $P < 0.05$). Eight weeks of both training protocols were able to soften the increase on body weight, improve the insulin sensitivity and increase aerobic capacity, promoting increasing in the gene expression of proteins involved in muscle metabolism.

(1) Seraphim, P.M., et al (2001). Braz J Med Biol Res 34:1353-62.

Hardie, D.G. & Sakamoto K. (2005) Physiology 21: 48–60.

Jornayvaz, F.R. & Shulman, G.I. (2010). 47:69-84.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC321

The contribution of motor commands to position sense differs between wrist and elbow

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Proprioception is critical to movement control and it relies on information from multiple sources, including receptors in muscles, joints and skin. Recently it was shown that motor commands contribute to perceived position and movement at the wrist (e.g. Walsh et al., 2010). In contrast, at the elbow when the arm was loaded, no evidence emerged for a contribution from motor commands to position sense (e.g. Ansems et al., 2006). However, these two sets of experiments were performed at different joints using different methods to indicate joint position. The present study aimed to resolve the discrepancy by using both methods at both joints.

Two experiments, each with 20 human subjects, were performed one at the wrist, the other at the elbow, each with two sessions. In the 'single wrist' session subjects were seated at a table with their right arm strapped to the table and the hand in a device that restricted movement to the wrist joint. A 50% of maximum voluntary conditioning contraction of wrist flexor or extensor muscles controlled for contraction history. After relaxation, the wrist was moved to a test angle. Position was indicated with a pointer when the hand was relaxed or when the conditioned wrist muscles were contracting isometrically at 30% of maximum. In the 'double wrist' session both arms were strapped into manipulanda, both arms performed the

conditioning contraction and instead of using a pointer, the subject indicated the perceived position of the right wrist by matching its position with the left wrist. In a further 20 subjects a similar experiment was performed on the elbow joint using the same experimental design.

In the single wrist session there was an effect of muscle contraction. This effect significantly (ANOVA, $p < 0.001$) changed subjects' perception of limb position by 7° [3, 12] (mean [95% confidence interval]) during a flexion contraction and 9° [4, 14] during an extension contraction. During the bilateral matching task in the 'double wrist' session, contractions also had a significant (ANOVA, $p < 0.01$) effect, changing perceived limb position by 13° [9, 16] during flexion and by 5° [2, 8] during extension. Contraction of elbow flexors or elbow extensors did not significantly alter the perceived angle of the elbow. The results of the present study are consistent with the previous studies on the wrist and the elbow, cited above. Thus while an effect of muscle contraction on perceived joint position at the wrist has been confirmed, a similar effect is not detectable at the elbow joint. Furthermore, the method of indicating joint angle (pointing or matching) also seems to influence the size of the effect of muscle contraction. The findings confirm that centrally-generated motor command signals can contribute to joint position sense, but the importance of the contribution may depend on the joint.

Ansems GE, Allen TJ & Proske U. (2006). Position sense at the human forearm in the horizontal plane during loading and vibration of elbow muscles. *J Physiol* 576, 445-455.

Walsh LD, Gandevia SC & Taylor JL. (2010). Illusory movements of a phantom hand grade with the duration and magnitude of motor commands. *The Journal of Physiology* 588, 1269-1280.

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PC322

Voluntary vs electrical muscle activation reveals the resonant nature of physiological finger tremor

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Human physiological finger tremor is usually described as having two main frequencies, one at ~ 20 Hz and one at ~ 10 Hz. The origin of these frequencies is uncertain. Two main views exist: one attributing tremor to neural oscillations and one attributing tremor to mechanical resonance (McAuley and Marsden, 2000). We recently showed the presence of a resonant component in hand tremor that could be reproduced by a very simple model driven by a white noise input (Lakie et al, 2012). This suggests tremor would be similar if the muscle is activated voluntarily by muscle activation or artificially by white noise input (i.e. direct electrical muscle stimulation). In this study, with ethical permission and the subjects' informed consent we examined tremor of the splinted middle finger with voluntary activation or artificial white noise stimulation at five different mean levels. With voluntary activation, surface EMG of the m. extensor digitorum communis was recorded. With electrical activation, white noise sequences of 50 μ s current pulses were applied to the skin over this muscle. During both methods of activation, we recorded tremor in two ways. In isometric conditions the finger pressed upwards against a rigid strain gauge device above the nail plate. In isotonic conditions a miniature accelerometer was attached to the finger. The fre-

quency spectra of the isometric tremor showed an exponential decline of force with higher frequency (Fig 1). The effect of different levels of muscular activation changed the amount of force produced, but did not change the shape of the spectrum. However, spectra of the isotonic tremor showed a specific peak frequency, and both frequency and amplitude were dependent on level of activation (Fig 1). For low levels of activation, a peak at ~ 20 Hz was produced. Increased levels of activation not only increased the acceleration amplitude, but also decreased the peak frequency to ~ 10 Hz. Importantly, isotonic and isometric tremor spectra show similar profiles with voluntary activation and white noise electrical activation. This implies that the voluntary activation and the artificial random input are equivalent – that is, there is nothing “special” about central drive. We suggest that the peak in the isotonic tremor spectrum represents a resonance of the limb. The resonant frequency decreases with increased activation due to a movement dependent reduction in muscle stiffness. We have recently shown that a drop in muscular stiffness with increased movement leads to a lower tremor frequency in the hand (Reynolds and Lakie, 2010) and we propose the same mechanism to hold for finger tremor. The results show that tremor frequencies of the finger ranging from 10 – 20 Hz can be produced by mechanical resonance and it is not necessary to invoke other causes.

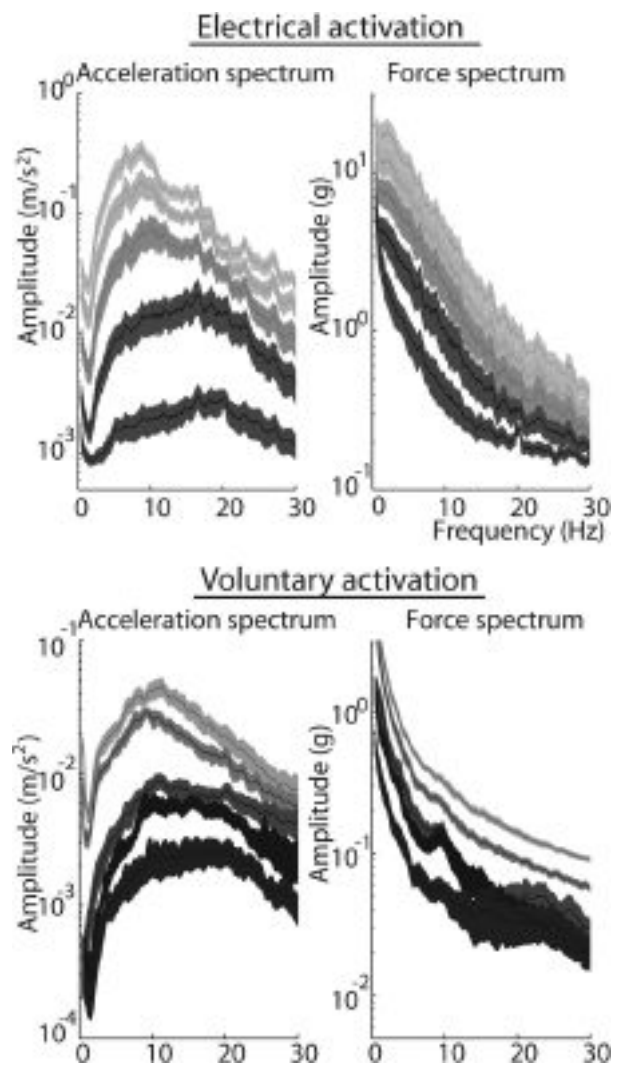


Fig 1. Frequency spectra of tremor acceleration in isotonic conditions (left panels) and tremor force in isometric conditions (right panels). Lighter grey colours represent higher levels of muscular activation. Shaded areas represent standard error.

McAuley, J. H., & Marsden, C. D. (2000). Physiological and pathological tremors and rhythmic central motor control. *Brain*, 123(8), 1545-1567.

Lakie, M., Vernooij, C. A., Osborne, T. M., & Reynolds, R. F. (2012). The resonant component of human physiological hand tremor is altered by slow voluntary movements. *The Journal of Physiology* (in press). Doi: 10.1113/jphysiol.2011.226449

Reynolds, R. F., & Lakie, M. (2010). Post-movement changes in the frequency and amplitude of physiological tremor despite unchanged neural output. *Journal of neurophysiology*, 104(4), 2020-2023.

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PC323

Multi-sensory contributions to balance control in pure cerebellar disease: Translating neuroscience from laboratory to living room

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INTRODUCTION: Balance impairment has long been associated with cerebellar damage but the cause of the impairment remains ill-defined[1-2]. SCA6 is a genetically inherited type of cerebellar disease known to feature Purkinje cell loss in the cerebellum but free from extra-cerebellar disease pathology[3]. SCA6 therefore provides an ideal human model in order to explore the cause of balance impairment in cerebellar disease. This study involves investigation of sensory control of balance in people with SCA6 in order to design future therapies targeting balance impairment.

METHOD: Sixteen subjects with SCA6 and 16 matched healthy controls underwent whole-body motion analysis using integrated Codamotion and Kistler force plate systems. Subjects balance was perturbed using moving visual scenery (MVS), vibration of postural muscles (VIB) and galvanic vestibular stimulation (GVS); targeting visual, proprioceptive and vestibular sensory channels respectively. Trials were randomly delivered and intermixed with no-stimulation trials. Ten trial repeats per stimulation type were collected. Force responses were analysed 0.2-0.4 seconds (s) following stimulation onset. Whole body sway responses were analysed between 0.2 and 1.0s following stimulation onset. Conditions were averaged over multiple trial repeats and compared between groups using t-tests in SPSS. Habituation effects were explored per stimulation type using ANOVAs (group x trial repeat).

RESULTS: All subjects generated measurable responses to all types of sensory stimuli. SCA6 response magnitudes to MVS were significantly larger than healthy controls (force, $p=0.002$; trunk sway, $p<0.001$). SCA6 response magnitudes to VIB trunk sway but not force were larger (force, $p=0.169$; trunk sway, $p=0.020$). No significant group differences were detected in response magnitudes were detected following GVS (force, $p=0.964$; trunk sway, $p=0.142$). A significant positive correlation between SCA6 response magnitude and disease severity (SARA score) was reported according to Pearson's correlation coefficients (MVS: $r=0.536$, $p=0.018$). Correlations were not significant between other stimulation types and disease severity (GVS: $r=0.021$, $p=0.940$; VIB: $r=0.378$, $p=0.149$). Habituation to the stimuli was not found following any type of sensory stimulation in either group.

IMPLICATIONS: Findings do not provide support for disrupted processing of proprioceptive or vestibular information for balance control in SCA6. Over-responses to visual stimuli appear closely associated with disease severity. Further investigation of visual control of balance in SCA6 is indicated and training avoidance of fast moving visual cues for optimisation of balance has begun to be trialled in the home environment.

Bunn L. 2010. Thesis, UCL libraries, London.

Maurtiz K. et al. 1979. *Brain*. 102:461-82.

Stevanin G. et al. 1997. *Neurology*. 49:1243-6.

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PC324

The effects of localised temperature changes on neuromuscular properties in hereditary and spontaneous Spastic Paraparesis

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Introduction

Spastic Paraparesis is caused by axonal degeneration of the corticospinal and spinocerebellar tracts and dorsal columns1. People with Spastic Paraparesis (pwSP) often report that walking ability is worse in cold weather. This study explored the effects of localised temperature changes on neuromuscular function2 and walking ability

Methods

Twenty-two pwSP were compared to 18 healthy controls. On two separate occasions water at either 7 or 37°C was pumped for 30 minutes around a wrap applied to the shank of the clinically most affected leg. Before and after the intervention measurements were taken of (a) skin and core temperature (b) maximal walking speed (c) tibial nerve conduction velocity (d) central motor conduction time (e) passive and stretch-evoked muscle stiffness of the ankle plantarflexors assessed using 150 perturbations at either 5 or 175 o/s (f) the rate of rise (MVCdt) and amplitude (MVCamp) of a maximal voluntary contraction of the ankle plantar- and dorsi-flexors.

Analysis

Results were analysed with a between groups repeated measures ANOVA with factors being temperature (cold Vs warm) and time (pre-post intervention).

Results

Compared to the control group, pwSP had a significantly slower walking speed; higher passive and stretch-evoked stiffness, lower MVCamp and MVCdt and a more prolonged central motor conduction time. Cooling and warming the limb resulted in a 12.1 ± 11.1 °C decrease and a 8.9 ± 9.9 °C increase in skin temperature respectively but no change in core temperature. In both groups cooling led to a decrease in walking speed, reduction in conduction velocity and decrease in MVCamp and MVCdt, whilst warming had the opposite effect ($P<0.05$). Greater reductions in walking speed with cooling were seen in pwSP ($P<0.05$). Reductions in MVCamp and MVCdt with cooling were significantly greater in the control group. Reductions in passive and stretch-evoked ankle stiffness were more marked with warming.

In pwSP changes in walking speed with cooling and warming were strongly related to changes in tibial nerve conduction

velocity in pwSP ($R^2=0.71$ $p<0.001$) whilst only a weak relationship was seen in the control group ($R^2=0.13$ $P=0.05$).

Conclusion

The effects of localised temperature changes on nerve conduction velocity were similar in pwSP and controls. However, these changes had a more marked impact on walking speed in pwSP. Control participants may be able to adapt their walking to local alterations in neuromuscular function to minimize the effects on walking speed whilst this adaptive ability may be less in pwSP due to the multiple impairments affecting the lower limbs in this patient group.

Salinas et al *Lancet Neurology* 2008;7:1127-1138.

Rutkove et al *Muscle and Nerve* 2001;24:867-882.

Marsden et al *Gait and Posture* 2012;35(2) 260-7

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PC324a

The ability of humans to discriminate binary taste mixtures is reduced by noradrenaline reuptake inhibition and α -2 adrenoceptor agonism

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Serotonin (5HT) and noradrenaline (NA) reuptake inhibitors increase taste sensitivity in healthy humans (1). Both 5HT and NA are hypothesised to have paracrine effects in taste buds (2,3), for example 5HT released from Type III taste cells (sour, salt) inhibits Type II taste cells (sweet, bitter, umami). The functions of paracrine actions of 5-HT and NA in the taste bud are not understood. This study investigated whether modulation of 5HT and NA altered taste contrast of Type III (salt) and Type II (sweet) taste cell modalities in healthy young adults.

Ethical approval for the study was given by the University of Bristol Faculty of Medical and Veterinary Sciences Ethics committee. Healthy subjects ($n=10$, age range 20-22yrs, female:male 7:3) gave informed consent for inclusion in the study. Taste contrast was measured by determining the participant's ability to rank 8 mixtures of increasing NaCl (0-0.6%) and constant sucrose (2%) solution (R index (4)). This task was performed before, and 1 and 2 hours after double-blind administration of placebo (lactose), serotonin specific reuptake inhibitor (paroxetine; 20mg), noradrenaline reuptake inhibitor (NARI; reboxetine, 4mg), or α -2 adrenoceptor agonist (lofexidine, 200 μ g). The cumulative R index for each participant was calculated, and linear regression analysis used to compare cumulative R index before and after drug administration (elevation of regression lines compared using ANCOVA). Stress, affect and anxiety were assessed using standard questionnaires, and participants divided into two groups on median scores for comparison of taste discrimination.

Participants were less able to correctly rank salt/sucrose mixtures compared to salt alone. After 1 hour, the ability of participants to discriminate salt in a salt/sweet mixture was only reduced by NARI ($p=0.0016$). After 2 hours, only the α -2 agonist reduced discrimination ($p=0.0087$). Less anxious participants performed better in the discrimination task than more anxious participants ($p=0.02$). Affective measures did not change with drug showing that reduced discrimination after

drug administration is not due to an acute drug-induced mood change.

Our findings suggest that NA acts to reduce the ability of healthy individuals to discriminate between Type II and III taste modalities, possibly through α -2 adrenoceptors. Furthermore, these findings suggest that reduced taste contrast may contribute to taste disturbance in anxiety.

Heath et al (2006) *J Neurosci* 26(49), 12664-71

Huang YA et al (2009) *J Neurosci* 29(44), 13909-18

Herness S et al (2002) *J Physiol* 543, 601-14

Fushan et al (2009) *Current Biology* 19(15),1288-93

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PC325

Combination of methanolic extract of mushroom (*Pleurotus pulmonarius*) and mineral/vitamin supplement reversed hyperglycemia and ameliorated the pathophysiology of diabetes mellitus in albino rats

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Diabetes mellitus is a metabolic disorder with greivous pathophysiological complications which are the major cause of mortality attributed to the disease. Various herbal remedies recommended for its treatment have however shown little effects on the pathophysiological complications caused by the disease. This study therefore investigated the hypoglycemic potentials of methanolic extract of mushroom; *Pleurotus pulmonarius* in combination with vitamins/minerals supplements and their effects on the pathophysiological complications of the diabetes mellitus in male albino rats (Sprague dawley). There were six groups ($n=5$ rats each). Diabetes was induced in five of the groups single intraperitoneal injection of 150mg/kg alloxan monohydrate dissolved in normal saline after an overnight fast, while the last group served as the control. Diabetic groups were treated orally with single and combinations of 200mg/kg methanolic extract of *P. pulmonarius* and vitamins/minerals for five days. Blood was collected by cardiac puncture into anticoagulant bottles. The effects of the treatment on blood glucose, heamatology, blood chemistry, lipid profile, liver and kidney functions were thereafter investigated using standard methods. Treatment of alloxan-induced diabetic rats with single and combinations of *P. pulmonarius* and vitamins/minerals caused significant decrease ($p<0.05$) in blood glucose concentration. Hyperglycemia was however reversed faster in rats treated with combination of both treatments compared to groups treated either of the treatments. Acute anemia, hypercholesterolemia and hypertriglyceridemia recorded in the diabetic rats were milder in rats treated with combinations of the treatments than those treated with either treatments. Ionoregulatory disruptions (hypernatremia, hyperkalemia, hypomagnesemia and hypercalcemia) were also milder in rats treated with the combination of the treaments. Indices of liver and kidney functions revealed improved phys-

iological functions of these organs in rats treated with both treatments. Result of this study affirmed the conclusion that treatment with *P. pulmonarius* supplemented with vitamins/minerals supplements ameliorated the pathophysiological complications caused by the diabetes mellitus.

Key words: diabetes mellitus, pathophysiology, treatment, protocols

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PC326

Contribution of endogenous positive and negative modulators of the human calcium-sensing receptor to its extracellular calcium sensitivity

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The calcium-sensing receptor (CaR) controls parathyroid hormone (PTH) secretion and thus extracellular calcium (Ca^{2+}_o) homeostasis. CaR studies invariably employ simple buffers lacking most of the human plasma constituents capable of modulating CaR activity. These include aromatic amino acids and spermine (positive modulators) and albumin and phosphate that bind ionised Ca^{2+} *in vivo* thus inhibiting CaR. Since their serum concentrations change under various physiological and pathological conditions, here we aimed to model the likely CaR effects of such changes. CaR activity was determined as intracellular calcium (Ca^{2+}_i) mobilisation in Fura2-loaded CaR stably-transfected HEK-293 cells, by epifluorescence microscopy. Experimental Buffer contained (mM) 20 HEPES (pH 7.4), 125 NaCl, 4 KCl, 1 PO_4 , 0.5 CaCl_2 , 0.5 MgCl_2 and 5.5 glucose.

Chronic kidney disease (CKD) involves hyperphosphataemia and raised PTH secretion, but the causal mechanism of secondary hyperparathyroidism (SHPT) remains controversial. Here 3mM Ca^{2+}_o elicited robust, oscillatory Ca^{2+}_i mobilisation in the absence of phosphate, whereas co-treatment with 2mM phosphate abolished the response. Interestingly, in Ca^{2+}_o -free buffer where CaR was activated with neomycin (100 μM), 2mM phosphate still inhibited CaR activity.

Next, CaR calcium sensitivity was tested in the absence and presence of supplementation with 4% (w/v) bovine serum albumin, 1mM phosphate, 1mM MgCl_2 and 100 μM each of L-His, L-Phe, L-Trp and spermine (each equivalent to physiological concentrations). The EC_{50} for the Ca^{2+}_o -induced CaR response in experimental buffer ($3.0 \pm 0.3\text{mM}$, S.E.M) was unchanged following supplementation ($2.9 \pm 0.1\text{mM}$; N=4) suggesting that the endogenous supplements may have, in effect, cancelled each other out. However, further increasing the phosphate concentration to 2mM (as seen in CKD), caused a rightward shift in the concentration effect curve for Ca^{2+}_o ($3.9 \pm 0.3\text{mM}$; $P < 0.05$ by ANOVA, N=5). In contrast, doubling the spermine concentration to 200 μM failed to alter CaR Ca^{2+}_o sensitivity ($3.0 \pm 0.2\text{mM}$; N=3) suggesting that physiological variations in blood spermine concentration may not affect CaR activity.

In conclusion, while CaR experiments should take greater account of endogenous modulators, the current use of simple salt solutions produces appropriate EC_{50} values. In addition, while SHPT is often linked to low vitamin D levels or direct effects of phosphate on parathyroid cells, these data suggest that decreased CaR activation resulting from the hyperphosphataemia of CKD may play a much greater role than previously realised.

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PC327

Effects of chronic peripheral administration of Apelin-13 water intake, food intake and body weight in rats

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Apelin (AP), a novel peptide, is an endogenous ligand for the G-protein-coupled receptor, APJ. AP and APJ mRNA have been found to have a widespread distribution in both the central nervous system and the peripheral tissues. AP and its receptor has also been detected in the arcuate and paraventricular nuclei of the hypothalamus, which are involved in the control of feeding behavior and energy expenditure. It is reported that intracerebroventricular (icv) injection of AP-13 caused a decreased food intake in fed but not in fasted rats, while daytime administration of AP-12 stimulated feeding. Considering the controversy, the physiological significance of these findings is unclear. The present study was designed to investigate the effects of chronic peripheral administration of AP-13 on food intake, water intake and body weight in rats. The animals (Sprague Dawley rats, male, 180-200g) were adapted to metabolic cages for a period of 5 days before the experiments. AP-13 [30, 100 and 300 $\mu\text{g/kg}$ (per group, n=8)] or vehicle was administered i.p. for 10 days at the onset of the dark cycle. Metabolic measurements were performed at 24 hours after vehicle or AP-13 administration. All data are reported as mean values $\pm$ SEM, compared by ANOVA. Metabolic measurements revealed that AP administered groups had significant increase food intake (17.2 ± 0.2 , 18.0 ± 0.2 , $18.2 \pm 0.2\text{g}$. per 100g body weight, respectively; AP30, AP100, AP300) compared with the vehicle (16.2 ± 0.3) but not in body weights. Additionally AP treated groups had increased water intake (8.2 ± 0.1 , 8.0 ± 0.1 , $8.1 \pm 0.1\text{ml}$ per 100g body weight, respectively) compared the vehicle (7.3 ± 0.1). Our data suggest that AP-13 is a peptide that stimulates food and water intake in rats. AP may have an important role between peripheral and central mechanisms involved in feeding. So that, the understanding the exact role of AP-13 in regulation of food intake and body weight control is fundamental to establishing effective therapies for obesity.

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PC328

The developmental priming of severe fatty liver disease involves alterations in core clock gene and mitochondrial Sirtuin gene expression

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The on-going obesity epidemic has seen an increase in the prevalence of non alcoholic fatty liver disease (NAFLD). We have previously shown that maternal high fat diets (mHFD) can increase the susceptibility of developing a severe form of NAFLD (non-alcoholic steatohepatitis or NASH) in adulthood, through increased lipogenesis gene expression and mitochondrial dysfunction. Components of the endogenous molecular "clock" network transcriptionally regulate genes involved in mitochondrial and fatty acid metabolism through time-of-day dependant negative feedback loops. Therefore we investigated whether early HF exposure in rodents could disrupt the expression of hepatic clock genes (Cry1, Cry2) and genes important for mitochondrial fatty acid metabolism (Sirt3), to cause metabolic changes that promote NASH development in adulthood. Rodent dams were fed a control (C) or HF diet before and during gestation and lactation. Offspring were fed either the C or HF diet after weaning to generate 4 offspring groups: HF/HF, HF/C, C/HF and C/C. Livers of 15 week old male offspring were obtained during the day (ZT8 = 3 pm) and night (ZT20 = 3 am). Expression of the genes: Cry1, Cry2 and Sirt3 were measured by quantitative PCR. We observed a significant reduction (3.3-fold decrease) in night-time Cry1 expression in the HF/C offspring ($p < 0.05$). In addition, mHFD exposure (HF/C offspring) appeared to reverse the canonical day-night gene expression pattern in these offspring, and there was a trend towards elevated night-time Cry2 expression ($p = 0.1$). Moreover, there was a significant reduction (12.5-fold decrease) of night-time Sirt3 gene expression in the HF/HF offspring ($p < 0.05$). In summary, these results illustrate that exposure to a mHFD induces day-night changes in hepatic core clock gene expression. We suggest that lower expression of Cry2 during the day in offspring exposed to a mHFD may result in altered transcriptional regulation of other clock components resulting in downstream perturbations in hepatic metabolism. In addition, mHFD exposure coupled with HF exposure in later life decreased Sirt3 expression. This may provide a potential mechanism underlying the developmental priming of mitochondrial impairment leading to NASH onset.

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Brain c-Fos-like immunoreactivity in rats anticipating a palatable food reward

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In order to adapt to their environment animals need to anticipate when and to what extent food will be available. Using c-

Fos-like immunoreactivity (Fos-ir) we have previously shown that a variety of rat brain regions are activated before and during access to bland food under a restricted feeding schedule. Here we conditioned rats to expect a palatable food reward in addition to their normal bland food. We mapped neural activity using Fos-ir in two experimental groups - a group that received the expected reward and one that did not.

Two groups of adult male Sprague Dawley rats were conditioned to expect daily restricted access to a palatable food reward. The temporary removal of normal food and water 30 min prior acted as a cue for the reward. Rats had access to the reward for 30 min then bland food and water was replaced 30 min after the reward was removed. This protocol was repeated daily for 10 days. On the 11th day, group 1 received the expected reward and group 2 did not. 60 min after receipt or non-receipt of the reward, the rats were anaesthetised (sodium pentobarbitol, 400mg/kg ip) and perfused transcardially with histological fixative. Brains were removed, post-fixed, sectioned and processed for identification of Fos-ir.

In the expectation-receipt group, Fos-ir was observed in brain regions involved in the control of food intake, reward and spatial memory. Fos-ir was observed in the hypothalamus, specifically in the supraoptic nucleus (SON), the arcuate nucleus, the lateral hypothalamus and the ventromedial hypothalamus, but not the paraventricular nucleus (PVN). In regions associated with reward detection and processing, Fos-ir was observed in the substantia nigra, the nucleus accumbens, the medial forebrain bundle, the Islands of Calleja, olfactory tubercle and the medial amygdala. In regions associated with memory, Fos-ir was observed in the mammillary bodies and the dentate gyrus. In rats expecting but not receiving the reward the distribution of Fos-ir was similar to that observed in rats receiving the reward. However, in the SON and mammillary bodies of these rats there was a trend to a reduction in Fos-ir compared to rats that received the expected reward (SON: 26.9 ± 13.8 v. 4.8 ± 3.3 Fos-ir cells/section, $p = 0.16$; mammillary bodies: 53.6 ± 20.4 v. 19.2 ± 5.7 Fos-ir cells/section, $p = 0.14$, both $n = 5$).

In summary, rats anticipating a palatable food reward in addition to their normal bland food show widespread neural activity in brain regions associated with the control of feeding, reward and memory. Furthermore, receipt of the reward results in increase in Fos-ir in brain regions associated with satiety and memory.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC330

Acetate reduces NEFA release under basal and isoproterenol stimulated conditions in 3T3-L1 cells

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Background:

Lipolysis is the pathway responsible for triacylglycerol breakdown into glycerol and non-esterified fatty acids (NEFA) in adipose tissue. Perturbation of this process has been observed in vivo in studies examining the oral administration of short

chain fatty acids (SCFA) (3). SCFA, such as acetate, can also increase the concentration of plasma acetate after the consumption of alcohol and carbohydrate-rich meals (1-2), but there is limited tissue-specific evidence for the effects of SCFA on adipocyte lipolysis.

Aim:

The aim of this study was to examine the effects of the short chain fatty acid, acetate, on the release of NEFA in adipocytes.

Methods:

The adipose cell line 3T3-L1 was used in all experimental conditions at 7 days post-differentiation. Cells were incubated in a basic media of DMEM with 2% fatty acid-free BSA (Control) for 180 min with either, the β -adrenergic receptor activator isoproterenol (ISO) (5 μ M), acetate (4mM) and acetate (4mM) plus insulin (1 μ g/ml). In the conditions where acetate was present cells were pre-incubated for 30 min with acetate. Samples of the media were collected every 60 min and analysed for NEFA concentration and all samples were normalised to total protein.

Results:

In the presence of acetate, NEFA release decreased and was lower at 120 and 180min compared with the control (Figure 1). A similar effect was observed in the presence of insulin plus acetate where NEFA release was lower compared with the control at 120 and 180 min (Figure 1).

Under the conditions of stimulated lipolysis, ISO increased NEFA release by ~20 fold compared with the basal NEFA release over a 3 hour period (Figure 2). In the ISO-stimulated cells acetate inhibited NEFA release, and by 180 min this had been reduced to about 40% compared with ISO alone (acetate, 0.78; ISO, 1.33 μ mol/mg protein). Insulin also inhibited NEFA release in the ISO-stimulated cells at 120 and 180 min.

Conclusions:

These data demonstrate that acetate has a small inhibitory effect on the basal release of NEFA. Under conditions of adrenergic stimulation the effect of acetate is similar to insulin in inhibiting NEFA release. Future work identifying the mechanism(s) of action would lead to a greater understanding of adipocyte metabolism and function and provide further insight into the abnormal metabolism associated with disorders, such as obesity and type II diabetes.

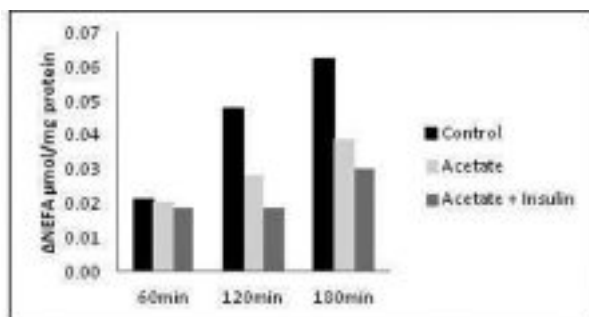


Figure 1 The change in NEFA concentration (μ mol/mg protein) in the control, acetate only and acetate plus insulin conditions. Preliminary Data, n = 2 in triplicate

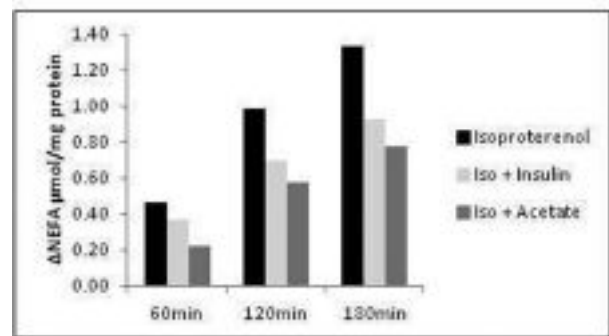


Figure 2 The change in NEFA release (μ mol/mg protein) in stimulated cells with ISO plus insulin and ISO plus acetate. Preliminary data, n = 2 in triplicate.

1. Bloemen JG, Venema K, van de Poll MC, Olde Damink SW, Buurman WA, and Dejong CH. Short chain fatty acids exchange across the gut and liver in humans measured at surgery. *Clinical Nutrition* 28: 657-661, 2009.
2. Peters SG, Pomare EW, and Fisher CA. Portal and peripheral blood short chain fatty acid concentrations after caecal lactulose instillation at surgery. *Gut* 33: 1249-1252, 1992.
3. Smith GI, Jeukendrup AE, and Ball D. Sodium Acetate Induces a Metabolic Alkalosis but Not the Increase in Fatty Acid Oxidation Observed Following Bicarbonate Ingestion in Humans. *The Journal of Nutrition* 137: 1750-1756, 2007.

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PC331

Short chain fatty acids stimulate the release of gut hormone peptide YY from human primary enteroendocrine L cells

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Obesity and its associated metabolic diseases are a major public health burden. Consumption of dietary fibre is associated with appetite suppression, reduced weight gain and elevated plasma levels of the anorectic gut hormones peptide YY (PYY) and glucagon-like peptide-1 (GLP-1). Bacterial fermentation of dietary fibre in the colon yields short-chain fatty acids (SCFAs) including acetate (C2), propionate (C3) and butyrate (C4). In humans, luminal concentrations of SCFAs are low in the small intestine but higher than 100mmol/L in the colon, and rise even further following increased fibre consumption. SCFAs have recently been shown to stimulate GLP-1 release from mouse primary enteroendocrine L cells via the FFAR2 receptor. The effects of SCFAs on gut hormone release from human L cells have not been demonstrated.

To investigate the effect of SCFAs on PYY release from human L cells, a primary human colonic cell model was developed. Colonic crypts were isolated from healthy human colonic tissue obtained from patients undergoing diagnostic colonoscopy. All participants provided informed, written consent (2000/5795). Colonic crypts were isolated following repeated digests using 0.4mg/ml collagenase at 37°C. The colonic crypt cultures were incubated for 2 hours with increasing concentrations (0, 100, 200 and 400mmol/L) of SCFAs. PYY levels in supernatants and lysed cells were measured by radioimmunoassay and percentage PYY release was calculated. Cell viability following the treatments was confirmed using a

lactate dehydrogenase cytotoxicity assay. Values represent means $\pm$ S.E.M. ($n=4-6$), compared by one-way ANOVA. All three SCFAs significantly increased PYY release from human colonic L cells compared to control (400mmol/L acetate and butyrate vs. control, $P<0.05$; 400mmol/L propionate vs. control, $P<0.01$). At 200 and 400mmol/L, propionate increased PYY secretion 2-fold and 3-fold compared to control, respectively ($7.4\pm1.2\%$ and $13.8\pm1.3\%$ PYY released vs. $4.1\pm0.5\%$). Similarly, 200 and 400mmol/L acetate also increased PYY secretion 2-fold and 3-fold, respectively, compared to control ($6.5\pm1.6\%$ and $10.5\pm1.9\%$ PYY released vs. $3.6\pm1.3\%$). These data, obtained using a novel human colonic cell model, demonstrate for the first time that SCFAs act directly on human primary colonic cells to stimulate the release of the anorectic gut hormone PYY. These results offer a mechanism by which dietary fibre may suppress appetite and reduce food intake in humans.

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PC332

Hypoxia-inducible factor-mediated enhancement of angiogenesis reduces the delay of bone defect healing caused by mechanical unloading

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The delay of fracture healing may expose elderly persons to the higher risk of osteoporosis or, in the worst case, put them into a bedridden state. Angiogenesis, a requisite for fracture healing, is regulated by angiogenic factors such as vascular endothelial growth factor (VEGF), and its expression is mediated by hypoxia inducible factor (HIF). This study was undertaken to test the hypothesis that stabilizing HIF expression reduces the unloading-induced delay of bone repair through the enhancement of angiogenesis. Angio- and osteogenesis within a rat cortical bone defect at early healing stages were examined by k-edge subtraction CT using synchrotron light and a newly developed zirconia-based vascular contrast-casting agent (Zr-CA). Under pentobarbital anesthesia (40 mg/kg ip), rats ($\varnothing$, 12 wk) receiving a drill-hole surgery on a tibial diaphysis were divided into three groups undergoing no treatment (C), hindlimb unloading by tail suspension (HU), and HU with desferrioximine administration (HU-DFO). Desferrioximine, an agent to stabilize HIF, was injected close to the defect site (200 μ M, 20 μ L) from postoperative day 1 on alternate days. At postoperative day 5 (DAY5: C, $n=10$; HU, $n=13$; HU-DFO, $n=11$) or 10 (DAY10: C, $n=10$; HU, $n=11$; HU-DFO, $n=11$), each rat was anesthetized again (pentobarbital, 40 mg/kg ip) and perfused with Zr-CA from the abdominal aorta. The rats were then euthanized with KCl administration (3 M, 0.5 ml iv) and immersed in ice-cold water for solidifying Zr-CA. The defect site was scanned at SPring-8 (Harima, Japan) with 17.9 and 18.1-keV X-ray, below and above the zirconium k-edge, respectively, and the two scan data sets were reconstructed with 2.74- μ m voxel resolution. Taking advantage of contrast

enhancement of vascular image at 18.1 keV, vascular and bone images were obtained separately by subtraction between 17.9 and 18.1-keV images after 3D-registration based on mutual information. Volume fractions of bone (B.Vf) and vasculature (V.Vf) were calculated, and values (means $\pm$ S.E.M.) were compared between groups at each healing stage by the Kruskal-Wallis test followed by Dunn's multiple comparison test. At DAY5, B.Vf [%] was higher in HU-DFO (1.50 ± 0.38) than in HU (0.28 ± 0.08) ($p<0.05$) although both values were lower than B.Vf in C (3.60 ± 0.78) ($p<0.05$). V.Vf [%] tended to be lower in HU (4.48 ± 1.31) than in C (7.37 ± 2.00) but did not differ between HU-DFO (8.30 ± 1.17) and C. At DAY10, bone occupied a larger space than vasculature. B.Vf was lower in HU (26.0 ± 2.14) than in C (43.9 ± 1.93) ($p<0.05$) but similar between HU-DFO (39.5 ± 1.21) and C. V.Vf did not differ between HU-DFO (6.22 ± 0.78) and HU (5.01 ± 0.74) and both values were lower than V.Vf in C (8.10 ± 0.92) ($p<0.05$). These results suggest that poor angiogenesis at a very early stage of healing is involved in the delay of bone defect filling under hindlimb unloading and that the enhancement of angiogenesis through the HIF-VEGF pathway reduces this delay of bone defect healing.

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PC333

Tibolone has anti-inflammatory effects in estrogen deficient state

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Cardiovascular and immune system abnormalities have been reported in females with estrogen deficiencies. To control these disorders in post-menopausal women, hormone replacement therapy (HRT) has been used. In the present study, we investigated the effects of HRT using estradiol or tibolone on the natriuretic peptide system and pro-inflammatory cytokines in ovariectomized rats. The procedures were carried out in compliance with the guidelines for the ethical use of animals in scientific research and were approved by the Ethical Committee of the Federal University of Espírito Santo. All of the surgical procedures were carried out under ketamine (70 mg/kg i.p.) and xylazine (10 mg/kg i.p.) anesthesia. Female rats were divided into four groups ($n=7$): SHAM, ovariectomized (OVX), OVX treated with 17 β -estradiol (OVX+E: 14 days 0.5 mg/kg/day) and OVX treated with tibolone (OVX+T: 14 days 1.5 mg/kg/day), 21 days after ovariectomy. On day 35, blood was collected to determine atrial natriuretic peptide (ANP) and cytokine (IL-6 and TNF- α) levels. In addition, selected tissues were collected for determining ANP, natriuretic peptide receptor type-A (NPR-A) and type-C (NPR-C) gene expression levels by RT-PCR. Values are means $\pm$ S.E.M., compared by ANOVA. The cytokine levels of IL-6 and TNF- α were increased in ovariectomized rats (OVX, IL-6: 32.80 ± 5.00 and TNF- α : 31.00 ± 5.00 vs SHAM, IL-6: 19.30 ± 6.00 and TNF- α : 20.30 ± 2.00 pg/ml). Here, we show that treatment with estradiol or tibolone decreased cytokine levels, except the level of IL-6 in the OVX+T group. In the ovariectomized, vehicle-treated rats, the plasma ANP concentrations were lower than in the SHAM

group (OVX: 263.30 ± 53.30 pg/ml vs SHAM: 92.50 ± 19.50 pg/ml, $P < 0.05$). Treatment with estradiol or tibolone significantly increased the levels of ANP in the plasma of ovariectomized rats (OVX+E: 247.30 ± 29.90 ; OVX+T: 285.70 ± 60.40 pg/ml, $P < 0.05$ vs. OVX). Left atrium tissue (SHAM: 5.68 ± 0.35 vs OVX: 3.48 ± 0.40 microg/mg protein, $P < 0.05$) and plasma (OVX: 263.30 ± 53.30 pg/ml vs SHAM: 92.50 ± 19.50 pg/ml, $P < 0.05$) ANP concentrations were decreased after ovariectomy, as well as, ANP mRNA levels in the left atrium (OVX 40.00 ± 10.10 AU vs SHAM: 91.30 ± 4.40 AU) and the NPR-A mRNA levels in the kidney (OVX: 0.68 ± 0.11 vs SHAM group 12.00 ± 5.20 AU; $P < 0.05$). HRT normalized these parameters, except the levels of atrial ANP in the OVX+T group. The NPR-C mRNA levels in the kidney did not change. These data indicate that ovariectomy impairs the natriuretic peptide system and what appears to be one of the pathways that induces increased the levels of pro-inflammatory cytokines in this experimental model. Furthermore, our data suggest for the first time that treatment with tibolone has anti-inflammatory effects by influencing the natriuretic peptide system and the levels of TNF- α .

CNPq, CAPES and FAPES

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC334

Differential contribution of microglia activation to neuropeptide-Y and pro-opiomelanocortin neurons of the arcuate nucleus

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It has been recently demonstrated that inflammatory responses in the brain are associated with a number of metabolic diseases, including high fat diet-induced obesity. Still, the precise cellular sources and targets during the inflammatory processes in the brain are poorly understood. Microglia cells, the main immune resident cells in the brain have been shown to be activated in the arcuate nucleus (ARC) of high fat diet-induced obesity, producing pro-inflammatory mediators. However, the downstream consequences on ARC neuronal activity have not yet been investigated. In this study, we evaluated the effects of microglia TLR4 (toll-like receptor 4) activation (by LPS, lipopolysaccharide) on NPY (hypothalamic neuropeptide Y) and POMC (pro-opiomelanocortin) ARC neurons firing activity. Conventional whole-cell patch-clamp recordings in brain slices were obtained from ARC neurons (lateral-POMC neurons and medial-NPY neurons portion of ARC), or from NPY neurons at ARC, obtained from control rats and eGFP-NPY transgenic mice, respectively. Current-clamp measurements of mean membrane potential and firing rate were assessed in period before, during and after bath-applied LPS (10ug/ml, an agonist of TLR4) and minocycline (100uM, an agent that inhibits activated microglia). All values are expressed as means $\pm$ SEM (n=cells). Results were compared using paired t tests or ANOVA. Differences were considered statistically significant at a $P < 0.05$. In the current study, we report that microglial activation with bath-applied LPS induced an increase in the firing activity in most 56% of neurons at lateral ARC, which was underlied by membrane depolarization ($P < 0.05$, $n = 16$). These effects were not change when microglia cell were inhibited (58%,

$n = 19$). On the other hand, LPS induced a decrease in the firing discharge in most 53% of neurons at medial ARC, which was underlied by membrane hyperpolarization ($P < 0.05$, $n = 15$). Moreover, the inhibition of activated microglia cell reverts the effects of LPS showing increase in the firing activity in most 48% of neurons at medial ARC, which was underlied by membrane depolarization ($P < 0.05$, $n = 23$ cells). Similar results were observed in 77% of eGFP-NPY neurons showed a decrease in the firing activity ($n = 18$). In the majority of these cases, LPS effects on NPY neurons were prevented by the microglia inhibitor minocycline (33%, $n = 9$). These results indicate that activation of TLR4 by LPS induced contrasting effects on ARC neuronal activity. Thus, while TLR4 activation inhibited NPY neuronal activity, likely via activation of microglia cells, an excitatory effects was observed in putative POMC neurons, which was independent on microglia activation. These contrasting effects mediated by TLR4 activation would contribute to an overall anorexigenic effect during an inflammatory process.

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PC335

Reproductive parameters and oxidative status of male Sprague Dawley rats treated with low and high salt diet

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Diet and nutrition is a crucial part of reproductive health. Deficiency of minerals and micronutrients has been reported to impair the process of spermatogenesis (Smith and Akinbamijo, 2000). Historically, salt has been used by women on their husbands to increase their libido (Moiner and Drueke (2008). The present study was designed to determine the effect of low salt diet and high salt diet on sperm parameters, oxidative status and reproductive hormone levels of male rats. Eighteen healthy Sprague Dawley male rats weighing between 120–130g were used. The rats were divided into three groups of six rats each. Group I: (control) received 0.3% salt diet, Group II: low salt (received 0.14% salt diet), and group III: high salt (received 8% salt diet). All animals were treated for six (6) weeks after which they were sacrificed by cervical dislocation. Sperm analysis was done on sample derived from the cauda epididymis by conventional methods. Sperm motility was done and expressed as percentage (Zemjanis 1977). The reduced glutathione (GSH) content of the testis and epididymis homogenate were determined using the method described by Van Dooran (1978). Malondialdehyde (MDA) was determined based on its interaction with thiobarbituric acid (TBA). The activity of the superoxide dismutase (SOD) enzyme in the testis homogenate was determined according to the method described by Sun and Zigman (1978). Serum samples were also collected and an enzyme linked immunoassay (EIA) system was employed to determine testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels. Values are means $\pm$ S.E.M., compared by ANOVA.

This study reports a decreased sperm count in the low salt diet rats (44.06 ± 1.3) and increased sperm count in the high salt diet treated rats (59.6 ± 2.3) Vs control (51.45 ± 2.2). Both low salt and high salt diet treated rats had a significant increase in percentage abnormal sperm cells when compared with con-

trol values at $P < 0.05$ (12.6 ± 1.2 , 9.9 ± 1.3 respectively Vs 5.46 ± 0.8)

The level of MDA significantly increased while GSH significantly decrease in the epididymis of both low and high salt treated diet ($P < 0.05$). Also a significant decrease in the activities of SOD and CAT in both the testes and the epididymis were observed in both treated groups. The increased MDA levels in the epididymis and decrease in antioxidant status in both salt diets indicates the presence of oxidative stress thus both low and high salt diet might play a negative role in male rats reproduction/fertility.

Table 1: LH, FSH and Testosterone levels in control, Low and High Salt treated Rats.

Parameter	Control	Low salt	High salt
FSH (mIU/L)	7.2 ± 0.21	7.25 ± 0.22	$99.9 \pm 0.57^*$
LH (mIU/L)	9.85 ± 0.29	9.39 ± 0.62	$6.28 \pm 0.38^*$
Testosterone (nmol/L)	9.9 ± 0.82	12.1 ± 0.45	$18.7 \pm 2.9^*$

1. Smith OB and Akinbamijo OO. Micronutrients and reproduction in farm animals. Anim. Reprod. Sci. 2000; 60-61:549–560.

2. Moirer BM and Druke TB. Aphrodite, sex and salt – from butterfly to man. Nephrol Dial Transplant 2008; 0: 1-8.

Zemjanis R. Collection and evaluation of semen. In: Diagnostic and Therapeutic Techniques in Animal Reproduction. William and Wilkins company 1977, Baltimore, USA, p. 242

Sun M and Zigman S. An improved spectrophotometric assay for Super-oxide dismutase based on epinephrine autooxidation. Anal Biochem. 1978; 247(10): 82-89.

Van Dooran R, Liejdekker CM and Handerson PT. Synergistic effects of phorone on the hepatotoxicity of bromobenzene and paracetamol in mice. Toxicol. 1978; 11: 225-233.

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PC336

Assessment of inflammatory status in the islets of Langerhans of solid Ehrlich carcinoma bearing- mice

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The cancer cachexia syndrome, which affects a large proportion of patients with solid tumors, is associated not only with decreasing quality of life but also with a shorter survival time and poor therapy response (1). The imbalance in glycemic homeostasis and the increase of inflammatory response caused by tumoral development are among the main factors involved in the establishment of the cachetic syndrome (1). However, there are no studies that evaluate changes in the endocrine pancreas that may interfere with the imbalance of glycemic control in individuals with cachexia. In previous studies we noted the establishment of cachexia in solid Ehrlich carcinoma bearing-mice. These animals have reduced insulin secretion and decrease in expression of key proteins in insulin signaling pathway, like PKA- α , M3 receptor and AKT. The purpose of this study was to evaluate components of islet inflammatory response of cachetic solid Ehrlich carcinoma bearing-mice that could be involved on decrease in insulin secretion. For this, 40 male adult Swiss mice were divided in two groups. The first group was subcutaneously inoculated with solid Ehrlich tumor (SET) and sacrificed 14 after tumor implantation whilst control group received saline alone (CTL). Real time PCR was performed in islets to evaluate gene expression of toll-like recep-

tors 3, 4, and 9, and Western blotting for determination of proinflammatory cytokines expression such as TNF- α , IL-1 β , IFN- α , IFN- γ , IL-6 and IL-8 (replicated 3 times). Western blotting was expressed in arbitrary units and real time PCR was expressed as % of cycles. The results were expressed as Media $\pm$ SE. Significance of the differences was evaluated by t test followed by Tukey's test. The significance level was $P < 0.05$. The results revealed that tumor bearing-mice showed a marked increase in islets protein expression of all proinflammatory cytokines analyzed when compared with CTL group (IFN- α – CTL: 3749.7 ± 628.5 vs. SET: 6752.2 ± 672.0 ; IFN- γ – CTL: 5427.5 ± 219.2 vs. SET: 7699 ± 371.0 ; IL-6 – CTL: 3332.7 ± 742.9 vs. SET: 7382.2 ± 1239.0 ; IL-8 – CTL: 4856.2 ± 703.1 vs. SET: 7792.2 ± 661.7 ; TNF- α – CTL: 5486.6 ± 430.0 vs. SET: 7078 ± 372.9). Further, the results also showed that islets of tumor bearing-mice had increase in gene expression of toll like receptors 3 and 4 but not 9 when compared with CTL group (TLR3 - CTL: 100 ± 1.04 vs. SET: 145 ± 1.52 ; TLR4 - CTL: 100 ± 1.03 vs. SET: 142 ± 1.43). Based on the present data, we can suggest an inflammation status on Islets of Langerhans of SET animals that could be involved in the decrease on insulin secretion previously demonstrated in cachetic tumor-bearing mice.

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Control of iron homeostasis during chronically elevated erythropoiesis

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The hepcidin-ferroportin axis crucially determines systemic iron homeostasis. Hepcidin (Hpc), a 25-aminoacid peptide secreted by the liver, negatively regulates the only known iron exporter ferroportin (Fpn) to reduce iron absorption and mobilization, this way balancing iron demands (1). Liver Hpc mRNA expression is modulated in response to hypoxia, anaemia, iron levels, inflammation and erythropoiesis (2), but the interactions between these signals are not well understood. This study aims to elucidate the primary signal(s) regulating Hpc expression during chronically elevated erythropoiesis. To this end, we investigated iron homeostasis in male wild type (Wt, C57BL/6, n=4) animals and in transgenic mice (Tg6, (TgN(PDGFBEPO)321ZbZ), n=5) that chronically overexpress human erythropoietin (12-fold compared to Wt) resulting in haematocrit values of about 80%. In addition, we treated Wt (Wt_DXT, n=4) and Tg6 (Tg6_DXT, n=3) animals with a single dose of iron dextran (5 mg administered intraperitoneally), and reduced erythropoiesis in Tg6 (Tg6_SPL, n=2) by splenectomy (3). Surgery was performed under isoflurane anaesthesia (5% induction, 2% maintenance, 100% oxygen) and animals

were treated with 0.075 mg/kg of buprenorphin to provide analgesia. All the procedures were approved by the Kantonal Veterinäramt. Real time-rtPCR showed that liver Hpc mRNA expression was significantly suppressed in Tg6 ($p<0.001$ vs. Wt). Iron administration resulted in a 5-fold increase in Hpc expression in Wt_DXT mice ($p=0.024$ vs. Wt) and enhanced Hpc expression in Tg6_DXT animals to levels comparable to untreated Wt mice. In addition, Hpc expression was elevated in Tg6_SPL compared to Tg6 ($p<0.01$), but remained low compared to Wt ($p=0.002$). Western blotting for the divalent metal transporter-1 revealed no changes in protein levels at the mucosal side of the duodenum. On the other hand, at the basolateral side, Fpn was upregulated in Tg6 mice (3-fold vs. Wt, $p=0.047$) and reduced to normal levels in Tg6_DXT and Tg6_SPL. Iron mobilization from liver and spleen was not altered, though Wt_DXT showed a decrease of Fpn level in the spleen ($p=0.048$ vs. Wt). The potential to acquire iron, as measured by western blotting of the transferrin receptor-1 (TfR1), showed a dramatic increase in Tg6 vs. Wt mice in liver ($p<0.001$) and spleen ($p=0.003$). In Tg6_DXT animals TfR1 was reduced to normal levels in liver ($p<0.001$ vs. Tg6), but remained elevated in the spleen ($p=0.007$ vs. Wt; ns vs. Tg6). On the other hand, splenectomy decreased TfR1 expression in the liver ($p=0.006$) whereas it remained elevated compared to Wt ($p=0.026$). Finally, L and H ferritin (Ft) subunits were increased in Wt_DXT and Tg6_DXT, whilst in Tg6_SPL only H-Ft was elevated ($p=0.04$ vs. Tg6; ns vs. Wt). Additionally, L-Ft but not H-Ft increased in Tg6_DXT in the spleen ($p<0.001$ vs. Tg6; ns vs. Wt). Overall, the data we obtained so far suggest that the body's iron content overrides the erythropoietic signal regarding Hpc regulation and, thus, is the primary signal affecting iron homeostasis during chronically elevated erythropoiesis.

Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*. 2004 Dec 17;306(5704):2090-3.

Nicolas G, Chauvet C, Viatte L, Danan JL, Bigard X, Devaux I, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. *J Clin Invest*. 2002 Oct;110(7):1037-44.

Vogel J, Kiessling I, Heinicke K, Stallmach T, Ossent P, Vogel O, et al. Transgenic mice overexpressing erythropoietin adapt to excessive erythrocytosis by regulating blood viscosity. *Blood*. 2003 Sep;102(6):2278-84.

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PC338

Valproic acid does not affect decreased insulin secretion in WFS1-deficient pancreatic islets

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Wolfram syndrome, an autosomal recessive disorder, is caused by mutations in the *WFS1* gene. *WFS1* regulates endoplasmic reticulum (ER) stress and has a role in diseases involving ER stress as in case of β -cell death in diabetes. Valproic acid (VPA) is an anticonvulsant and mood-stabilizer. There are contradictory findings whether VPA increases insulin secretion (Luef et al., 2003) or changes its metabolism in liver (Pylvänen et al.,

2006). In the study by Terasmaa et al. (2011) acute pre-treatment with VPA improved glucose tolerance in *Wfs1* knockout (*Wfs1*KO) mice, but had no effect in wild-type (WT) mice. The aim of current study was to study insulin secretion and evaluate the effect of VPA in isolated pancreatic islets of *WFS1*-deficient mice.

WT, heterozygous (*Wfs1*HZ) and *Wfs1*KO mice were used, 6 animals in each group. All studies were performed in male 5-6 months old mice. Pancreatic islets were isolated as previously described (Shimomura et al., 2009). Islets were preincubated for 1 h in Krebs-Ringer solution (KRBH) containing 2 mM glucose followed by 1 h at 37°C and 5% CO₂ in selected assay solution (KRBH + 2 mM, 10 mM or 20 mM glucose or 200 μ M tolbutamide and 2 mM glucose). Each assay media group contained 5 islets in duplicate per genotype. The supernatant was collected and insulin was measured with Ultra Sensitive Mouse Insulin ELISA Kit (Crystal Chem Inc., USA). To determine total insulin content, insulin was extracted using 95:5 ethanol:acetic acid solution (Shimomura et al., 2009). VPA was added to each assay solution with final concentration of 500 μ M. Data are presented as mean $\pm$ SD. For comparisons ANOVA followed by Bonferroni post-test was used. A *P* value of <0.05 was considered statistically significant ($P<0.05$).

WFS1-deficient mice had considerably less pancreatic islets than *Wfs1*HZ or WT mice ($P<0.001$) (Fig. 1). *Wfs1*HZ mice had also statistically fewer pancreatic islets compared to WT mice ($P<0.001$) (Fig. 1). *Wfs1*KO pancreatic islets secreted less insulin after stimulation with 2 and 10 mM glucose and with tolbutamide solution compared to WT and *Wfs1*HZ islets, but after stimulation with 20 mM glucose there was no statistically significant difference ($P>0.05$) (Fig. 2). Although VPA has been shown to increase insulin secretion in vitro (Luef et al., 2003), no such effect was seen in present study (Fig. 2).

WFS1-deficient mice have impaired insulin secretion and may therefore be a diabetes model. Current study is in accordance with studies referring that VPA does not influence insulin secretion directly, but may have an effect upstream of insulin exocytosis.

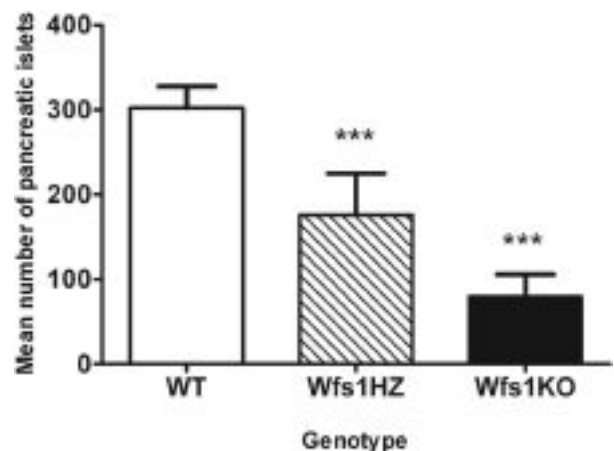


Figure 1. *Wfs1* mutant mice have significantly fewer number of isolated pancreatic islets compared to other genotypes (***) ($P<0.001$).

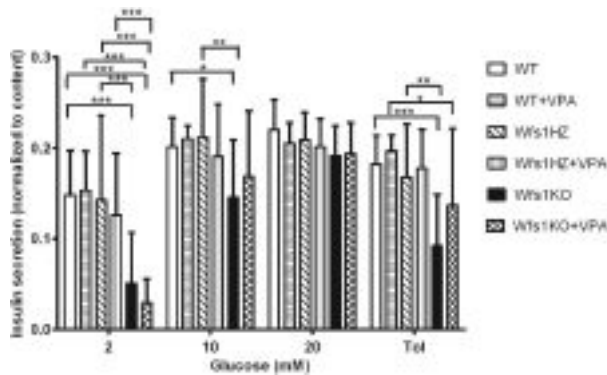


Figure 2. Insulin secretion from islets of Wfs1KO, Wfs1HZ and WT mice in response to glucose or 200 μ M tolbutamide (Tol) and in the presence of 500 μ M VPA normalized to total insulin content. (Two-way ANOVA, * P <0.05, ** P <0.01 and *** P <0.001).

Luef GJ et al. (2003). *Epilepsy Res* **55**, 53-58.

Pylvänen V et al. (2006). *Epilepsia* **47**, 1460-1464.

Shimomura K et al. (2009). *Methods Enzymol* **457**, 451-480.

Terasmaa A et al. (2011). *J Physiol Biochem* **67**, 381-390.

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PC339

Absence of melatonin alters the expression pattern of the glucocorticoid signaling pathway proteins and enzymes of hepatic energy metabolism

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The regulation of energy balance and metabolism are related to signals of circadian and seasonal rhythmicity. In this respect, it is essential the actions of melatonin and glucocorticoids in the liver, the central organ of systemic regulation of energy metabolism. Our objective was to investigate, by Western blot analysis, the main changes in the glucocorticoid pathway and the expression of enzymes of energy metabolism in the liver of male Wistar rats ($\pm$ 200g, n=8/group) subjected to surgical removal of the pineal gland after sodium thiopental (50 mg/kg, ip) anesthesia (PIN), and replaced with nocturnal melatonin in drinking water (1mg/5ml water/100g of body weight - PM) compared to control animals (CT) measured every 3 hours around the clock, starting at ZT 3 (lights on-ZT0, lights off-ZT12). Circadian variation of the results (ANOVA/ cosinor) showed that expression of glucocorticoid receptor presented ultradian rhythm and varies over a period of 8 hours in CT and PM groups, whereas the period of variation changed to 12 hours in PIN. The transcription factor DexRas and the enzyme 11 β HSD1 presented increased expression in pinealectomized animals at ZT3 (DexRas: 0.63 $\pm$ 1 for CT vs 0.9 $\pm$ 0.1 for PIN vs 0.95 $\pm$ 0.1 for PM; 11 β HSD1: 11 $\pm$ 1.5 vs 24 $\pm$ 2 vs 27 $\pm$ 5). In addition, we observed at ZT15 and 21 the lowest expres-

sion of DexRas (0.5 $\pm$ 0.1 vs 0.6 $\pm$ 0.1 vs 0.3 $\pm$ 0.1 and 0.7 $\pm$ 0.05 vs 0.6 $\pm$ 1.7 vs 0.5 $\pm$ 0.06, respectively) and highest expression of 11 β HSD1 (52 $\pm$ 3 vs 37 $\pm$ 3 vs 40 $\pm$ 3 and 41 $\pm$ 1.5 vs 51 $\pm$ 3 vs 27 $\pm$ 2 respectively), which indicates an inverse correlation between the expression of these proteins. Regarding enzymes of energy metabolism, PIN group at ZT24 showed increased expression of phosphoenolpyruvate carboxykinase (1.80 $\pm$ 1 vs 2.40 $\pm$ 1 vs 1.82 $\pm$ 1), demonstrating changes on gluconeogenesis in pinealectomized animals. Glucose 6-phosphatase (G6Pase) presented an expression peak at ZT6 in PIN (1.4 $\pm$ 0.7 vs 0.75 $\pm$ 0.1 vs 0.7 $\pm$ 0.1). At nocturnal ZTs, the expression of G6Pase in PIN and PM is diminished in all ZTs (mean of 0.7 $\pm$ 0.3 for both groups vs 1.3 $\pm$ 0.2 for CT). The expression of glucokinase follow the same patterns in all groups over the ZTs evaluated (mean of 0.6 $\pm$ 1), however there is decreased expression in PIN (0.7 $\pm$ 0.1 vs 0.4 $\pm$ 0.1 vs 0.6 $\pm$ 0.1) in the period between ZT18 and 0. Values are expressed as mean $\pm$ SEM of arbitrary units of optical densitometry and were analyzed by two-way ANOVA. In all cases, β -actin expression was used for data normalization and p $\leq$ 0.05 was adopted. These preliminary results indicate correlation between the signals of melatonin and glucocorticoids, suggesting that absence of melatonin alter the circadian pattern of protein expression of the glucocorticoids pathway as well as the enzymes of energetic metabolism.

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PC340

Novel oestradiol-sensitive microRNAs may contribute to colon carcinoma tumour promotion after loss of oestrogen receptor beta

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Colorectal carcinoma (CRC) is the third most common malignancy worldwide, and displays a lower incidence in women than in men (1). The protective role of oestrogen in CRC is associated with oestrogen receptor (ER) β expression and the loss of ER β correlates with worse prognosis (2). It is not known whether oestrogen can promote tumour progression following the loss of ER β . microRNAs (miRNAs) have the ability to repress gene expression and are believed to play an important role in development, differentiation, proliferation, survival, and oncogenesis. Pre-miRNA precursor transcript and mature miRNA can be modulated within minutes by transcription factors and represent a novel class of molecules rapidly activated by steroid hormones. miRNA expression is modulated by oestrogen, therefore, ER α and/or G-protein coupled ER (GPER)-regulated miRNAs may be detrimental in CRC.

We have investigated the ER isoform abundance and 17 β -oestradiol (E2)-responsive miRNAs, and their target mRNAs, in colon carcinoma cells. Real-time quantitative PCR analysis revealed both ER α and ER β mRNA were absent in HT29 cells (n=3). GPER mRNA was, however, abundantly expressed in T84, HT29, RKO and DLD-1 colon carcinoma cells with a decrease in GPER expression correlating with a more aggressive cell phenotype (n=3). GPER was down-regulated in colon carcinoma tumour specimens compared to normal colonic tissue (R2 analysis). Microarray analysis of 750 miRNAs and

24,000 mRNAs revealed novel E2-modulated miRNA and mRNA transcripts in HT29 cells. Eleven E2-sensitive miRNAs were significantly differentially expressed (>2-fold, $n=3$, $p<0.05$). By *in silico* analysis, E2-modulated miRNAs were predicted to target significantly differentially expressed mRNAs (>2-fold, $n=3$, $p<0.05$). In particular, tyrosine kinase substrate 4 (*SH3PXD2B*), a recently documented regulator of colon carcinoma cell invasion (3), was significantly up-regulated (>2.4-fold, $n=3$, $p=0.003$) after E2 (10nM) treatment and showed significant enrichment for E2-repressed miRNA target sites ($p=0.012$). This study shows, for the first time, that GPER mRNA is expressed in colon carcinoma cells and, by meta-analysis, correlates with colon carcinoma tumour progression. E2 modulates miRNA expression in colon carcinoma cells. Novel E2-responsive mRNAs, including known regulators of tumourigenesis, were also identified and are predicted targets for E2-modulated miRNAs. We hypothesise that miRNA induction through GPER may contribute to tumour promotion following loss of ER β expression in colon carcinoma. This work will direct future research of oestrogen-sensitive miRNAs to establish their potential value as early diagnostic and therapeutic targets in colon carcinoma.

1. Ries L, Wingo P, Miller D, Howe H, Weir H, Rosenberg H, et al. The annual report to the nation on the status of cancer, 1973-1997, with a special section on colorectal cancer. *Cancer*. 2000;88:2398-424.

2. Jassam N, Bell S, Speirs V, Quirke P. Loss of expression of oestrogen receptor beta in colon cancer and its association with Dukes' staging. *Oncol Rep*. 2005;14:17-21.

3. Gianni D, Taulet N, DerMardirossian C, Bokoch G. c-Src-mediated phosphorylation of Nox1 and Tks4 induces the reactive oxygen species (ROS)-dependent formation of functional invadopodia in human colon cancer cells. *Mol Biol Cell*. 2010;21:4287-98.

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R2 microarray analysis and visualisation platform (<http://hgserver1.amc.nl/cgi-bin/r2/main.cgi>)

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PC341

Impact of a maternal low protein diet during pregnancy on fetal amino acid availability and kidney development in sheep

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Introduction: Maternal malnutrition leads to offspring with a greater risk of developing chronic kidney disease. We have previously shown that a maternal low protein, but isoenergetic, diet impacts upon early developmental processes (decreased angiogenesis, increased apoptosis) in the fetal kidney to reduce nephron number in the mature kidney (a 15% reduction). In the current study we used this nutritional paradigm to characterise the nutritional environment of the dam and fetus at 0.44 dGA and relate the nutritional pattern to macro- and microvascular development in the fetal kidney.

Methods: Pregnant twin-bearing ewes were fed either a control diet providing adequate dietary protein (control protein, CP [16.9 g/kg/MJ DM]; $n=15$), or a low protein diet (LP, 8.7g/kg/MJ DM; $n=16$) from the day of artificial insemination with semen from a single ram (day 0) to day 65 gestation (term,

147 days). Maternal blood samples were taken at fortnightly intervals until day 65 when the ewes and conceptuses were euthanised (barbiturate overdose, 150 mg/kg BW). Tissues were fixed (4% PFA in 0.1M PBS), snap-frozen or a fetal renal vascular corrosion cast was made. Casts were scanned by computed tomography (40 μ m, Nanotom) and volumetric data quantified. Maternal and fetal plasma and amniotic amino acids were measured by GC-MS. Data are mean [s.e.d] and were analysed by linear mixed-effects models (Genstat v14, VSNi, UK).

Results: LP sheep had significantly increased plasma concentrations of glucose, lactate, albumin, total protein and NEFA relative to controls, but decreased urea (e.g. in amniotic fluid; 3497 vs. 6916 [489] μ mol/L for LP vs. CP). Maternal plasma osmolality was increased in LP vs. CP (e.g. at day 14 gestation, 320 vs. 285 [10] mosmoles/kg water). The maternal amino acid profile was unaffected by diet but changed with time; however, in fetal plasma and amniotic fluid ornithine concentration was significantly decreased by a maternal LP diet (e.g. in amniotic fluid, 74.2 vs. 109 [$\pm$ 12] μ mol/L for LP vs. CP). Fetal kidney vascular casts were not different between treatment groups, but indices of the fetal kidney microvasculature were significantly ($P=0.02$) reduced in LP vs. CP (e.g. VEGF protein abundance in nephrogenic zone; 0.79 vs. 1.32 [0.14] units).

Conclusions: A maternal low protein diet has little effect on maternal or fetal macronutrient balance but has marked effects on specific micronutrients; urea is reduced in the maternal and fetal compartment and this translates to markedly reduced plasma ornithine concentration in fetal plasma and amniotic fluid. A nutritional pathway from maternal low protein, through decreased urea-ornithine to reduced polyamine synthesis and blunted cellular growth is inferred, but remains to be tested.

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PC342

The effect of cyclic monoterpene menthol on blood glucose, water and electrolyte excretion in rats

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Rowachol (Rowa Ltd., Bantry, Eire), a proprietary choleric containing the purified mono- and bicyclic monoterpenes menthol (32% w/v), pinene (17% w/v), menthone (6% w/v), borneol (5% w/v), camphene (5% w/v), and cineole (2% w/v) in olive oil, has been shown to cause dissolution of cholesterol gallstones in man (Doran et al., 1979) and to inhibit hepatic HMG-CoA reductase in rats (Middleton et al., 1979) and man (Ellis et al., 1981) when administered *in vivo*. The present study was therefore designed to investigate the effect of menthol on water with electrolyte excretion and blood glucose concentration in rats.

Menthol was administered in olive oil by gastric tube in a volume of 2ml/kg. In determining the dose-response relation for menthol, it was given at 1.0, 3.0 and 6.0 mmol/kg of body weight in a volume of 2 ml/kg. The oral route using oro-gastric tube was used in administering the menthol. Cardiac puncture method was used in obtaining the blood samples which

were sampled only once at the end of treatment period. Water together with electrolyte excretion and blood glucose concentration were investigated using flame photometric and colorimetric techniques in rats ($n=48$). Values are means $\pm$ S.E.M. and compared by one-way ANOVA using Graph Pad prism 4. The treatment of rats with menthol resulted in a decrease in blood glucose concentration (73.0 ± 1.39 , 67.5 ± 1.41 and 63.9 ± 2.06 mg/dl) that was significant ($p < 0.05$) in all the menthol treated animals (1.0, 3.0 and 6.0 mmol/kg) compared to that of the olive oil treated control group (75.4 ± 1.10 mg/dl). There was a significant dose ($p < 0.05$) dependent increase in urine Na^+ ion level (420 ± 15.00 , 450 ± 8.86 , 480 ± 10.52 mmol) and urine output volume (150 ± 5.67 , 200 ± 9.82 , 280 ± 11.02 ml) in menthol treated animals compared to their control groups 400 ± 12.82 mmol and 135 ± 6.55 ml respectively, while urinary K^+ excretion showed no significant change ($p > 0.05$) in all the treated animal groups (86 ± 2.08 , 87 ± 2.38 , 89 ± 2.09 mmol) with respect to the control (81.5 ± 1.75 mmol). The data obtained indicates that menthol increases water intake, urine output and urine Na^+ excretion, and decreases blood glucose concentration.

Doran, J., Keighley, M.R.B., and Bell, G.D. 1979. Rowachol - a possible treatment for cholesterol gallstones. *Gut* 20, 312-317

Ellis, W.R., Bell, G.D., Clegg, R.J., Middleton, B., and White, D.A. 1981. Mechanisms for adjuvant cholelitholytic properties of the monoterpene mixture Rowachol. *Gastroenterology* 80, 1141

Middleton, A., Middleton, B., White, D.A., and Bell, G.D. 1979. The effects of monocyclic terpenes on hepatic S-3-hydroxy-3-methylglutaryl-Coenzyme A reductase in vivo. *Biochem. Soc. Trans.* 7, 407-408

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PC343

Apelin induces myometrial contractions through the involvement of protein kinase c mechanism in rat

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Apelin induces myometrial contractions through the involvement of protein kinase c mechanism in rat

Apelin, is a recently discovered peptide hormone and its receptor were found in the hypothalamus, uterus, ovary and some other peripheral tissues. We have previously demonstrated that apelin-13 has stimulatory effect on myometrial contractility in rats. Moreover, this effect turns out in calcium free condition. The purpose of this study was to investigate the possible involvement of protein kinase C (PKC) pathway in stimulation of myometrium contractility by apelin. Additionally we investigated plasma apelin levels in pregnant and lactating rats.

Adult Wistar rats were anaesthetized with ketamine (60 mg/kg, intraperitoneally) and myometrium strips were removed following decapitation. Strips placed in a jacked tissue bath containing Krebs solution at 37°C and pH 7.4, constantly bubbled with 95% oxygen and 5% carbon dioxide. They were allowed

to contract under 1g tension and isometric contractions were measured by force displacement transducer. After equilibration of spontaneous contractions, calcium free condition was constitute. Silent period (no contraction) was recorded and protein kinase C inhibitor agent, chelerythrine chloride (10 μ M), added to the organ bath. Apelin-13 at a concentration of 20 μ M was also applied 5 mins later.

In the other experimental protocol, we collected blood samples via tail vein of Wistar rats in dioestrus and in different stages of pregnancy and lactation under light ether sedation. Plasma apelin-13 concentration was determined by ELISA. Hormone concentrations were calculated as mean $\pm$ SEM and One Way ANOVA with posthoc Student-Newman-Keuls test was used for statistical analysis.

Apelin has stimulatory effect on myometrial contraction in calcium free condition. However, chelerythrine chloride pretreatment blocked the effect of apelin in same status. In the other experimental protocol, plasma apelin levels were 120.2 ± 10.9 ng/ml, 101.9 ± 14.5 ng/ml, 151.1 ± 31.7 ng/ml and 235.8 ± 46.5 ng/ml in dioestrus, and 12th, 18th and 21th day of pregnancy groups, respectively. Blood apelin concentration in 21th day of pregnancy was significantly high compared to both dioestrus and first pregnant groups ($p < 0.05$). There was a significant decrease in plasma apelin level in 2th day of lactation group (111.4 ± 19.2 ng/ml) compared to 21th day of pregnancy group ($p < 0.05$). The 10th day of lactation group had no different hormone level compared to the others (143.3 ± 13.2 ng/ml).

The present data indicate that apelin induce myometrium contraction through the possible involvement of PKC mechanism. According to the results of this study and our previous data, apelin can stimulate uterus contractions by inducing Ca^{2+} release from intracellular stores. Additionally, the elevation of plasma apelin concentration at the end of pregnancy may reveal a facilitatory role for apelin on myometrium contraction during parturition.

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PC344

The effect of heating on spontaneous and agonist-evoked contractions in rabbit mucosa-intact and denuded preparations

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The bladder may be exposed to mild heating from either external devices that deliver microwave therapies for cancer or during fever (1). The aims of this study were to determine if: 1) heating from 37°C to 41°C for 15 minutes decreased spontaneous activity of mucosa-intact bladder; 2) heating had a direct effect on detrusor muscle contractility or activation of motor nerves.

All procedures accorded with current UK legislation. Two male and ten female rabbits (4.5 ± 0.6 kg) were sacrificed. The urinary bladder was placed in gassed (95% O₂/5% CO₂) and chilled (4°C) Tyrode's solution and opened longitudinally. Strips of rabbit mucosa-intact and denuded preparations (approximate length 10 x diameter 2 mm) were mounted in a horizontal superfusion trough and tied to an isometric tension transducer connected to bridge amplifier. A thermocouple (0.2 mm diameter) was inserted into the sub-urothelial layer, and the prepa-

ration stretched to 20mN resting tension. For mucosa-intact strips a heating device was positioned <5 mm above the mucosa, perpendicular to the preparation and calibrated prior to the experiment. Denuded mucosa preparations were exposed to electrical field stimulation (EFS: 0.1 ms pulses, 8 Hz) or 0.1 μ M carbachol whilst the Tyrode's solution was heated from 37°C to 41°C and back down to 37°C. Spontaneous contraction amplitude and frequency were measured during 15 minute heat exposures. Data are medians [25, 75% quartiles], data sets were compared by ANOVA, with Bonferroni post-hoc tests, the null hypothesis was rejected at $p < 0.05$.

During 15 minutes of exposure to 41°C the mucosa-intact preparations exhibited significantly reduced spontaneous contraction amplitude (83 [48,94]%) when compared to control preparations maintained at 37°C (94 [83,119]%). Post-heating, the amplitude of spontaneous contractions returned to control within 30 minutes. During this recovery period there was a reduction in the frequency of contractions in the heated preparations in comparison to the control preparations. Carbachol (0.1 μ M) generated a rise in contraction tone of denuded preparations at 37°C and 41°C ($n=5$). The magnitude of the carbachol-induced contractions was significantly reduced at 41°C (0.7 [0.1, 1.3]mN) compared to control at 37°C (1.1 [0.3, 3.1]mN) and recovered completely on return to 37°C. (1.3 [0.4, 2.9]mN). The EFS contraction amplitude did not significantly alter during and post-heating, indicating there was no evidence of nerve block.

Mild local heating suppressed spontaneous activity of mucosa-intact tissue and global heating suppressed carbachol (0.1 μ M)-induced contraction of denuded tissue. These data suggest that mild heating affects both detrusor contractility and mucosal-dependent mechanisms that generate spontaneous contractile activity.

(1) Stelfox HT, Straus SE, Ghali WA, Conly J, Laupland K, Lewin A. (2010) Temporal Artery versus Bladder Thermometry during Adult Medical-Surgical Intensive Care Monitoring: An Observational Study. BMC anaesthesiology, 10:13.

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PC345

The impact of vitamin E supplementation on urinary bladder contractility in streptozotocin-induced diabetic rats

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Objective: To determine whether vitamin E has protective effects or not on streptozotocin-induced diabetic rats in diabetic urinary bladder dysfunction and discover its possible mechanism. **Material and Method:** A total of 40 rats were randomly divided into four groups: a control group (A), a diabetic group (B), a group given vitamin E only (C), and a diabetic group given vitamin E therapy for 8 weeks (D). Diabetes was induced in the rats by 65 mg/kg STZ via an intraperitoneal (i.p.) injection. Vitamin E was given 50 mg/kg/day i.p. Under urethane anaesthesia (1.2 g/kg) subcutaneously, contractile responses to carbachol of detrusor strips in all groups were studied *in vitro*. The levels of nitrite, nitrate, malondialdehyde (MDA), glutathione (GSH), superoxide dismutase, (SOD), cata-

lase (CAT), and glutathione peroxidase (GPx) were detected in bladder tissues homogenates. Apoptosis studies were performed by detection levels of caspase 3 and cell death detection. **Results:** The bladder weights were significantly increased ($p < 0.001$) in diabetic groups compared to the other studied groups. Contractile responses increased in the diabetic group to carbachol than in the other groups ($p < 0.001$). Vitamin E improved the contractile responses and improved them nearly to that of the control group but still significantly higher ($p < 0.05$). Vitamin E treatment decreased the tissue MDA, nitrite, nitrate and GSH levels of group D which were in group B significantly higher than those of group A and C groups ($p < 0.001$). All enzymes activities of group B were significantly lower than those of the other groups, although they increased significantly in group D but still lower than those of A and C groups. However, No significant differences were detected between the levels of GPx and SOD of group D and those of A and C groups. **Conclusions:** These data suggests that vitamin E supplementation may be beneficial in delaying the progression of diabetic cystopathy in experimental animal model.

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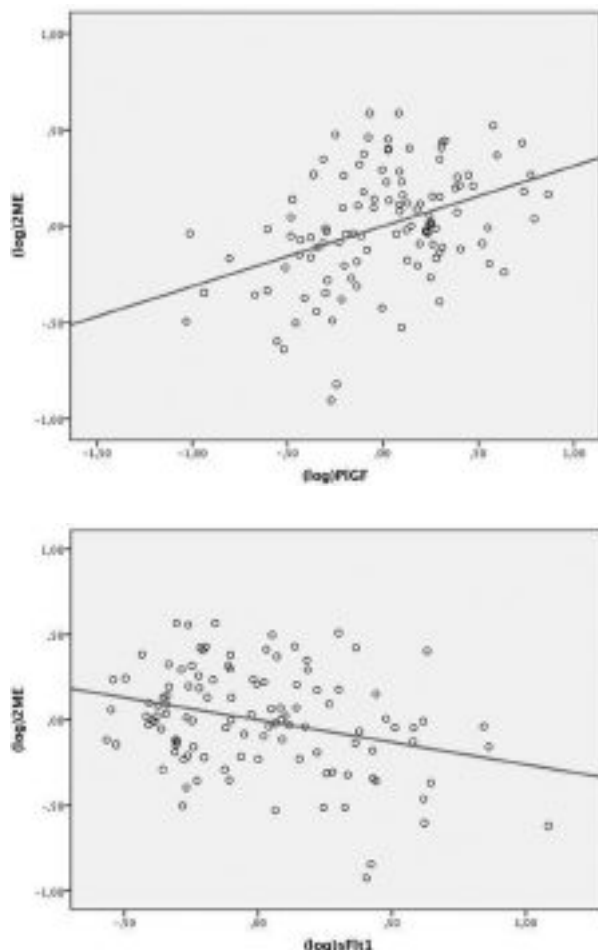
Risk of preeclampsia and COMT Val158Met polymorphism

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BACKGROUND: A functional single-nucleotide polymorphism of the gene for catechol-O-methyltransferase (COMT) results in a valine to methionine mutation at position 158 (Val158Met). The valine variant catabolizes the introduction of a methyl group to 2-hydroxyestradiol to form 2-methoxyestradiol (2ME) at a higher rate of its methionine counterpart. Preeclampsia (PE) is a major obstetric problem and a significant source of maternal and fetal morbidity and mortality (Sibai, 2005), complicating an estimated 2% of all pregnancies (Duley, 2003). Several studies have suggested that the main features of PE are consequences of endothelial dysfunction related to excess circulating anti-angiogenic factors, most notably, soluble sVEGFR-1 (sFlt-1) and as well as to decreased placental growth factor levels (PlGF) (Munaut, 2012). A key factor connecting the angiogenic imbalance with generalized endothelial dysfunction in PE has been suggested to be a reduction in the synthesis of 2ME (Kanasaki, 2008). **AIM:** We investigated the relationship between COMT Val158M polymorphism and the risk of preeclampsia. **METHODS:** A total of 54 preeclamptic women and 72 normotensive women were enrolled in a prospective study. We gathered information on maternal history with both maternal and placental COMT Val158Met polymorphism and blood biomarkers of pregnancy hypertensive disorders. COMT Val158Met polymorphism was genotyped by quantitative fluorescent-polymerase chain reaction. Serum levels of maternal PlGF and sFlt-1 and plasmatic levels of 2ME were measured by ELISA. Student's t-test was used to compare 2ME means levels between case and control

subjects. Multiple logistic regression analysis was used to estimate the adjusted risk of preeclampsia according to levels of 2ME and genotypes. Multiple lineal regression was used to explore the adjusted association between 2ME and both PIGF and PLGF levels respectively. Covariates taken into account in all the analyses: smoking habit, body mass index, maternal age and nulliparity. RESULTS: Controls had a significantly higher levels of plasmatic 2ME than preeclamptics ($t=-4.45$, $p<0.001$) (2697.03 ± 188.34 vs 1878.26 ± 183.52 pg/m). The adjusted odds ratios (adjOR) was 0.05 [95%, Confidence interval (CI):0.01,0.25] with a p -value <0.001 . The adjOR for maternal met/met vs not-met/met was 1.54 [95% CI: 0.57,4.17] with a p -value=0.390. Fetal met/met vs not-met/met was 3.27 [95%CI: 1.06,10.09] with a p -value=0.039. 2ME levels were significantly negatively associated with sFlt-1 [$B=-0.26$, [95% CI: -0.42,-0.11)]; $p=0.001$] and significantly positively associated with PIGF levels [$B=0.31$, (95% CI: 0.18,0.45); $p<0.001$]. CONCLUSION: These findings suggest that homozygosity for fetal COMT met/met genotype and reduced 2ME levels may increase susceptibility to preeclampsia.



Sibai B, et al. (2005). Lancet; 365(9461):785-99.

Duley L. (2003). Br Med Bull.; 67:161-76.

Munaut, (2012) PLoS One;7(3):e33475.

Kanasaki K, et al. (2008) Nature.; 453(7198):1117-21.

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A role for large conductance calcium activated potassium channels (BK_{Ca} channels) in L-arginine transport and vasorelaxation induced by insulin in human feto-placental unit

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Insulin increases nitric oxide (NO) synthesis via the endothelial NO synthase (eNOS), and L-arginine transport via the cationic amino acid transporters (hCATs) in human umbilical vein endothelial cells (HUVECs) (González *et al.* 2004). Ex vivo experiments demonstrated that insulin causes relaxation of human umbilical vein by an endothelium- and hCATs-dependent mechanism, under acute (30 min) or chronic (8 h) incubation (González *et al.*, 2011). We here investigated the involvement of large conductance calcium activated potassium channels (BK_{Ca} channels) in the acute effects of insulin in the vascular reactivity of human chorionic veins, and on effects of insulin on L-arginine transport in HUVECs. Chorionic vein rings (368±18 µm diameter) and HUVECs were isolated from normal pregnancies (ethics committee approval and informed patient consent were obtained). Vessel rings were mounted on a wire myograph using standar condition (Wareing *et al.*, 2006). Contractions were standardized using 90 mM KCl. Vessels were washed, stabilized and pre-treated (30 min) with insulin (10 nM) and/or tetraethylammonium (TEA, 5 µM, K⁺ channels blocker) or iberiotoxin (IbTx, 100 nM, BK_{Ca} channels blocker). Then vessels were constricted by adding hydrogen peroxide (H₂O₂) (0.01-1 mM). HUVECs were isolated by collagenase digestion (37 celsius degree) and cultured in medium 199 (M199) supplemented with 20% newborn and fetal calf sera. After incubation (1-30 min) with insulin (1 nM), TEA and/or IbTx, L-arginine transport (100 µM L-arginine, 2 µCi/mL [³H]-arginine, 1 min, 37 celsius degree) was measured. Significant (ANOVA unpaired Student's t test, $P<0.05$, $n=5-10$) contraction (94±11% KCl response) was obtained with 100 µM H₂O₂; contraction decreased with insulin pre-treatment (48±12% KCl response). The insulin effect on H₂O₂-induced contraction was reversed with TEA and IbTx co-incubation. In HUVECs, insulin increased L-arginine transport in a time-dependent manner, with a maximal effect (2.4-fold) after 30 min of incubation. This effect on L-arginine transport was blocked by TEA and IbTx co-incubation.

In conclusion, insulin pre-treatment attenuated H₂O₂-induced contraction of human chorionic plate veins. Insulin pre-treatment also significantly increased L-arginine transport in umbilical vein endothelial cells. Selective potassium channel blockade partially reversed these effects suggesting a role for BK_{Ca} channels in insulin's actions on human placental vascular tissues.

González *et al.* (2004). Pflugers Arch. **448**, 383-394.

González *et al.* (2011). J Cell Physiol. **226**: 2916-2924.

Wareing *et al.* (2006). Placenta **27**: 42-48.

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PC348

Tyramine-evoked release of endogenous noradrenaline constricts *in situ* vasa recta diameter via its action at contractile pericytes

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Pericytes reside at regular intervals on vasa recta capillaries and are known to regulate *in situ* vasa recta diameter following exposure to vasoactive agents including the co-transmitters noradrenaline (NA) and ATP [1,2]. The aim of this study was to (i) co-localise vasa recta pericytes with sympathetic nerves in the renal medulla of fixed kidney tissue slices, (ii) to demonstrate that tyramine-stimulated release of endogenous NA can similarly evoke pericyte-mediated regulation of *in situ* vasa recta diameter in live kidney slices and (iii) to investigate whether the constriction evoked by the co-transmitters ATP and NA was additive.

Kidney slices (200µm) were obtained from adult male Sprague Dawley rats, following cervical dislocation and maintained in oxygenated physiological saline solution (PSS). For live imaging experiments, slices were secured in an open bath chamber on the stage of an upright microscope and continually superfused with oxygenated PSS. Video-imaging techniques were used to capture pericyte-mediated changes in vasa recta diameter following exposure to Tyramine (1µM) plus and minus the co-transmitter ATP (100µM). Pericytes and sympathetic nerves were labelled in fixed kidney slices using anti-NG2 and anti-TH antibodies (respectively), and the appropriate fluorescently-conjugated secondary antibodies. Fluorescence images of sympathetic nerves and pericytes in both the inner and outer medulla were taken with a Zeiss LSM 510 confocal microscope and the distance between sympathetic nerves and the nearest pericyte measured.

Bath application of tyramine (1µM) alone evoked an $11.9 \pm 2.9\%$ constriction of vasa recta capillaries at pericyte sites, which was significantly greater than at non-pericyte sites ($3.0 \pm 0.6\%$, $P < 0.05$, $n = 7$). Co-application of tyramine and ATP caused a significantly greater constriction of vasa recta at pericyte sites ($26.5 \pm 4.6\%$) than at non-pericyte sites ($1.7 \pm 0.5\%$, $P < 0.05$, $n = 5$), an additional 55% decrease in vessel diameter compared with tyramine alone ($P < 0.05$). Sympathetic nerves were identified in both the inner and outer medulla of fixed kidney slices. Mean distance between sympathetic nerves and the nearest pericyte in the outer medulla ($1.6 \pm 0.4 \mu\text{m}$) is significantly reduced compared to the inner medulla ($4.8 \pm 1.1 \mu\text{m}$, $P < 0.05$, $n = 19$).

Here we demonstrate tyramine-stimulated release of endogenous NA causes pericyte-mediated constriction of *in situ* vasa recta. The effect on pericyte-mediated constriction of vasa recta was additive when kidney slices were exposed to ATP with tyramine. Given the identification of sympathetic nerves in close proximity to vasa recta pericytes we hypothesize that sympathetic nerves are an endogenous source of the co-transmitters NA and ATP and that local release of these vasoactive agents is one of the mechanisms involved in regulation of vasa recta blood flow.

Crawford C et al. (2009). *Proc Physiol Soc* **15** PC149

Crawford C et al. (2011). *Acta Physiol* **202**(3):241-251

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PC349

PGE₂ modulation of *in situ* vasa recta diameter is mediated by pericytes: mechanism for NSAID induced nephrotoxicity

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It is well known that non-steroidal anti-inflammatory drugs (NSAIDs) are nephrotoxic and indomethacin has been shown to reduce medullary blood flow (MBF) by up to 20 % [1]. Since NSAIDs inhibit cyclooxygenase enzymes, the NSAID-evoked reduction in MBF is thought to be due to inhibition of cyclooxygenases and the subsequent reduction of vasodilatory prostaglandin E₂ (PGE₂) synthesis. Here we use a live kidney slice model to investigate (i) the role of contractile pericyte cells in the indomethacin-induced reduction in MBF and (ii) to localise the cyclooxygenase (COX) enzymes and E-prostanoid 2 and 4 (EP 2, EP 4) receptors in the medulla. Kidney slices (200 µm thick) containing intact medulla were obtained from adult (~300 g) male Sprague-Dawley rats and maintained in physiological saline solution (PSS), bubbled with 95% O₂/5% CO₂. Real-time images of vasa recta were recorded using video imaging techniques and vasa recta diameter at pericyte and non-pericyte sites was measured off-line. Immunohistochemistry techniques were used to localise COX enzymes and EP 2 and 4 receptors in the medulla of fixed kidney slices. It has previously been demonstrated that PGE₂ dilates isolated perfused descending vasa recta (DVR) capillaries [2]. Application of PGE₂ (10 µM) to live kidney slices evoked a significantly greater vasodilation of vasa recta at pericyte sites ($8.60 \pm 1.58\%$) than at non-pericyte sites ($1.00 \pm 0.83\%$) ($P < 0.01$). PGE₂ (10 µM and 50 µM) significantly attenuated the pericyte-mediated vasoconstriction evoked by ET-1 (10 nM, $P < 0.001$) and Ang-II (10 nM, $P < 0.001$) respectively. We also investigated the ability of a range of NSAIDs (indomethacin, SC-560 meloxicam and celecoxib) to regulate vasa recta diameter and measured a significant reduction in vasa recta diameter specifically at pericyte sites ($P < 0.001$) in response to all compounds tested. Indomethacin significantly attenuated the pericyte-mediated vasodilation of vasa recta evoked by PGE₂ ($P < 0.001$), bradykinin ($P < 0.01$) and the NO donor S-Nitroso-N-Acetyl-D,L-Penicillamine (SNAP) ($P < 0.001$). In addition to functional studies an enzyme immune assay (EIA) kit was used to monitor PGE₂ concentration in our experimental perfusate and was used to determine whether indomethacin treatment inhibited production of PGE₂ specifically. COX enzymes were localised to the apical membrane of medullary tubular structures and EP 2 and 4 receptors were localised with medullary vasa recta. All data are expressed as mean $\pm$ s.e.m., $n \geq 5$ animals.

Collectively data presented here indicate that i) PGE₂ is key in attenuating vasa recta diameter via its action at contractile pericytes and ii) NSAID-evoked constriction of vasa recta occurs at pericyte sites and is likely to be due to a reduction in PGE₂. Hence, pericytes are likely to be key in NSAID-evoked reduction in MBF and the ensuing reduced kidney function.

Dobrowolski L, Sadowski J. 2004. Renal medullary infusion of indomethacin and adenosine. Effects on local blood flow, tissue ion content and renal excretion. *Kidney Blood Press Res.*, 27: 29-3

2Silldorff, E., P., Yang, S. & Pallone, T. L. 1995. Prostaglandin E2 Abrogates Endothelin-induced Vasoconstriction in Renal Outer Medullary Descending Vasa Recta of the Rat. *J. Clin. Invest.*, 95, 2734-2740.

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PC350

Identification of Anoctamine-1, Ca²⁺ activated chloride channel in porcine urinary bladder and characterization of its functional role using niflumic acid

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Interstitial cells (ICs), analogous to the interstitial cells of Cajal in the gut, may generate phasic activity (PA) in smooth muscle tissues, including the bladder. An established marker of the ICs is c-kit [1]. However, recent studies have shown that Anoctamin-1 (Ano1, Tmem16a), a Ca²⁺ activated chloride channel (CaCC), influences generation of pacemaker activity in gut ICs and therefore can be used as a novel marker for these cells [2]. CaCC blocking drugs such as niflumic acid were able to alter the pacemaker activity of the ICs in the gut and thus may be important modulators of these cells in other tissues. Thus, the aim of this study was to investigate whether Ano1 is expressed in porcine bladder and to explore the role of niflumic acid in modulating the PA of bladder tissue.

Methods: Female pig (~6months old) bladders were obtained from the local abattoir, hence ethical approval was unnecessary. PCR was carried out on the c-DNA synthesized from total RNA isolated from bladder mucosa (composed of the urothelium, lamina propria and muscularis mucosa) and denuded detrusor. Primers were designed for *Sus scrofa* Ano1 mRNA (XM_003122417.2). PCR products were separated by electrophoresis and sequenced. Longitudinal strips of denuded detrusor (n=7) or mucosa (n=47) were mounted in perspex microbaths and superfused with Krebs' solution at 37°C. Isometric tension was measured via UF1 force transducers connected to a Powerlab system using the LabChart software. Denuded detrusor strips were superfused constantly with 0.1µM carbachol (CCh) solution to induce PA. The effect of increasing concentrations of niflumic acid (1-30µM added cumulatively, 10-min exposure for each concentration) or drug vehicle (DMSO) on spontaneous and CCh-stimulated PA was investigated by measuring the amplitude and frequency of PA. All data is expressed as mean±SEM. Statistical analysis was carried out by using repeated measure ANOVA followed by Dunnett's post hoc test.

Results: Ano1 mRNA expression was found in both mucosal and detrusor layers of porcine bladder. Niflumic acid did not have a significant effect on the amplitude or the frequency of CCh-stimulated PA in the denuded detrusor strips at all concentrations. However, the amplitude of basal PA in mucosal strips was significantly reduced (p<0.001) with 10µM (22.3±4.6% inhibition) and 30µM (26.6±4.4% inhibition) niflumic acid. The frequency of basal mucosal contractions was reduced only at 30µM (25.0±7.7% inhibition) niflumic acid (p<0.001). Drug vehicle had no effect on PA.

Discussion: Inhibition of mucosal PA but not cholinergic-induced detrusor PA by niflumic acid, may suggest a role of ANO1 channels in mediating the PA of ICs found in the sub-urothelial layer of mucosal strips, while not affecting direct muscle stimulation by CCh.

1.Torihashi S.et al (1995).Cell Tissue Res.280,97-111

2.Hwang SJ.et al (2009).J Physiol.587,4887-904

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PC351

RhoA pathway is not responsible for the bronchial hyperresponsiveness induced by maternal protein restriction

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A poor maternal environment is known to predispose offspring to non-communicable diseases in later life, including asthma (Martino & Prescott, 2011). Bronchial hyperresponsiveness (BHR) is a feature of asthma, which has been linked to alterations in the RhoA pathway (Chiba et al., 1999). We have recently demonstrated BHR in the male offspring of protein restricted dams (Davis et al., 2012) and the aim of the current study was to investigate the role of the RhoA pathway in this response.

Pregnant Wistar rats were fed either a control diet (C, 18% casein) or a protein restricted diet (PR, 9% casein) from conception to term. At term, dams and pups were returned to standard chow. At 150 days male offspring were sacrificed by cervical dislocation and bronchi were dissected and snap frozen in liquid nitrogen. mRNA was extracted from bronchi and reverse transcribed to cDNA. mRNA levels of genes RhoA, ROCK-1 and ROCK-2 were quantified using qPCR. Data is expressed as mean ± SEM and was analysed by student's t-test. Significance was accepted at p<0.05.

In bronchial segments from 150 day old offspring of control and PR dams, mRNA levels of RhoA were similar between the groups (C, 0.59 ± 0.03, n=5; PR 0.60 ± 0.02, n=4 p=0.75). Similarly, mRNA levels of ROCK-1 and ROCK-2 were also not different between the two groups (ROCK-1; C, 0.62 ± 0.11, n=5, PR, 0.65 ± 0.05, n=4, p=0.84; ROCK-2, C, 0.78 ± 0.04, n=5, PR, 0.94 ± 0.13, n=4, p=0.24).

Increased mRNA levels of RhoA or ROCK does not appear to be responsible for the BHR in this model as the results showed that there was not a significant difference between the control and PR bronchi. However, this does not rule out whether post-translational modification may be responsible for the previously reported BHR.

Chiba et al., 1999 Br J Pharmacol, 127, 597-600

Davis et al., 2012 Proceedings of the British Pharmacological Society 9 (3) 018P

Martino & Prescott 2011, Chest 139, 640-647

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PC352

Role of antioxidant vitamin c on hypoxia-induced alteration of VEGF gene expression in diabetic rats

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Background: There are major gaps in our knowledge regarding the short- and long-term effects of intermittent hypoxia, especially when it comes to linking genomic and cellular responses with the physiological adaptation. It is also not clear whether Chronic Sustained Hypoxia (CH) and Chronic Intermittent Hypoxia (CIH) have any impact in hyperglycemia.

Objectives: To find out the effect of Chronic Sustained Hypoxia or Chronic Intermittent Hypoxia in diabetic rats on cellular transcriptional and Gene expression pathways (HIF 1 α , i-NOS, e-NOS, Nitrite/Nitrate (NOx) & VEGF) with or without supplementation of Vitamin C.

Methods: Rats were divided into twelve groups in two parts: Part I consists of Control, Vitamin C treated, Chronic Sustained Hypoxia exposure, Chronic Sustained Hypoxia + Vitamin C, Chronic Intermittent Hypoxia exposure, Chronic Intermittent Hypoxia + Vitamin C. Part II consists of Diabetes, Diabetes + Vitamin C, Chronic Sustained Hypoxia + Diabetes, Chronic Sustained Hypoxia + Diabetes + Vitamin C, Chronic Intermittent Hypoxia + Diabetes and Chronic Intermittent Hypoxia + Diabetes + Vitamin C. Rats were made diabetic by injecting alloxan monohydrate, 150 mg/kg b.wt; i.p. single dose. Rats were exposed to hypoxia for 20 days.

The hypoxic environment was established in the hypoxic chamber (10 % O₂ and 90% N₂) with the inflow of a mixture of room air and nitrogen that was regulated by an oxygen analyzer (model 175518A, Gold Edition, Vacuum Med). CO₂ was absorbed by soda lime 27 granules, and excess humidity was removed by desiccator. Temperature was maintained at 24-26°C. The chamber was opened twice a week for 1 h to clean the cages and replenish food and water.

Serum HIF-1 α , serum i-NOS, serum e-NOS and serum VEGF concentration were evaluated by ELISA technique and serum nitrate/nitrite concentration were estimated by using UV-Visible spectrophotometer.

Results: Chronic Sustained Hypoxia, Chronic Intermittent Hypoxia increases and DM decreases serum HIF 1 conc. as compared to control. Chronic Sustained Hypoxia and Chronic Intermittent Hypoxia increases serum i-NOS, e-NOS and NOx conc. as compared to control. Diabetes M. causes increase in i-NOS and NOx but decreases e-NOS activities. DM with CH and DM with CIH shows significant elevation of serum i-NOS and reduction of serum e-NOS level as compared to only CH & CIH respectively. Vitamin C is found to be beneficial to reduce serum i-NOS and NOx conc. (but not in e-NOS) in all the experimental groups. Serum VEGF concentration increases in all the experimental groups but in case of CIH, the rise of VEGF is lesser as compared to VEGF concentration in CH group. VEGF concentration is lesser in DM which is remarkably improved after Vitamin C supplementation. **Conclusion:** CH or CIH definitely alters HIF-1 α transcriptional pathways as well as NOS activities and changes VEGF gene expression by modulating its concentration in serum and provide hypoxia induce adaptability in physiological system. DM just oppose this adaptive mechanism which become worst in case of simultaneous exposure with CH or CIH. But interestingly supplementation of Vitamin C is found to be beneficial to combat both CH and CIH. It is also found to be an effective non enzymatic antioxidant to fight against alteration of hypoxia adaptive pathways in diabetic condition.

Tam Tin Lap. The influence of acute, chronic or chronic intermittent hypoxia on NO release from the renal circulation. <http://hdl.handle.net/10722/31768>. 2005, pp26-27

Lin YM, Huang SK, Wang HF, Chen LM, et al. Short-term versus long-term intermittent hypobaric hypoxia on cardiac fibrosis and fas death receptor dependent apoptotic pathway in rat hearts. Chinese Journal of Physiology 2008; 51(5): 308-316

http://www.icmr.nic.in/bioethics/Guidelines_medicalcollege.pdf

Taylor C T, McElwain J C Physiology 2010;25:272-279

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PC353

Role of Ca²⁺ influx and diffusion in the initiation and propagation of calcium waves in ICC freshly isolated from the rabbit urethra

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Interstitial cells of Cajal (ICC) act as pacemaker cells in the rabbit urethra to stimulate smooth muscle to contract and maintain myogenic tone during bladder filling (Sergeant et al., 2000). Pacemaker electrical activity in urethral ICC results from propagating Ca²⁺ waves that stimulate Ca²⁺ activated Cl⁻ channels. The purpose of the present study was to examine the role of Ca²⁺ influx and diffusion in the initiation and propagation of the waves. All the experiments described were approved by DkIT Animal Care Committee. New Zealand white rabbits were humanely killed and strips of urethral smooth muscle were dissected and enzymatically dispersed to release single ICCs. These were allowed to settle on a glass bottomed dish, loaded with Fluo - 4 AM (500 nM) and imaged using a spinning disk confocal microscope. Cells were superfused with Hanks solution at 37°C. Under control conditions spontaneous increases in fluorescence (indicating calcium release from the endoplasmic reticulum, ER) occurred at various distances along the cell length. These varied in magnitude (from 1.3 to 14.0 F/F₀, mean 5.5 $\pm$ SEM 0.2, n=146 events in 6 cells). The length of spread varied in a continuum from 1.6 μ m in the case of transient events to 183 μ m in the case of full propagating waves with a mean value of 17.6 $\pm$ 2.4. Sometimes waves arose simultaneously at opposite ends of the cell and when they collided this caused mutual annihilation.

The development of propagating waves depended on three factors: influx of calcium from the extracellular medium; diffusion of calcium within the cell and the level of IP₃ within the cell. Thus in a cell that was firing regular propagated waves, removal of extracellular calcium abolished these leaving only short-lived calcium transients which did not propagate. Similarly when diffusion of calcium within the cell was inhibited, by adding 3 μ M EGTA-AM to the external solution, propagated waves were blocked, leaving short-lived calcium transients. Conversely in cells that exhibited only transient calcium increases these events developed into propagated waves when agonists (such as phenylephrine, which increased intracellular IP₃ levels) were added. Furthermore increasing the sensitivity of calcium release from ryanodine receptors (RyR) by adding 1 mM caffeine to cal-

cium-free external solution caused transient calcium events to develop into propagated waves. We conclude from these results that transient calcium events result from spontaneous release of Ca^{2+} from the ER. These can develop into propagated waves when sufficient cytoplasmic calcium and IP_3 exists to cause sensitization of adjacent release sites. Wave propagation occurs when calcium diffuses to an adjacent sensitized release site and stimulates regenerative release.

Sergeant GP, Hollywood MA, McCloskey KD, Thornbury KD, McHale NG. *J Physiol.* 2000 Jul 15;526 Pt 2:359-66.

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Effects of prostaglandin $\text{F}_2\alpha$ on Ca^{2+} -release Ca^{2+} -entry in rat pregnant uterine smooth muscle

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Introduction: Prostaglandin $\text{F}_2\alpha$ ($\text{PGF}_2\alpha$) is a myometrial stimulant, both $\text{PGF}_2\alpha$ and its receptor are reported to increase towards parturition. The mechanism by which $\text{PGF}_2\alpha$ exerts its effects on uterine excitation-contraction coupling is unknown. We examined $\text{PGF}_2\alpha$ effects on Ca^{2+} signalling and force in pregnant rat myometrium.

Methods: $\text{PGF}_2\alpha$ was examined on both longitudinal strips and isolated myometrial cells from pregnant Wistar rats. Strips were loaded with the calcium sensitive indicator Indo-1AM and simultaneous recording of $[\text{Ca}^{2+}]_i$ and force were made using a photometric system combined with force measurements. Cells were isolated using Liberase Blendzyme 3 (Roche), loaded with Fluo-4AM, and $[\text{Ca}^{2+}]_i$ recorded using Nipkow Disk based confocal imaging system. Data were analysed using T-tests, and results shown as mean $\pm$ SEM.

Results: Application of $\text{PGF}_2\alpha$ on myometrial strips in the presence of external Ca^{2+} initiated bursts of Ca^{2+} spikes associated with phasic contractions which were fully abolished in Ca^{2+} free solution ($n=5$). In the absence of external Ca^{2+} , $\text{PGF}_2\alpha$ produced a transient increase in $[\text{Ca}^{2+}]_i$ 2.74 ± 0.8 and force 9.00 ± 3.2 expressed as a percentage of high- K^+ induced response taken as 100% ($n=6$, $p=0.002$). Ca^{2+} appeared as propagating Ca^{2+} waves. Application of $\text{PGF}_2\alpha$ in the absence of external Ca^{2+} resulted in the depletion of the sarcoplasmic reticulum (SR) and re-admission of external Ca^{2+} following SR Ca^{2+} depletion resulted in a sustained rise in basal $[\text{Ca}^{2+}]_i$ to $94.35 \pm 0.8\%$ of high- K^+ ($n=6$), and activation of Ca^{2+} bursts superimposing the raised $[\text{Ca}^{2+}]_i$. Ca^{2+} was associated with activation of phasic contractions superimposed on tonic force. Nifedipine (L-type Ca^{2+} channel blocker) caused inhibition of the bursts of Ca^{2+} spikes and phasic contractions, but had little effect on the sustained component of both Ca^{2+} and force. Basal $[\text{Ca}^{2+}]_i$ and tonic force in the presence of nifedipine were: 87.11 ± 10.7 and $65.71 \pm 15.5\%$ of high- K^+ ($n=5$, $p=0.25$). The raised $[\text{Ca}^{2+}]_i$ and tonic force were significantly reduced by La^{3+} (non-specific blocker of store operated Ca^{2+} entry (SOCE)), $[\text{Ca}^{2+}]_i$ was reduced from 94.35 ± 0.8 , to $91.54 \pm 1.4\%$ of high- K^+ ($n=5$, $p=0.05$).

Conclusion: $\text{PGF}_2\alpha$ caused release from the SR which appeared as a propagating Ca^{2+} wave causing depletion of the store. Re-admission of external Ca^{2+} resulted in activation of a

nifedipine-resistance Ca^{2+} influx sensitive to La^{3+} . This suggests that the stimulant action of $\text{PGF}_2\alpha$ is associated with activation of a Ca^{2+} -release Ca^{2+} -entry coupling mechanism leading to opening of the SOCE pathway.

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PC355

Potential role for a potassium current with slow kinetics in regulating uterine smooth muscle action potential configuration: a modelling prediction

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During pregnancy the uterus undergoes considerable remodelling in preparation for the substantial contractile effort of parturition. We have recently developed a mathematical model for the uterine smooth muscle cell (USMC) in late pregnancy which describes all published, biophysically detailed excitation-contraction coupling parameters from membrane electrophysiology to intracellular Ca^{2+} dynamics and force production [1]. The model is capable of reproducing many experimental observations including several recorded action potential (AP) forms of variable duration ~ 0.5 -60 secs. However, a restriction of the model is a difficulty in computationally reproducing long duration spike APs that have been experimentally noted [2]. We here use a theoretical modelling approach to investigate whether altered potassium current characteristics may underlie a transition from short- to long duration spike APs.

We have systematically searched the parameter space of the 14 USMC electrogenic currents of the model to identify potential components that can change the electrophysiological behaviour of the USMC into longer duration spike APs. No single intrinsic parameter, such as altering individual channel conductance, could do so. However, a combinatorial approach of increasing the activation (2X) and inactivation (10X) time constants, and the conductance (from 0.65 pS/pF to 0.8 pS/pF), of a 4-AP-insensitive voltage-gated K^+ current did reproduce experimentally recorded long bursting-type APs [2].

Although this prediction may seem counter intuitive, there are supporting evidences: (i) mRNA expression encoding a protein that functions as a slowly activating potassium current, similar to the cardiac I_{Ks} current, has long been suggested to increase in late pregnancy [3]; (ii) I_{Ks} consists of the KCNQ voltage-gated channel and KCNE subunits and USMC KCNQ/KCNE transcripts are gestationally regulated [4]; (iii) KCNQ activation time constants and conductance can be increased by co-expression of different KCNE subunits [5]; (iv) our simulations present similar changes in the kinetic properties and conductance of a native voltage-gated K^+ current to produce long duration bursting APs frequently observed in USMCs [2]. This leads us to speculate that KCNQ/KCNE channels, or similar, may be important regulators of USMC AP form.

[1] Tong WC *et al.* (2011). *PLoS ONE* **6**, e18685

[2] Inoue Y *et al.* (1999). *Can J Physiol Pharmacol* **77**, 447-453

[3] Boyle MB *et al.* (1987). *Nature* **330**, 373-375

[4] McCallum LA *et al.* (2011). *J Cell Mol Med* **15**, 577-86

[5] Bendahhou S *et al.* (2005). *Card Res* **67**, 529-538

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PC356

The role of imatinib mesylate (Glivec) in mediating the cholinergic-induced phasic contractions of the isolated whole pig urinary bladder

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Interstitial cells (ICs) may generate phasic activity (PA) in smooth muscle tissues including the bladder (1). ICs express c-kit and signalling via the tyrosine kinase gene product, kit, is essential for development of the IC phenotype. Imatinib mesylate (Glivec), a c-kit inhibitor has been widely used for studying the role of ICs in generating PA in the bladder (2 & 3). The aim of this study was to identify c-kit positive cells in pig urinary bladder using molecular, immunohistochemical (IHC) and functional techniques. The effect of imatinib on cholinergic-induced PA of the isolated pig bladder was also investigated.

No ethical approval was required as female pig (~6months old) bladders were obtained from the local abattoir. Primers were designed for the *Sus scrofa* c-kit mRNA and polymerase chain reaction (PCR) carried out on the c-DNA synthesized from total RNA isolated from pig bladders. PCR products were separated by electrophoresis and sequenced. For IHC studies, formalin-fixed paraffin-embedded bladder tissue was analysed using IHC staining for c-kit.

Whole bladders (n=6) and their associated vasculature were surgically excised and maintained under physiological conditions, perfused with Krebs' solution as previously described (4). The effect of intravascular administration of increasing concentrations of imatinib (1-50µM added cumulatively, 20 min exposure for each concentration) or drug vehicle on carbachol (CCh)-induced (0.1µM) whole bladder PA was monitored by recording the intravesical pressure (cmH₂O).

Sequencing of the PCR product confirmed the expression of c-kit mRNA in both the mucosa and detrusor layers of the pig bladder. Expression of c-kit-antigen was detected in the suburothelial and muscle layers of bladders by positive immunoreactivity to c-kit antibodies. Isolated pig bladders developed an increase in baseline pressure (tonic contraction) with superimposed PA in the presence of 0.1µM CCh. Intravascular imatinib had no effect on PA frequency and only the 50µM concentration significantly inhibited (41.2±8.3% inhibition, p<0.01) the amplitude of PA. However, imatinib significantly reduced (5µM: 25.5±10.7%, 10µM: 45.9±8.6 & 50µM: 95.8±8.4% inhibition, p<0.05-0.001) the tonic contraction of the isolated whole bladder.

We have demonstrated c-kit expression in pig bladders using both PCR and IHC techniques. Imatinib significantly reduced the amplitude of the cholinergic-induced PA and the tonic contraction of the pig bladder with no effect on the frequency of PA. This may indicate that c-kit positive cells may play an important role in modulating the phasic contractions and the tone of the bladder in the pig.

McCloskey KD, Gurney AM. Kit positive cells in the guinea pig bladder. J Urol 2002; 168: 832-6

Kubota Y, Biers SM, Kohri K, Brading AF. Effects of imatinib mesylate (Glivec) as a c-kit tyrosine kinase inhibitor in the guinea-pig urinary bladder. NeuroUrol Urodyn 2006; 25: 205-10

Vahabi, B. McKay, NG, Lawson, K, Sellers, DJ. The role of c-kit-positive interstitial cells in mediating phasic contractions of bladder strips from streptozotocin-induced diabetic rats. BJU international 2011; 107: 1480-1487

Parsons BA, Drake MJ, Gammie A, Fry CH, Vahabi B. The validation of a functional, isolated pig bladder model for physiological experimentation. Frontiers in Pharmacol 2012; 3:1-8

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PC357

Lactate significantly decreases in vitro myometrial contractility

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Weak, uncoordinated uterine contractions (labour dystocia) accounts for 20% of all caesarean sections (CS) in the UK. The only treatment available is application of oxytocin, which only works in 50% of cases. Lactate has been shown to be significantly increased in myometrial capillary blood during labour dystocia suggesting it may be impairing force production, but there are no functional data on the effect of lactate on the myometrium. We have therefore investigated its effect on contractility. Methods Myometrial strips were taken from term pregnant rats (22 day gestation) or biopsies obtained with written consent for women either not in labour (elective) or in labour, term CS. The effects on spontaneous contractility in response to sodium lactate (1-20 mM) and other weak acids: sodium butyrate, propionate and pyruvate (5-20 mM) were recorded. Oxytocin-stimulated contractions (0.1 nM rat, 0.5 nM human) were also investigated. In some experiments, tissue was loaded with Indo-1 AM to simultaneously measure force and intracellular Ca²⁺ signalling. Statistical differences were tested using non-parametric tests and significance taken as P<0.05. Results Lactate significantly decreased spontaneous contractility (n=6 rat, n=5 human). A dose dependent decrease in the integral of force (area under the curve, AUC) was seen, which was significant at concentrations of 5 mM (rat=36%±14%, human=44%±17%) and above (relative to control). Other weak acids also significantly reduced contractions in a dose dependent manner. The effects of lactate were significantly reduced in the presence of oxytocin (n=6 rat, n=4 human); 5mM lactate reduced AUC on oxytocin-driven contractions (rat=59% ± 15%, human=64% ± 15%, n=5). Because of this we determined the effects of lactate (5 mM) in biopsies from labouring myometrium. Significant reductions in force in the presence of oxytocin, were also found (amplitude, 81 ± 17 %, n= 6). Lactate inhibited Ca²⁺ transients and its effects on intracellular Ca²⁺ mirrored those of force (n=3). Conclusions Lactate in the physiological range potently decreases spontaneous contractility in both rat and human myometrium. The effects of lactate were influenced by physiological condi-

tions, and while still significant, were reduced in the presence of oxytocin or labour. Other weak acids produce similar effects to lactate suggesting its mechanism of action is not via metabolism. Lactate inhibited Ca^{2+} transients, which could be due to a fall of intracellular pH. We suggest that differences in myometrial lactate production/efflux in women can lead to accumulation of extracellular lactate, which as we have shown, will reduce myometrial contractions and could therefore contribute to labour dystocia.

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PC358

Effects of erythropoietin on spontaneous and oxytocin induced myometrial contractions in the nonpregnant rat

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Erythropoietin (EPO) is a glycoprotein hormone that principally regulates erythropoiesis. EPO activity has been detected in various tissues including the female and male reproductive organs. It has been shown that EPO causes relaxation of smooth muscle in cardiovascular system. In the present study, it was aimed to investigate effects of EPO on spontaneous and oxytocin induced contractions of non-pregnant rat myometrium. Myometrial strips were prepared from virgin Wistar rats (weighing 200-250g) at oestrous stage. They were placed in an isolated organ bath chamber containing Krebs' solution at 37 °C and pH 7.4, constantly bubbled with 95% oxygen + 5% carbon dioxide, and isometric contractions were recorded by using BIOPAC MP35 Data Acquisition System. Epoetin beta (rEPO) was added cumulatively at 0.1, 1 and 10 IU/ml concentrations to the tissue bath using myometrial samples showing regular spontaneous contractions for periods of 30 minutes. Effects of 1 IU/ml concentration of rEPO were also studied on oxytocin-induced myometrial contractions. This study was approved by the local ethics committee.

rEPO inhibited both area under curve and frequency of spontaneous contractions at 1 and 10 IU/ml concentrations (ANOVA, $n_1=2$, $n_2=9$, $F_{1,20}=20.938$, $F_{2,20}=20.492$, $p_{1,2}=0.000$). However, this inhibitory effect was not statistically significant at 0.1 IU/ml rEPO level (Tukey HSD, $p_1=0.051$, $p_2=0.581$). On the oxytocin stimulated myometrial samples, a single dose of 1 IU/ml rEPO was studied. The area under curve and frequency values of these samples were inhibited by rEPO (Student t test, $n=9$, $t_1=4.776$, $p_1=0.000$; $t_2=2.835$, $p_2=0.012$, respectively). In conclusion, our findings demonstrate that rEPO inhibited spontaneous and oxytocin induced rat myometrial contractions in vitro. The effect was dose dependent. Considering physiological levels of EPO, these results suggest that uterine contractility may be affected in women with chronic renal disease. Further studies are needed to elucidate the mechanism(s) of this inhibition as well as the possible effect of EPO on pregnant myometrium.

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PC359

The effect of ex vivo radiation on the contractility of guinea-pig bladder strips

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Bladder dysfunction occurs after radiation therapy for pelvic malignancies (1) where normal bladder tissue is unavoidably irradiated causing urinary tract symptoms including urgency, frequency and incontinence, adversely affecting quality of life. Understanding the mechanisms underpinning radiation bladder dysfunction is important to achieve maximum tumour kill with minimal normal tissue damage.

The purpose of the present study was to examine the effect of radiation on spontaneous contractility or neurogenic-evoked contractions in bladder strips.

Bladders strips from male guinea-pigs (250-300g) killed by cervical dislocation under UK Schedule 1 regulations and local ethical approval were prepared as: (1) full thickness (mucosa and detrusor) or (2) detrusor-only (mucosa-free) from the body and trigone regions. Strips received sham or 20Gy irradiation and were studied with in vitro tension recordings. Electrical field stimulation generated neurogenic contractions (0.3ms pulse width, 70V, 10s duration, 0.5, 1, 2, 4, 8 and 16 Hz).

Spontaneous contractions were measured as force integral (area under curve, AUC, g.min) and neurogenic contractions as amplitude (g). Data are expressed as mean $\pm$ S.E.M. with N and n referring to number of animals and number of strip preparations respectively. Unpaired t-tests were used with $p<0.05$ considered as significant.

Spontaneous contractions in full thickness strips from bladder body were significantly reduced by radiation ($N=15$, $n=19$, $p<0.05$) whereas detrusor-only strips were not affected ($N=14$, $n=19$, $p>0.05$). Trigone strips exhibited low-amplitude changes in baseline tension; activity in full thickness strips was not affected by radiation ($N=10$, $n=15$, $p>0.05$) whereas it was significantly reduced in detrusor-only strips ($N=8$, $n=13$, $p<0.05$). Neurogenic contractions in full thickness strips from bladder body were significantly reduced by radiation at all frequencies tested ($N=7$, $n=11$, $0<0.05$) whereas detrusor-only strips were not affected ($N=6$, $n=8$, $p>0.05$). The opposite was found in trigonal strips where radiation did not affect neurogenic contractions in full thickness strips ($N=7$, $n=8$, $p>0.05$) but significantly reduced those from detrusor-only strips ($N=7$, $n=9$, $p<0.05$). Radiation-generated differences in neurogenic contraction amplitude persisted in the presence of atropine ($1\mu\text{M}$). Subsequent addition of PPADS ($100\mu\text{M}$) reduced neurogenic contractions in sham and irradiated tissues so that these were not significantly different from each other.

In conclusion, irradiation reduced spontaneous and neurogenic contractions in the bladder in a region-specific and tissue layer-specific fashion. The presence or absence of the mucosal layer was found to be an important factor.

Marks LB et al, (1995) Int J Radiat Oncol Biol Phys. 31(5): 1257-80.

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PC360

Dilation of rat pulmonary arteries by the Kv7 activator zinc pyrithione

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Pulmonary artery tone is partly determined by the membrane potential (E_m) of pulmonary artery smooth muscle cells (PASMCs). The Kv7 family of K^+ channels, encoded by KCNQ genes, has been implicated in the regulation of E_m in rat PASMCs, such that retigabine, an activator of Kv7 channels, promotes pulmonary vasodilation (Joshi et al, 2009). Zinc pyrithione (ZnPy) is a recently identified Kv7 channel activator with a site of action distinct from retigabine (Xiong et al, 2008). This study aimed to determine if ZnPy could activate pulmonary artery Kv7 channels. Its effects on E_m and whole-cell K^+ currents were investigated in acutely isolated rat PASMC using the whole-cell patch clamp technique. Data are given as mean $\pm$ s.e.m. of n cells and were compared using students unpaired t-test, $p < 0.05$ considered significant. At 10 μ M, ZnPy consistently hyperpolarized PASMCs by 11 ± 1 mV ($n=12$, $p < 0.001$). Its effect on E_m was unaffected by the presence of 10 μ M glibenclamide (11 ± 1 mV, $n=60$, $p < 0.001$), indicating that it did not involve activation of K_{ATP} channels. In contrast, in the presence of 10 mM tetraethylammonium ions (TEA), ZnPy (10 μ M) did not cause significant hyperpolarisation ($n=10$), suggesting that it might involve TEA-sensitive Kv7 or BK_{Ca} channels. Involvement of the latter is unlikely, because in the presence of 50 nM iberiotoxin to selectively block BK_{Ca} channels, the hyperpolarisation induced by ZnPy remained at 14 ± 3 mV ($n=5$, $p=0.01$). Kv7 channels are more likely to underlie the response to ZnPy, because it was abolished by the Kv7 blocker XE991 (10 μ M, $n=10$). ZnPy (10 μ M) was also found to activate K^+ current, but with different pharmacology. The current activated at 0 mV, by brief (200ms) steps from a holding potential of -80 mV, was enhanced nearly 4-fold from 532 ± 84 pA to 2000 ± 331 pA ($n=33$, $p < 0.001$). This increase was unaltered by 50 nM iberiotoxin and 10 μ M XE99, but was greatly reduced by 10 mM TEA: ZnPy increased current from 547 ± 183 pA to only 617 ± 171 pA ($n=6$). When cells were clamped at 0 mV for at least 5 min to inactivate delayed rectifier K^+ channels, ZnPy (10 μ M) still induced outward current, from 41 ± 6 pA to 261 ± 79 pA ($n=31$, $p < 0.01$). This effect was prevented in the presence of either 10 mM TEA ($n=5$) or 10 μ M XE991 ($n=4$). Thus ZnPy activated a current with Kv7-like properties, but also induced an inactivating current mediated by distinct, TEA-sensitive, but XE991-insensitive channels. Taken together, the results support the idea that ZnPy hyperpolarises PASMC by opening non-inactivating, Kv7 channels. The hyperpolarisation is expected to inhibit voltage-gated Ca^{2+} influx, leading to muscle relaxation and ultimately pulmonary artery dilation.

Joshi S, Sedivy V, Hodcy D, Herget J, Gurney AM (2009) *J Pharmacol Exp Ther*. 329: 368-76.

Xiong Q, Sun H, Zhang Y, Nan F, Li M (2008) *Proc Natl Acad Sci USA*. 105: 3128-33.

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PC361

Augmented sarcoplasmic reticulum Ca^{2+} leak attenuates P2X-mediated $[Ca^{2+}]_i$ transients in renal microvascular myocytes in primary hypertension

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Activation of P2X receptors (P2XRs) in renal vascular smooth muscle cells (RVSMCs) mediates sympathetic control and autoregulation of renal circulation triggering RVSMC contraction via elevation of $[Ca^{2+}]_i$ resulting from Ca^{2+} entry via P2XRs and voltage-gated Ca^{2+} channels, and ryanodine receptor (RyR)- and inositol 1,4,5-trisphosphate receptor (IP₃R)-mediated Ca^{2+} release from the sarcoplasmic reticulum (SR) [1]. We have recently demonstrated that attenuation of P2X-mediated $[Ca^{2+}]_i$ transients in RVSMCs from spontaneously hypertensive rats (SHR) is caused by P2X1 downregulation and decrease in the SR Ca^{2+} load [2]. Here we analysed the mechanisms of the decrease in the SR Ca^{2+} load in primary hypertension by comparison of: (i) expression of genes encoding RyRs, IP₃Rs and phospholipase C β (PLC β) using real-time PCR analysis; (ii) basal PLC β activity using myo-D-[³H]inositol-based measurements of IP₃ turnover; (iii) rate the SR Ca^{2+} leak using confocal detection of spontaneous Ca^{2+} -release events in fluo-3 loaded RVSMCs from SHRs and their normotensive control, Wistar Kyoto (WKY) rats. Data are presented as mean $\pm$ S.E.M. and compared using Student's t-test. We found that in SHR RVSMCs RyR2 and Plcb1 were upregulated 24.5 $\pm$ 8.6- and 22 $\pm$ 5.8-fold, respectively ($p < 0.01$), while there was no significant difference in Itpr1 expression ($p=0.166$). The peak of $\alpha\beta$ -meATP-induced $[Ca^{2+}]_i$ transient was reduced in SHR RVSMCs ($p < 0.001$) from 746 ± 58 nM (WKY, $n=76$) to 270 ± 23 nM (SHR, $n=55$). The peak of caffeine-induced $[Ca^{2+}]_i$ transients was reduced in SHR RVSMCs ($p < 0.001$) from 688 ± 32 nM (WKY, $n=47$) to 246 ± 19 nM (SHR, $n=21$). Ryanodine-sensitive fraction of $\alpha\beta$ -meATP-induced response increased in SHR RVSMCs ($p < 0.01$) from 19 $\pm$ 4 % (WKY, $n=7$) to 42 $\pm$ 5 % (SHR, $n=8$), while cyclopiazonic acid- or 2-APB-sensitive fractions remained unchanged ($p=0.77$ and $p=0.818$, respectively). Basal level of IP₃ production increased in SHR RVSMCs 3.8 times ($p < 0.001$): [³H]inositol phosphates' counts per minute elevated from 42.5 ± 2.7 (WKY, $n=6$) to 163.3 ± 20.6 (SHR, $n=3$). Spontaneous Ca^{2+} -release events induced by the SR Ca^{2+} overload were sensitive to both ryanodine and 2-APB and had 4.4 times higher frequency in SHR RVSMCs ($p < 0.001$): 0.38 ± 0.08 Hz (WKY, $n=9$) and 1.67 ± 0.2 Hz (SHR, $n=16$). Rate of the SR Ca^{2+} leak ($\Sigma(\Delta F/F_0) \times s^{-1}$) was 9 times higher ($p < 0.001$) in SHR RVSMCs. Thus, increased expression and activity of RyR2 and PLC β 1 in SHR RVSMCs augments the SR Ca^{2+} leak leading to decrease of the SR Ca^{2+} load and P2XR-mediated signals and may impair sympathetically driven and autoregulatory responses in renal vasculature in hypertension.

Povstyan OV et al. (2011) *Br J Pharmacol* 162, 1618-1638.

Gordienko DV et al. (2011) Proc Physiol Soc 25, PC22.

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PC362

Hyperglycaemia potentiates vasoconstrictor responses in coronary arteries

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In patients with acute myocardial infarction (MI) it has been demonstrated that a worsened prognosis is associated with the degree of hyperglycaemia on admission to hospital (1,2). Furthermore, coronary artery reperfusion on balloon angioplasty after MI is often impaired in such hyperglycaemic patients worsening outcome. We have previously demonstrated that glucose activates PKC to suppress hyperpolarising Kv channels in smooth muscle(3). We hypothesise that glucose-induced, PKC-mediated vasoconstriction, coupled to a potentiation of vasoconstrictor activity imparts a marked vasoconstriction in the coronary vessels limiting perfusion of the myocardium. Inhibition of the PKC isoforms activated by hyperglycaemia may be an effective therapeutic target for improving outcome after MI.

Wire-myography was used to measure vasoconstriction in porcine coronary arteries sourced from a local abattoir. Arterial rings mounted on a myograph were bathed in a solution containing 5 mM (control), 10, or 20 mM (hyperglycaemic) glucose. All solutions were osmotically balanced to 20 mM with mannitol. Vasoconstrictors were added to the bathing solution as appropriate.

Exchanging the control bathing solution (5 mM glucose) for one containing 20 mM glucose caused a marked and sustained vasoconstriction in coronary arterioles ($8.2 \pm 0.7\%$ ***, $n=16$) which was further enhanced if extracellular potassium was raised to 20 mM ($27 \pm 4\%$ ***, $n=18$)(Contractions all measured against a 60 mM K⁺ depolarisation, *** $P < 0.001$, t-test). This was found to be PKC-dependent using the pharmacological PKC classical isoform inhibitor Gö6976 (300 nM) which attenuated the increase on exchange solutions ($2 \pm 1\%$, $n=12$).

In 10 and 20 mM glucose there was a marked potentiation of the magnitude of contraction ($14 \pm 5\%$, $36 \pm 7\%$ and $114 \pm 6\%$ *** of 60 mM K⁺ response in 5, 10 and 20 mM glucose respectively, *** $P < 0.001$, $n < 15$ for each) and the potency of the thromboxane A2 agonist U46619 (-7.0 ± 0.3 , -7.4 ± 0.1 and -8.1 ± 0.3 *** EC50 value (Log M) for 5, 10 and 20 mM glucose respectively, $n < 15$ for each). This enhanced vasoconstriction in 20 mM glucose was reversed by pre-treatment with the classical PKC isoform inhibitor Gö6976 ($114 \pm 6\%$ and $12 \pm 3\%$ in 20 mM glucose with and without Gö6976 respectively, $n=6$) Our data suggest that there is a significant, and acute, effect of hyperglycaemic concentrations of glucose on coronary artery function. A vasoconstriction in response to an elevated glucose and an enhanced response to U46619 could both lead to hypercontractile arteries therefore exacerbating the deleterious effects of MI. These findings highlight the importance of good glycaemic control and also suggest that classical PKC isoforms may be a beneficial therapeutic target in improving the prognosis after myocardial infarction.

Kosiborod M, Inzucchi SE, Krumholz HM, Xiao L, Jones PG, Fiske S, Masoudi FA, Marso SP, Spertus JA: Glucometrics in patients hospitalized with acute myocardial infarction: defining the optimal outcomes-based measure of risk. Circulation 2008;117:1018-1027

Squire IB, Nelson CP, Ng LL, Jones DR, Woods KL, Lambert PC: Prognostic value of admission blood glucose concentration and diabetes diagnosis on survival after acute myocardial infarction: results from 4702 index cases in routine practice. Clinical Science 2010;118:527-535

Rainbow RD, Hardy MEL, Standen NB, & Davies NW (2006). Glucose reduces endothelin inhibition of voltage-gated potassium channels in rat arterial smooth muscle cells. Journal of Physiology 575, 833 – 844

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PC363

The effects and potential mechanism of Simvastatin on human spontaneous myometrial contractility

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Myometrial regulation throughout pregnancy is key to understanding the processes involved in preterm labour and birth. Cholesterol depletion perturbs the membrane microenvironment by altering cell permeability and fluidity. Previous studies have also shown that cholesterol is able to modify myometrial contractility (Buxton & Vittori, 2005; Wray et al., 2005). Our hypothesis is that the cholesterol depleting agent simvastatin is able to inhibit myometrial contractions, accompanied by effects on lipid-raft associated proteins. The aim of this study was to investigate the effects of simvastatin on myometrium and the potential mechanism underlying any modulation.

Myometrial biopsies were collected with written informed consent from patients undergoing elective caesarean section at term (> 37 weeks). Tissue strips were mounted for isometric tension recordings and effects of simvastatin ($3.3 \text{ nM} - 0.1 \text{ mM}$, $n=20$) on spontaneous myometrial contractility, both in the absence and presence of BayK 8644 ($1 \mu\text{M}$, $n=10$), determined. Simvastatin treated cultured human myocytes were studied using immunofluorescence to investigate whether there is any effect on the localisation of the lipid raft markers caveolin-1 and flotillin-1 ($n=4$). Total cholesterol and protein were measured in untreated and treated cultured myocytes, using the Amplex red and bicinchoninic acid protein determination assay kits respectively ($n=4$). In all experiments, 15mM methyl- β -cyclodextrin (MCD) was used as a control cholesterol-depleting agent for comparison. Data are presented as means $\pm$ SEM and compared using ANOVA.

Simvastatin significantly decreased myometrial contraction amplitude and frequency until a complete cessation was achieved at $100 \mu\text{M}$ ($p < 0.0001$). MCD treatment increased contraction amplitude and frequency ($p < 0.0001$). BayK 8644 significantly increased contraction frequency ($p < 0.001$), but was unable to abolish the effects of simvastatin in tissues preincubated with this calcium channel agonist. Immunofluorescence for both lipid raft markers was detected in cultured myometrial cell membranes. Time course (0-24hrs) analyses of cells incubated in simvastatin indicated a redistribution of the two raft markers, an effect not seen in myocytes treated with MCD. There was no difference in total cholesterol and protein concentration in simvastatin-treated myocytes (p

>0.05) compared with MCD treatment where a significant decrease in total cholesterol only ($p < 0.001$) was observed. These data demonstrating acute inhibition of human myometrial contractility by simvastatin and an altered distribution of raft associated proteins indicate mechanisms of statin action distinct from cholesterol depletion alone.

Buxton, I.L. & Vittori, J.C. (2005). 'Cholesterol depletion enhances both spontaneous and agonist-evoked uterine smooth muscle contractions in a reversible manner', *Proc West Pharmacol Soc* 48:126-8

Smith, R.D., Babiychuk, E.B., Noble, K., Draeger, A., Wray, S. (2005). 'Increased cholesterol decreases uterine activity: functional effects of cholesterol alteration in pregnant rat myometrium', *Am J Physiol, cell Physiol* 288(5): C982-8

Dr. Cyril Rauch & Dr. Raheela Khan

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PC364

Uterine relaxant effects of watermelon (*Citrullus lanatus*) extracts

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Watermelon (*Citrullus lanatus*) is a natural and rich source of the amino acid citrulline; a precursor of L-arginine, the substrate for nitric oxide synthase in the production of NO. Recent work has shown that watermelon consumption increases plasma arginine in adults and that increased watermelon ingestion improved aortic haemodynamics. While several studies have also examined the in vitro effects of citrulline on vascular smooth muscle, there appears to be no studies of its effect on the myometrium. As relaxants of the myometrium are needed to help reduce preterm deliveries, the aims of the present study were therefore, to determine the effects on the myometrium of watermelon extracts and to investigate their mechanisms of action. Watermelon flesh and rind were ethanolic extracted. Rats were humanely killed with CO₂, and longitudinal uterine smooth muscle was dissected. The animal procedures were conducted in accordance with the Institutional Animal Care and Use Committee, Suranaree University of Technology, Thailand. Isometric force was measured and the effects of extracts of watermelon flesh and rind were evaluated on three types of contractile activity; spontaneous, those elicited by KCl depolarization (40 mM) or oxytocin (10 nM) application. Inhibitors of NO and their mechanisms of action, L-NAME (100 μ M), LY83583 (1 μ M), and tetraethylammonium chloride (5 mM), as well as Ca signaling pathways, were determined. The results show that both flesh and rind extracts significantly decreased force produced by all three mechanisms, in a dose dependent manner. The extracts could also significantly decrease force under conditions of sustained, high Ca levels (depolarization and agonist) and when force was produced only by sarcoplasmic reticulum (SR) Ca release. Investigation of L-citrulline (64 μ M) demonstrated that it produced the same effects on force as watermelon extracts. Combination of L-citrulline and watermelon extracts, at their EC₅₀ doses produced additive effects. The inhibitory effects of extracts and L-citrulline were reversed upon addition of the inhibitors of NO, and pretreatment of tissues with these inhibitors prevented the actions of both extracts

and L-citrulline. Thus these data show that watermelon is a potent tocolytic, decreasing force produced by calcium entry and SR release and arising by different pathways, including oxytocin stimulation. Its major mechanism appears to be to stimulate the NO-cGMP relaxant pathway, which can be accounted for by its constituent, citrulline.

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PC365

Shatavari (*Asparagus racemosus*) extract can relax the myometrium

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Shatavari (*Asparagus racemosus*) is a traditional Ayurvedic herb and is often used for infertility and for women's health. Animal studies have been conducted in vivo and demonstrated that Shatavari may have tocolytic effects. However, the direct effects of Shatavari on the myometrium and its mechanisms of action have never been clarified. As relaxants of the myometrium are needed to help reduce dysmenorrhea, miscarriage and preterm labour, the aims of the present study were therefore, to determine the effects on the myometrium of Shatavari root extract and to investigate its mechanisms of action. Shatavari roots were ethanolic extracted. Non-pregnant and pregnant rats were humanely killed by asphyxiation with CO₂ and longitudinal uterine smooth muscles dissected. The animal procedures were conducted in accordance with the Institutional Animal Care and Use Committee, Suranaree University of Technology, Thailand. Isometric force was measured and the effects of Shatavari root extract studied. The effects of Shatavari root extract on spontaneous, high-K-depolarisation-induced and oxytocin-induced contraction in both non-pregnant and pregnant rats were particularly examined. The results show that Shatavari root extract significantly decreased force produced by all three mechanisms, in a dose dependent manner. However, the effects of the extract in pregnant rats were more potent compared to those of non-pregnant rats. The extract also significantly decreased force when oxytocin was added under depolarised conditions and when force was produced only by sarcoplasmic reticulum Ca release. Thus these data show that Shatavari is a potent uterine relaxant, decreasing force produced by both Ca-dependent and -independent pathways. The inhibition of uterine activity of Shatavari may be a useful source of uterine relaxant and its active ingredient on the uterus should be identified, as it may be helpful for dysmenorrhea, miscarriage and preterm labour.

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SA1

Myofilament protein changes in atrial fibrillation; passive bystander or active contributor to disease progression

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Background Chronic atrial fibrillation (cAF) is characterized by electric, structural, and contractile remodeling that leads to pronounced atrial contractile dysfunction. To determine the impact of sarcomere alterations on contractile dysfunction in cAF, comparisons were made between membrane-permeabilized cardiomyocytes, muscle strips, and myofibrils isolated from atrial samples of patients in sinus rhythm (SR) and patients with cAF. Measurements of tension development and ATPase activity were performed at various calcium concentrations.

Results Compared to SR, cAF muscle preparations showed (1) a reduction in maximum tension; (2) a reduction in the rates of tension activation and relaxation; (3) preserved economy of contraction; (4) an increase in myofilament Ca^{2+} -sensitivity; (5) a reduction in myofibril passive tension. The slow β -myosin heavy chain isoform (β -MHC) and the more compliant titin isoform N2BA were up-regulated in cAF compared to SR. Phosphorylation of multiple myofilament proteins was increased in cAF compared to SR atrial myocardium.

Conclusions The increased relative amount of the slow β -MHC in cAF directly accounts for the reduction in cross-bridge cycling kinetics of cAF compared to SR muscle preparations. The negative impact of the MHC isoform change on the power output and velocity of atrial contraction may contribute to atrial contractile dysfunction in cAF. The decrease in passive stiffness in cAF myofibrils was entirely explained by a shift in titin isoform composition from the stiff N2B to the compliant N2BA isoform.

Alterations in active and passive tension generation at the sarcomere level, explained by translational and post-translational changes of multiple myofilament proteins, are part of the contractile dysfunction of human cAF and may contribute to the self-perpetuation of the arrhythmia and the development of atrial dilatation.

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SA2

Sodium ions and atrial fibrillation

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Impaired intracellular Ca^{2+} handling in atrial fibrillation (AF) has attracted much scientific interest because of its relation to AF-associated contractile dysfunction and proarrhythmic mechanisms. However, less attention has been paid to the role of Na^+ despite the fact that cellular Ca^{2+} and Na^+ concentrations are closely interlinked via the Na^+ , Ca^{2+} exchanger (NCX). Na^+ enters cardiomyocytes via voltage-dependent Na^+ channels or Na^+ , Ca^{2+} exchanger (NCX). Here we wish to scrutinize AF-induced alterations in atrial Na^+ influx and discuss their potential as targets for pharmacologic interventions.

In chronic atrial fibrillation, peak Na^+ currents are about 20% smaller than in SR, but other electrophysiological parameters and sensitivity of Na^+ channels to blockers (flecainide, verapamil) are similar. It has recently been reported that human atrial cardiomyocytes from patients in AF develop a "late" Na^+ current ($I_{\text{Na,late}}$) that is selectively suppressed by the antiarrhythmic drug ranolazine at concentrations that do not affect peak I_{Na} . In search of evidence for $I_{\text{Na,late}}$ we studied atrial action potentials (APs) with low and high concentrations of ranolazine (30 μM versus 300 μM). SR and AF preparations exhibited the typical spike-and-dome versus triangular AP configuration, respectively. Low concentrations of ranolazine did not significantly alter AP shapes, whereas 300 μM markedly suppressed the upstroke, and prolonged APD₉₀. In voltage clamp experiments with 100 ms-long test pulses from -110 mV to +20 mV followed by a 100 ms repolarising ramp pulse we could not detect any $I_{\text{Na,late}}$ that was clearly distinguishable from leak currents. As a positive control, robust $I_{\text{Na,late}}$ could be induced with *Anemonia sulcata* toxin ATX II (30 nM to 1 μM).

NCX activity is up-regulated in AF, giving rise to an increased risk of delayed afterdepolarization and to enhanced Ca^{2+} removal from the cells possibly contributing to contractile dysfunction. Indeed, block of NCX with the selective compound SEA0400 produced a positive inotropic effect in rat atrial and ventricular myocardium. However, in SR and AF atrial trabeculae SEA0400 failed to increase force of contraction or alter the shape of the cardiac action potential. Interestingly, in isolated cardiomyocytes, NCX activated by elevation of extracellular Ca^{2+} concentration from 0 to 1 mM in the presence of extra- and intracellular Na^+ concentrations of 0 and 100 mM, respectively, was markedly blocked by SEA0400.

Our findings in human atrial preparations suggest that further research is necessary before $I_{\text{Na,late}}$ and NCX can be accepted as potentially useful drug targets in AF.

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SA3

Cellular and molecular mechanisms in atrial fibrillation

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Atrial fibrillation (AF), the most common sustained cardiac arrhythmia in clinical practice, is often associated with progressive dilatation and remodeling of the atria which constitute the substrate of the arrhythmia. This atrial remodeling is characterized by complex structural and function alterations of the atrial myocardium: short action potentials, heterogeneous refractory periods, dystrophic myocytes and interstitial fibrosis which act together to favor local conduction bloc, activation of ectopies and the formation of microreentries of the electrical excitation. However, the underlying mechanisms of the AF substrate are not yet fully understood. Beside alterations in myocytes excitability, fibrosis, myocytes hypertrophy and endothelial dysfunction are important features of the AF substrate. Atrial hemodynamic overload and local activation of the renin-angiotensin system is one well-established pathogenic factor. Thrombin that accumulates in dilated and fibrillating atria could be another important mediator of the myocardial structural alterations during AF. This peptide, by binding on its receptor PAR1, can modulate several signaling pathways regulating growth and survival of myocardial cells.

Accumulation of epicardial adipose tissue too has been associated with the risk of AF while the underlying mechanisms are unknown. During the session, data will be presented that could provide new clues to better understand how these pathogenic factors contribute to the formation of the AF substrate.

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SA4

Studies of pro-arrhythmic atrial remodelling in rat models of elevated afterload

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Atrial fibrillation (AF) is a rapid and irregular activation of the atria that represents by far the most common cardiac arrhythmia. Despite the filtering effect of the atrioventricular node, transmission of excitation to the ventricles results in an inappropriately high ventricular rate during AF while the pooling of immotile blood within the atrial chambers leads to an increased risk of thrombo-embolic events. In consequence, AF contributes to the development of heart disease, is a major cause of stroke and is thereby associated with increased mortality. AF is not only a factor in the development of heart disease but can also be caused by the existence of a predisposing cardiac condition: for example, the presence of systemic hypertension and left ventricular hypertrophy are good epidemiological indicators of the risk of developing AF. Valve disease and regurgitation are also risk factors for the development of AF. The association of AF with pre-existing structural heart disease has led to the suggestion that remodelling of the atria in heart disease, possibly involving increased haemodynamic load on the atrial wall, predisposes the atrium to AF. The mechanisms underlying this remodelling remain unclear and it is hoped that an improved understanding of the processes leading to the genesis of AF might lead to new therapeutic strategies in the management of at-risk patients. However, the aetiology of the disease is usually complex and it is extremely difficult to study the mechanisms underlying the pathogenesis of AF in patients. On the other hand, models of heart disease in small animals (e.g. rats) can afford an accessible and cost-effective means of studying in the laboratory the mechanisms underlying pro-arrhythmic atrial remodelling caused by a single disease process. Multiple electrode array recording of atrial electrograms from the epicardial surface of isolated perfused hearts allows the measurement of atrial effective refractory period and conduction velocity and the assessment of localised inhomogeneities in atrial conduction. The inducibility of AF can be assessed by recording the incidence of paroxysms of arrhythmia following burst pacing of the atrium. All animal procedures were considered by the research ethics committee of the University of Bristol and were in accordance with the Animals (Scientific Procedures) Act, 1986 of the United Kingdom. Data from studies of atrial remodelling in an in-bred model of systemic hypertension, the spontaneously hypertensive rat (SHR), and in a surgical model of elevated afterload involving banding of the ascending aorta in rats, in comparison with the appropriate controls demonstrate the importance of fibrosis and conduction abnormalities in forming a substrate for re-entrant tachyarrhythmia and

increased inducibility of AF (Choisy et al., 2007; Kim et al., 2011). In both models, the pro-arrhythmic atrial remodelling involved the long term elevation of afterload, being evident in hearts from 11 month old SHR but not at 3 months of age and being evident after 20, but not at 8, weeks post surgery in the aortic banding model. Notably, while treatment of SHR with the vasodilator, hydralazine (14 mg/kg/day), or the angiotensin receptor blocker, candesartan (3 mg/kg/day), via the drinking water for 14 weeks prior to experimentation led to the normalisation of arterial pressures and the regression of atrial and ventricular hypertrophy, fibrosis remained unchanged, suggesting that it may be difficult to reverse pro-arrhythmic structural remodelling. It is concluded that small animal models of heart disease represent a useful tool for the investigation of the mechanisms underlying pro-arrhythmic atrial remodelling in heart disease.

Choisy SCM, Arberry LA, Hancox JC, James AF (2007). Increased Susceptibility to Atrial Tachyarrhythmia in Spontaneously Hypertensive Rat Hearts. *Hypertension* 49(3): 498-505.

Kim S-J, Choisy SCM, Barman P, Zhang H, Hancox JC, Jones SA, et al. (2011). Atrial Remodeling and the Substrate for Atrial Fibrillation in Rat Hearts with Elevated Afterload. *Circ Arrhythm Electrophysiol* 4(5): 761-769.

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SA5

Atrial fibrillation induces central cellular Ca²⁺ signaling silencing in atrial myocytes

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Background: During atrial fibrillation (AF) the atrial activation rate is chronically increased 5-7fold. Unstable intracellular Ca²⁺ signaling (i.e. increased Ca²⁺-sparks and -waves), another characteristic of AF, is thought to contribute to AF perpetuation through cellular arrhythmogenic mechanisms like delayed afterdepolarizations. Also, failure of centripetal Ca²⁺ wave propagation might contribute to loss of atrial contractility and changes in Ca²⁺ dependent signaling in the nucleus. We therefore characterized Ca²⁺ signaling in atrial myocytes of patients with AF and in rabbits undergoing rapid atrial pacing.

Methods and Results: In atrial myocytes of rabbits undergoing rapid atrial pacing (RAP; 10 Hz, 5 days) global and central-cellular Ca²⁺ transients (CaTs) were reduced, while subsarcolemmal CaTs were unaltered. No change in the low abundance of t-tubules was found. However, fast [Ca²⁺]_i buffering capacity (CaBC) was increased by 180% which included troponin buffering, an increase in sarcoplasmic reticulum (SR) Ca²⁺ uptake and an increase in calmodulin protein expression (90%). However, SR Ca²⁺ load and Ca²⁺ spark and wave frequencies were unchanged as was Ca²⁺ spark-mediated Ca²⁺ leak. RAP did also lead to a decrease in ryanodine receptor (RyR2) expression (77%) but with an increase in RyR2 phosphorylation (7fold). Nuclear Ca²⁺ transient amplitude was also significantly decreased in RAP cells. 10 minutes perfusion with 10M L-Phenylephrine elevated both nuclear Ca²⁺ and perinuclear Ca²⁺ release in RAP but not in sham myocytes. Failure of centripetal Ca²⁺ wave propagation and unaltered t-tubular sys-

tem (low abundance) were confirmed in atrial myocytes of patients with AF.

Conclusion: During tachycardia-induced atrial remodeling Ca^{2+} signaling silencing in the central region of atrial myocytes occurs. This adaptive response to rapid myocyte activation counteracts intracellular Ca^{2+} overload in AF and hampers the development of Ca^{2+} dysregulation-based arrhythmogenic mechanisms. The results also suggest downregulation of nuclear Ca^{2+} signalling during RAP, but enhanced IP3-dependent regulation of the nuclear Ca^{2+} transients. This may contribute to Ca^{2+} -mediated and IP3-dependent alterations of gene transcription in tachycardia-induced remodelling.

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SA6

Mitochondrial ROS production – impact on neurodegeneration of Parkinson's disease and physiological calcium signalling

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Parkinson's disease is a prevalent and progressive neurodegenerative disorder characterized by motor deficits, loss of dopaminergic neurons in the substantia nigra pars compacta leading to dopamine depletion in the striatum. The mainstay of treatment is dopamine replacement therapy. We studied the effects of dopamine in PINK1 associated PD. Loss of PINK1 function causes mitochondrial dysfunction with calcium dysregulation and susceptibility to neuronal death. However the basis for dopaminergic neuronal vulnerability in sporadic and genetic forms of PD is not clear. We demonstrate that low concentrations of dopamine induce cell death in PINK1 deficient cells, but not WT cells, by mitochondrial depolarisation induced by mitochondrial permeability transition pore (mPTP) opening. Dopamine-induced mPTP opening was dependent on a combination of ROS production and calcium signalling. Dopamine induced cell death could be prevented by application of antioxidants, inhibition of ROS production and by respiratory chain substrates. Importantly, we also found that dopamine-induced calcium signal in astrocytes can be blocked by antioxidants. The mechanism of this calcium signal is initiated by metabolites of dopamine produced by monoamine oxidase, which generate hydrogen peroxide that lead to induction of lipid peroxidation. This stimulates the activation of phospholipase C, and subsequent release of calcium from the endoplasmic reticulum via the IP3-receptor mechanism. These findings demonstrate that reactive oxygen species can play a physiological role in healthy cells but can also induce pathology and cell death in cells with genetic forms of PD, thus helping us to better understanding the physiological role of dopamine and mechanisms of neurodegeneration.

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SA7

Coupling of the bioenergetic and antioxidant status of neurones

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Neurones are the most vulnerable brain cells to excess reactive oxygen species. Their survival is thought to exclusively rely on the antioxidant protection promoted by neighbouring astrocytes. Indeed, these glial cells are not only self-protected by a robust antioxidant equipment –coordinated by Nrf2; they also provide neurones with both energy substrates and antioxidant glutathione (GSH) precursors (1). However, whilst astrocytes are necessary for neuronal protection, recent data suggest that they may not be sufficient; neurones would be intrinsically equipped with a biochemical mechanism that couples glucose metabolism to antioxidant defence for full protection (2). Here, we would like to review recent data from our and other laboratories suggesting that this neuroprotective coupling is connected with neuronal activity.

Increasing body of evidence is now consolidating the antioxidant and antiapoptotic functions of glucose oxidation through the pentose phosphate pathway (PPP). In essence, PPP oxidatively decarboxylates glucose-6-phosphate (G6P) to ribulose-5-phosphate (R5P), conserving the redox equivalents as NADPH(H^+). Constantly maintaining this redox coenzyme in its reduced status is essential for cellular GSH regeneration from its oxidized form (GSSG). Neurones are deficient in total glutathione and in their capacity to *de novo* synthesize it. Accordingly, the antioxidant status and survival of these cells are particularly dependent on a very efficient PPP (3). How is this metabolic route constitutively up-regulated in neurones? Glycolysis and PPP are two interconnected metabolic pathways that exist in equilibrium. Thus, the rate of G6P conversion into pyruvate through glycolysis, and that into R5P through PPP, are reciprocally influenced. In neurones, the rate of glycolysis is normally very low due to continuous destabilization of PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3), a key glycolytic-promoting enzyme (4). Our laboratory identified APC/ C^{Cdh1} (anaphase-promoting complex/cyclosome-Cdh1) as the E3 ubiquitin ligase responsible for PFKFB3 destabilization (4). The high APC/ C^{Cdh1} activity in neurones hence accounts for their low glycolysis and high PPP rates; in contrast, astrocytes express very low APC/ C^{Cdh1} activity (4), hence PFKFB3 is stable. By keeping PFKFB3 unstable in neurones, APC/ C^{Cdh1} accounts for efficient PPP-mediated NADPH(H^+) regeneration and high GSH/GSSG redox status (4). Thus, neurones maintain their antioxidant status by consuming G6P through the PPP at the expense of lowering its use through glycolysis for energy purposes. How, then, neurones obtain their energy from?

The most plausible –but not the only– explanation to this apparent paradox is the astrocyte-neurone lactate shuttle (ANLS) (5). In essence, following neuronal activity, astrocytes remove excess glutamate from the synaptic cleft by an energy-dependent glutamate transport that stimulates glycolysis to lactate. Astrocytic-released lactate would then be taken up by neurones, which utilize it for oxidative mitochondrial metabolism (5). Our results demonstrating that APC/ C^{Cdh1} , by promoting PFKFB3 degradation, continuously inhibits glycolysis in neurones (4) would favour lactate to pyruvate conversion,

thus contributing to explain how neuronal activity is coupled to brain glucose utilization. Furthermore, the coordination of APC/C^{Cdh1} activity with ANLS supports the notion that the coupling of energy metabolism between astrocytes and neurones is necessary to maintain neuronal antioxidant redox status and survival. However, how is this bioenergetic-antioxidant axis physiologically regulated?

Previously, it was found that following NMDA receptor (NMDAR) activity, the APC/C protein adaptor, Cdh1, becomes hyper-phosphorylated, and inhibited, *via* a Ca²⁺-dependent activation of cyclin-dependent kinase 5 (Cdk5) in neurones (6). Recent results from our laboratory show that NMDAR stimulation, by inhibiting APC/C^{Cdh1}, stabilizes PFKFB3 leading neurones to an increased rate of glycolysis and a decreased rate of PPP (7). These results indicate that the control of the bioenergetic and antioxidant status of neurones by APC/C^{Cdh1} is amenable to regulation, at least in a glutamatergic *in vitro* setting. Further studies would be required to more precisely identify and characterize the molecular players involved in the tuning of glycolysis and antioxidant status under different physiological conditions. This would also provide a boost to our understanding of the molecular mechanisms underlying neurological disorders, as well as in our search for novel therapeutic strategies.

Shih AY *et al.* (2003). Coordinate regulation of glutathione biosynthesis and release by Nrf2-expressing glia potently protects neurons from oxidative stress. *J Neurosci* **23**, 3394-3406.

Fernandez-Fernandez S *et al.* (2012) Antioxidant and bioenergetic coupling between neurons and astrocytes. *Biochem J* **443**, 3-12.

Bolaños JP & Almeida A (2009). The pentose-phosphate pathway in neuronal survival against nitrosative stress. *IUBMB Life* **62**, 14-18.

Herrero-Mendez A *et al.* (2009). The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. *Nat Cell Biol* **11**, 747-752.

Pellerin L & Magistretti PJ (2011). Sweet sixteen for ANLS. *J Cereb Blood Flow Metab* (doi: 10.1038/jcbfm.2011.149).

Maestre C *et al.* (2008). Cdk5 phosphorylates Cdh1 and modulates cyclin B1 stability in excitotoxicity. *EMBO J* **27**, 2736-2745.

Rodriguez-Rodriguez P *et al.* (2012). Excitotoxic stimulus stabilizes PFKFB3 causing pentose-phosphate pathway to glycolysis shift and neurodegeneration. *Cell Death Differ* (doi:10.1038/cdd.2012.33).

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SA8

Antioxidative support provided by brain astrocytes

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Reactive oxygen species are continuously generated during oxidative metabolism and have to be efficiently detoxified in order to prevent oxidative damage. This process is especially important for the brain, since this organ consumes about 20% of the oxygen utilized by the human body. Brain astrocytes contribute to the antioxidative potential of the brain in multiple ways and provide antioxidative support to neurons. A key molecule for these processes is the antioxidative tripeptide glutathione (GSH). Astrocytes contain around 8 mM of GSH

which enables these cells to efficiently detoxify peroxides and drugs via GSH peroxidase and GSH S-transferase reactions, respectively, thereby protecting neighboring neurons against oxidants and toxins (Hirrlinger & Dringen 2010). In addition, astrocytes provide precursors for neuronal GSH synthesis. This metabolic cooperation involves the release of GSH from astrocytes via multidrug resistance proteins and the extracellular cleavage of GSH by astroglial gamma-glutamyl transpeptidase and neuronal aminopeptidase (Hirrlinger & Dringen 2010). Drugs and toxins can modulate this astrocytic supply of GSH precursors, for example by lowering or accelerating GSH export from astrocytes (Tulpule & Dringen 2011; Brandmann *et al* 2012). Furthermore, astrocytes are considered as important regulators in the metabolism of the redox active metals iron and copper in brain and to protect neurons against the toxic potential of metal ions and metal-containing nanoparticles. Astrocytes efficiently accumulate large amounts of iron, copper and of metal-containing nanoparticles (Tulpule *et al* 2010; Geppert *et al* 2011; Scheiber *et al* 2012) and store metal ions in proteins such as ferritin and metallothioneins, thereby protecting other brain cells against metal toxicity. In addition, astrocytes are considered to supply neurons with trace elements such as iron and copper which are essential for neuronal metabolism. For example, copper export from primary astrocytes is mediated by the Menkes protein ATP7A and the cellular localization of this protein depends on the copper availability (Scheiber *et al* 2012). The contribution of astrocytes in multiple antioxidative and supportive pathways in brain suggests that compromised astrocytic functions will severely affect normal brain metabolism.

Hirrlinger J & Dringen R (2010). *Brain Res Rev* **63**, 177-188.

Tulpule K & Dringen R (2011). *J Neurochem* **116**, 626-635.

Brandmann M *et al* (2012). *J Neurochem* **120**, 78-92.

Tulpule K *et al* (2010). *J Neurosci Res* **88**, 563-571.

Geppert M *et al* (2011). *Nanotechnology* **22**, 145101.

Scheiber IF *et al* (2012). *Neurochem Int* **60**, 292-300.

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SA9

Dynamic transcriptional control of intrinsic neuronal antioxidant enzyme systems by electrical activity

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Synaptic N-Methyl D-Aspartate Receptor (NMDAR) activity is required for neuronal survival in the developing forebrain, and blockade of NMDAR activity induced cell death associated with oxidative stress [1-4]. We previously showed that synaptic NMDAR activity supports antioxidant defences in part by promoting the capacity of the thioredoxin-peroxiredoxin system [2]. Here we have investigated the regulation of another key antioxidant system: that centred on glutathione.

We show that in rat neurons, Ca²⁺ signalling, particularly through NMDARs, is of critical importance to tune the capacity of the neuronal glutathione (GSH) system to the needs of an active neuron, and to guard against increased demand. Through a coordinated transcriptional program, synaptic activity enhances the capacity of the GSH system to combat oxidative insults. In particular, the transcriptional induction of GSH

reductase activity and glutamyl-cysteine ligase (GCL) activity, the rate-determining enzyme in GSH biosynthesis, ensures that GSH levels are maintained in response to oxidative insults, despite basal rates of utilization being higher in active neurons. These changes to the supply side of the GSH pathway are complemented up by elevated rates of glutathione peroxidase activity, which together facilitate rapid reactive oxygen species (ROS) detoxification which would otherwise trigger Puma induction-dependent apoptosis. In vivo blockade of NMDAR activity in P7 rats by the i.p. administration of the antagonist MK-801 suppresses GCL expression and activity, leading to reduced GSH levels and neuronal death. The deleterious effects of low NMDAR activity can be suppressed by supplying neurons with a cell-permeable form of glutamyl-cysteine (GCEE), which bypasses the need for GCL activity and maintains GSH levels, preventing Puma induction and neuronal death. Thus, the NMDAR-dependence of the GSH biosynthetic pathway ensures that antioxidant defences can adapt to reflect activity levels, but render neurons particularly vulnerable to NMDAR blockade.

Ikonomidou, C., F. Bosch, M. Miksa, P. Bittigau, J. Vockler, K. Dikranian, T.I. Tenkova, V. Stefovskaja, L. Turski, and J.W. Olney, Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science*, 1999. 283: p. 70-74.

Papadia, S., F.X. Soriano, F. Leveille, M.A. Martel, K.A. Dakin, H.H. Hansen, A. Kaindl, M. Siffringer, J. Fowler, V. Stefovskaja, G. McKenzie, M. Craigon, R. Corriveau, P. Ghazal, K. Horsburgh, B.A. Yankner, D.J. Wyllie, C. Ikonomidou, and G.E. Hardingham, Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. *Nat Neurosci*, 2008. 11(4): p. 476-87.

Hardingham, G.E. and H. Bading, Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat Rev Neurosci*, 2010. 11(10): p. 682-96.

Leveille, F., S. Papadia, M. Fricker, K.F. Bell, F.X. Soriano, M.A. Martel, C. Puddifoot, M. Habel, D.J. Wyllie, C. Ikonomidou, A.M. Tolkovsky, and G.E. Hardingham, Suppression of the intrinsic apoptosis pathway by synaptic activity. *J Neurosci*, 2010. 30(7): p. 2623-35.

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SA10

Activation of astrocytic Nrf2 as a powerful neuroprotective strategy

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Increased oxidative stress is associated with neuronal cell death during the pathogenesis of multiple chronic neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis. Transcriptional activation of protective genes is mediated by a cis-acting element called the antioxidant responsive element (ARE) that binds the transcription factor Nrf2 (NF-E2-related factor 2). Activation of this pathway protects cells from oxidative stress-induced cell death. We hypothesize that Nrf2-ARE activation is a novel neuroprotective pathway that confers resistance to a variety of oxidative stress-related neurodegenerative insults. We have ongoing studies using mouse models of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), nerve degeneration/regeneration, and amyotrophic lateral sclerosis (ALS). In recent studies, primary

neuronal cultures treated with chemical activators of the Nrf2-ARE pathway displayed significantly greater resistance to oxidative stress induced neurotoxicity. Similar cultures generated from ARE-hPAP (human placental alkaline phosphatase) reporter mice demonstrated selective activation of the Nrf2-ARE pathway in astrocytes suggesting that Nrf2 activation in the astrocyte somehow confers resistance to naïve neurons. Furthermore, in chemical models of neurodegeneration, Nrf2 knockout mice are significantly more sensitive to mitochondrial complex I and II inhibitors. Combining these observations with the results implying that the astrocyte is central to Nrf2-ARE mediated neuroprotection, we generated transgenic mice that selectively overexpress Nrf2 in astrocytes using the GFAP (glial fibrillary acid protein) promoter to drive Nrf2 expression. This presentation will discuss how the GFAP-Nrf2 mice affect acute and chronic models of neurodegeneration as well as current research efforts to identify novel natural product activators of Nrf2 isolated from marine invertebrates.

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SA11

Therapeutic modulation of adaptive responses to oxidative stress in astrocytes: Nrf-2 dependent and Nrf-2 independent mechanisms

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Activators of the transcriptional factor, Nrf-2 have been shown to diminish cell dysfunction and death secondary to a host of nervous system insults in vitro and in vivo. It is believed that Nrf-2 mediates its salutary effects in part, via the upregulation of genes that counteract oxidative stress in astrocytes, with concordant cell autonomous and non cell autonomous protective effects in the CNS. However, the upstream activators of Nrf-2 remain obscure. To address this important question, we developed a novel method to spatially and temporally manipulate levels of an important reactive oxygen species, hydrogen peroxide in mixed astrocyte-neuronal cultures. The method involves the heterologous expression of d-amino acid oxidase in astrocytes not neurons, followed by addition of the d-amino acid, d-alanine. Low levels of d-alanine addition (16 micromolar) lead to generation of 3.7 nM of peroxide per min/mg of protein only in astrocytes not untransfected neurons. 16 micromolar d-alanine for seven hours induces a state change in astrocytes that permits protection of adjacent neurons from oxidative death (Haskew-Layton et al., 2010). By contrast, higher concentrations of d-alanine (2 millimolar) lead to 130 nM/min peroxide produced/mg protein and toxicity of adjacent neurons. Unexpectedly, protective concentrations of peroxide neither activate Nrf-2 nor require Nrf-2 for their salutary effects; rather it appears that low peroxide can lead to inactivation of the phosphatase PTEN leading to modulation of gene expression including the enzyme transglutaminase 2 (TG2). Indeed, TG2 inhibition in astrocytes leads to protection of neurons from oxidative death (Basso et al., 2012). These

results demonstrate the importance of understanding the spatial and temporal regulation of peroxide in astrocytes, neurons and other cells of the CNS; and suggest that peroxide is not a dominant mediator of Nrf-2 activation.

While peroxide induces a protective “state” in astrocytes, the magnitude of the protection remains inferior to canonical activators of Nrf-2, including sulforafane and tert-butyl hydroquinone. However, a theoretical limitation in the application of canonical activators of the Nrf-2 pathway to neurological disease lies in the fact that most of these compounds are “electrophiles”. Under basal conditions, these electrophiles alkylate the cytoplasmic inhibitor Keap to activate Nrf-2; however, under pathological conditions where redox balance is disrupted, electrophiles would be more likely induce toxicity. To overcome this theoretical problem, we have developed a novel screening and monitoring tool to enable us to identify non toxic activators of the Nrf-2 pathway. Specifically, we fused the Neh2 domain of Nrf-2 to luciferase. The Neh2 domain is the putative negative regulatory domain that interacts with Keap1. The Neh2-luciferase construct was more sensitive with a wider dynamic range than currently available reporters for Nrf-2; further it is induced by molecular knockdown of Keap1 and suppressed by overexpression of Keap1 (Smirnova et al., 2011). We screened a library of 2000 compounds from the Microsource Library and identified a number of compounds that activated our reporter; secondary assays confirmed that these hits could activate Nrf-2 dependent gene expression in primary neurons. Based on kinetic studies, and assumption of equal bioavailability of our compounds, we have sub classified the activators into five groups. Among the groups identified, those activated via a switch or receptors were deemed to be the most effective and least toxic. Among this group, nordihydroguaiaretic acid, fisetin and gedunin represented the best hits in the screen. These hits activated Nrf-2 dependent gene expression in astrocytes or neurons and protected these cells from oxidative stress in an Nrf-2 dependent manner. Overall, our studies identify novel and safer activators of Nrf-2 that induce neuroprotection.

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SA12

Activity-dependent plasticity of fast after-depolarizing potentials: a route to persistent excitability changes in hippocampal pyramidal cells

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When depolarized, Hippocampal pyramidal cells (HPCs), typically fire an initial burst of a few action potentials at high frequency (circa 80-200 Hz) before accommodating to either fire at lower frequency or and stop firing altogether. Similar high frequency burst firing can be observed in vivo, for example in the in the activity of place cells, and is thought to be important for the generation of long-lasting synaptic plasticity (for example LTP) at the excitatory synapses HPCs form with various downstream neurones. Changes to such high frequency bursting can also be seen associated with models of human disease including epilepsy and Alzheimer's disease.

The ionic conductances that underpin the high frequency bursting of HPCs are also those which produce the fast after-depolarizing potential which follows the firing of a single action potential in these cells. Although the complement of ion channels that produce this ADP is not entirely understood, pharmacological studies have revealed that agents which enhance this ADP, for example Kv7 channel antagonists, promote burst firing (and can be pro-cognitive/pro-convulsant), whereas compounds which depress the ADP, for example retigabine, inhibit bursting and are anticonvulsant.

Our recent studies of hippocampal slice in vitro have revealed that the ADP amplitude in HPCs, and consequently the propensity of these cells to exhibit high frequency bursting, is subject to multiple activity-dependent forms of persistent intrinsic plasticity. In our first investigations (Brown and Randall, 2009) we demonstrated that various defined, brief patterns of activity elicited in the cell under investigation produced a long-lasting (>30 min) depression of the ADP, and an accompanying reduction in high frequency firing ability. This appeared to be due to the up-regulation of Kv7 channel activity. In a subsequent study (Brown, Booth and Randall 2011) we first demonstrated that in CA3 pyramidal cells a period of pharmacological activation of mGluR1 produced a long-lasting depression of the ADP and a parallel reduction in burstiness. We then demonstrated that this action could be mimicked by synaptically-mediated activation of mGluR1 produced by suitable electrical stimulation of the associational commissural pathway. Unlike the depression produced by cell-intrinsic conditioning stimuli, the persistent depression of the ADP produced by mGluR1 activation was not mediated by changes to Kv7 channels, since it still occurred in the presence of the Kv7 blocker XE-991. Furthermore, mGluR1-mediated depression of the ADP was not blocked by intracellular BAPTA, suggesting changes in intracellular Ca²⁺ levels are not involved in this process.

In combination, our work reveals that in HPCs the fast ADP, and thus ability to produce fast spike bursts, is under dynamic regulation through multiple pathways. This may have implications for both normal CNS function, for example the ability to produce LTP, and pathological states such as epilepsy, as discussed recently by others (Schorge and Walker 2009).

Brown, J.T. & Randall, A.D. (2009) *J. Physiol.* 587, 1265-81.

Brown, J.T., Booth, C.A. & Randall, A.D. (2011). *Eur. J. Neurosci.* 33, 879-89.

Schorge, S. & Walker, M.C. (2009). *J. Physiol.* 587, 1143-4.

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SA13

Nitric oxide - an activity-dependent regulator of neuronal intrinsic excitability

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Activity-dependent changes in synaptic strength are well established as mediating long-term plasticity underlying learning and memory, but modulation of target neuron excitability could complement changes in synaptic strength and thereby

regulate network activity [1, 2]. It is thought that homeostatic mechanisms match intrinsic excitability to the incoming synaptic drive, but direct evidence for signalling to voltage-gated conductances is sparse.

Regulation of neuronal excitability and synaptic strength must preserve or maintain the basic physiology and yet permit adaptive processes. How are these adaptations of target neuron excitability achieved? In addition to changes in excitatory synaptic strength (e.g. LTP/LTD), synaptic activity also regulates voltage-gated conductances in target neurons [3], implying mediation by glutamatergic signalling. Such regulation of postsynaptic neuronal excitability and network function is widely reported (e.g. hippocampus, cortex and auditory brainstem) and given the ubiquitous nature of glutamatergic synaptic transmission, it is likely to be of global significance in regulating neuronal network function.

Nitric oxide (NO) is involved in processes regulating LTP/LTD mechanisms [4] and produced via neuronal nitric oxide synthase (nNOS). nNOS is broadly expressed in the brain and associated with synaptic plasticity through NMDAR-mediated calcium influx. However, its physiological activation and the exact mechanisms by which NO influences synaptic transmission have proved elusive. Here, we use the calyx of Held synapse within the medial nucleus of the trapezoid body (MNTB) and hippocampal CA3 pyramidal neurons to characterize NO modulation of postsynaptic high-voltage activated K⁺ channels. We have previously shown that NO is generated in an activity-dependent manner by MNTB principal neurons receiving a calyceal synaptic input [5]. Generation of NO occurs in the active neuron but it can exert its actions in the target neuron itself or in adjacent inactive neurons via volume transmission. Diffusion of NO allows modulation of excitability and synaptic efficacy by inhibiting postsynaptic Kv3 potassium currents in a PKC-dependent manner (via phosphorylation) following moderate activation times (up to 25min). This reduction in Kv3 currents led to increasing action potential duration and reduced transmission fidelity.

Longer periods of glutamatergic synaptic activity (more than 60min) induce an additional NO-dependent potentiation of Kv2 channels. The net effect is to switch the basis of action potential repolarization from Kv3 to Kv2 potassium channel dominance, thereby adjusting neuronal signalling between low and high activity states, respectively. This time-dependent and NO-mediated signalling dramatically increases Kv2 conductances in both the auditory brain stem and hippocampus (>3-fold) transforming synaptic integration and information transmission but with only modest changes in action potential waveform [6]. Evidence that this signalling pathway is active under physiological conditions was gained from recordings made within 15 min of brain isolation, which showed enhanced Kv2 currents and the amplitude of which decayed within 30 min in vitro. This potentiated current was absent in neurons from Kv2.2 or nNOS KO mice.

The data suggest that NO exerts its actions as a volume transmitter and slow dynamic modulator, integrating spontaneous and evoked neuronal firing, thereby providing an index of global activity and regulating information transmission across a population of active and inactive neurons within the MNTB and hippocampus.

We conclude that NO is a homeostatic regulator, tuning neuronal excitability to the recent history of excitatory synaptic inputs over intervals of minutes to hours.

Nelson, S.B. and G.G. Turrigiano, Strength through diversity. *Neuron*, 2008. 60(3): p. 477-82.

Marder, E. and J.M. Goaillard, Variability, compensation and homeostasis in neuron and network function. *Nat Rev Neurosci*, 2006. 7(7): p. 563-74.

Frick, A., J. Magee, and D. Johnston, LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. *Nat Neurosci*, 2004. 7(2): p. 126-35.

Garthwaite, J., Concepts of neural nitric oxide-mediated transmission. *Eur J Neurosci*, 2008. 27(11): p. 2783-802.

Steinert, J.R., et al., Nitric Oxide Is a Volume Transmitter Regulating Postsynaptic Excitability at a Glutamatergic Synapse. *Neuron*, 2008. 60(4): p. 642-656.

Steinert, J.R., et al., Nitric oxide is an activity-dependent regulator of target neuron intrinsic excitability. *Neuron*, 2011. 71(2): p. 291-305.

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SA14

Kv2.1: a key regulator of hippocampal excitability

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The homeostatic regulation of membrane excitability provides stability to the neural network in the face of dynamic and plastic synaptic inputs. This is mediated by a number of ion channels expressed in different membrane domains of neurons. The Kv2.1 voltage-gated potassium channel is a delayed rectifier channel with a small single channel conductance (~10 pS), slow deactivation, and a relatively high-threshold for voltage-dependent activation ($G_{1/2} \approx +15$ mV). This channel is highly concentrated in the proximal domain of neurons, the soma and proximal dendrites, where it can effectively regulate the overall excitability of neurons. We have shown that increased neuronal activity results in dephosphorylation of Kv2.1 through a Ca²⁺-dependent signaling pathway and phosphatases. Dephosphorylation of the cytoplasmic C-terminus shifts the voltage-dependent activation of Kv2.1 to lower membrane potentials. Together with the slow deactivation of Kv2.1, the change in activation dramatically increases the availability of the K⁺ channel during a train of action potential firing. This in turn provides the activity-dependent suppression of neuronal excitability, which can be reversed by blocking Kv2 channels.

The efficient suppression of neuronal excitability by Kv2.1 is in part due to its localization in the proximal domain of neurons, where synaptic potentials are integrated to determine the rate of action potential firing. Protein trafficking of Kv2.1 in the proximal domain of neurons is mediated by unique molecular mechanisms. We recently found that Kv2.1 is transported in a specific population of transport vesicles, of which motion is driven by a myosin and actin filaments. Because of the organization of actin filaments, determined by a super-resolution microscopy, and the property of the motor, the transport of vesicles carrying Kv2.1 is restricted in the proximal domain. Furthermore, we recently found that the trafficking mechanism is regulated by neuronal activity through NMDA receptors. I will discuss the potential role of this phenomenon in the homeostatic regulation of neuronal excitability by Kv2.1 channel.

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SA15

M currents and control of sensory neuron excitability

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Pain is a somatic sensation that alerts the organism about the body damage. As such, pain is crucial for avoiding injury (and ultimately - for survival). However, in some disease states (e.g. arthritis, migraine, neuropathic pains etc.) the transmission within the pain pathways of peripheral and/or central nervous system can become partially or totally disconnected from the external stimuli causing pain which is no longer beneficial but may bring severe suffering and distress. These pains often are very difficult to treat.

Electrical excitation of the peripheral damage-sensing (nociceptive) neurones constitutes an initial phase of nociceptive transmission to the CNS, therefore, ion channels that define and control the excitability of nociceptive neurones are curtail determinants of pain signaling. One of the major mechanisms controlling tonic excitability of mammalian neurones is M current, a slow-kinetics K⁺ current conducted by Kv7 channels (coded for by the KCNQ1-5 genes). Most M channels in neurones are homo- or heteromultimers of Kv7.2, 7.3 and Kv7.5. Due to their distinctive biophysical properties, M channel activity maintains a strong control over neuronal excitability. Accordingly, loss-of-function mutations within KCNQ genes often result in epilepsy (reviewed in (1)).

Recently we and others identified functional M channels in sensory neurones (2-6). We demonstrated that pharmacological inhibition of M channels in peripheral nociceptive terminals produces excitation and causes pain. Thus, hind paw injection of a specific M channel blocker, XE991 induced nociceptive behaviour (3, 4, 6) while in cultured DRG neurones XE991 induced marked depolarisation and increased action potential (AP) firing (2-4). We have also demonstrated that inflammatory mediators such as proteases (3) and bradykinin (4) can cause acute nociception by inhibiting M channels in sensory fibres via the G protein coupled receptor signalling cascade utilising depletion of the plasma membrane phosphatidylinositol 4,5-bisphosphate and release of Ca²⁺ from intracellular stores as M channel inhibiting signals (2-4). On the contrast, a neuropeptide substance P, acting via a novel signalling cascade utilising mitochondrially-generated reactive oxygen species (ROS) as intermediates, augments peripheral M channel activity thus reducing peripheral fibre excitability (unpublished). We have also demonstrated that pharmacological augmentation of M current in sensory fibres has an antiexcitatory and antinociceptive effect (2, 4, 6).

In another recent study we identified a functional binding site (RE1) for the repressor Element 1-Silencing Transcription factor (REST, NRSF) within the KCNQ2, KCNQ3 and KCNQ5 genes (7). We demonstrated that REST can bind to KCNQ genes and repress their transcription. Overexpression of REST in cultured DRG neurones robustly suppressed M current density and increased tonic excitability of these neurones. Baseline REST expression in neurones is low but it increases greatly after the neuropathic injury (6). Accordingly, quantitative RT-PCR and immunostaining demonstrated that after the neuropathic injury produced by the partial sciatic nerve ligation (PSNL), the expression of KCNQ2 in rat dorsal root ganglion (DRG) was dramatically downregulated, an effect that was paralleled by the reciprocal upregulation of REST expression in DRG.

In sum, our data put forward a strong case for the M current as a key determinant of peripheral sensory fibre excitability and a peripheral target for pain therapeutics.

Brown DA & Passmore GM (2009) Neural KCNQ (Kv7) channels. *Br J Pharmacol* 156:1185-1195.

Linley JE, Pettinger L, Huang D, & Gamper N (2012) M channel enhancers and physiological M channel block. *J Physiol* 590:793-807.

Linley JE, et al. (2008) Inhibition of M current in sensory neurones by exogenous proteases: a signaling pathway mediating inflammatory nociception. *J Neurosci* 28:11240-11249.

Liu B, et al. (2010) The acute nociceptive signals induced by bradykinin in rat sensory neurones are mediated by inhibition of M-type K⁺ channels and activation of Ca²⁺-activated Cl⁻ channels. *J Clin Invest* 120:1240-1252.

Passmore GM, et al. (2003) KCNQ/M currents in sensory neurones: significance for pain therapy. *J Neurosci* 23:7227-7236.

6. Rose K, Ooi L, Dalle C, Robertson B, Wood IC, Gamper N (2011) Transcriptional repression of the M channel subunit Kv7.2 in chronic nerve injury. *PAIN* 152:742-754.

Mucha M, et al. (2010) Transcriptional control of KCNQ channel genes and the regulation of neuronal excitability. *J Neurosci* 30:13235-13245.

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SA16

Modulation of ion channels in the axon initial segment

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Our research has examined the generation of simple and complex spikes in inhibitory "cartwheel" interneurons of the dorsal cochlear nucleus, using slices of the auditory brainstem from P16-24 mice. The term 'complex spike' refers to a burst of Na⁺ spikes riding on a slower Ca²⁺ dependent depolarization. We find that both simple and complex spikes propagate down the axons of cartwheel cells and trigger the release of a mixture of GABA and glycine from nerve terminals. A variety of Ca²⁺ channels contribute to these spikes, but the lowest-threshold complex spikes are generated by T-type channels. Two-photon Ca²⁺ imaging experiments showed that T-type channels are found on the axon initial segments of cartwheel cells. Selective blockade of these channels inhibited both the Ca²⁺ signals in the initial segment (e.g., with 3 μ M mibefradil 58 $\pm$ 3% block, n=5) and the generation of complex spikes (e.g., the # of spikelets in evoked complex spikes reduced by half by 50 μ M Ni²⁺; n=9 cells). Because Ca²⁺ channels are often the targets of modulators, we explored candidate modulatory systems in the DCN for their effects on complex spikes and on initial segment Ca²⁺ channels. We found that as little as 500 nM dopamine, acting on a D2-family dopamine receptor (probably D3), altered the mode of spontaneous spiking in cartwheel cells, converting it from a bursting to a tonic firing pattern, effects similar to those of T-type blockers. Moreover, activation of this dopamine receptor also inhibited evoked complex spikes and initial segment Ca²⁺ signals. PKC may mediate these effects of dopamine, as a phorbol ester (phorbol 12-myristate 13-acetate, 10 μ M) mimicked the action of dopamine or the channel antagonists on Ca²⁺ signals and complex spikes. Although T-type currents were present in the soma-

todendritic domains as well as on the axon, the dopamine pathway only inhibited the initial segment T-type channels, as assessed by imaging. We conclude that T-type channels of the initial segment play an essential role in controlling the mode of spiking in these interneurons, and that they are selectively modulated by dopamine.

Bender, K.J., and Trussell, L.O. (2009). Axon initial segment Ca²⁺ channels influence action potential generation and timing. *Neuron* 61, 259-271.

Bender, K.J., Uebele, V.N., Renger, J.J., and Trussell, L.O. (2012). Control of firing patterns through modulation of axon initial segment T-type calcium channels. *J Physiol* 590, 109-118.

Bender, K.J., Ford, C.P., and Trussell, L.O. (2010). Dopaminergic modulation of axon initial segment calcium channels regulates action potential initiation. *Neuron* 68, 500-511.

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SA17

Activity- and developmental-regulation of hippocampal feedforward inhibition

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Hippocampal local circuit GABAergic inhibitory interneurons control Na⁺- and Ca²⁺-dependent action potential generation, regulate synaptic transmission and plasticity, and pace large-scale synchronous oscillatory activity. The axons of this diverse cell population make local, short-range projections and release the inhibitory neurotransmitter γ -aminobutyric acid (GABA) onto a diverse array of targets. An increasing appreciation of the roles played by interneurons in a number of mental health issues such as epilepsy, stroke, Alzheimer's disease and schizophrenia have placed this important cell type center stage in cortical circuit research.

Despite representing only 15–20% of the total hippocampal neuron population they are represented by >20 subtypes. Moreover, each interneuron subtype is unique in its proliferative history, migration during corticogenesis as well as post-natal integration into the hippocampal circuitry. Precision in this control relies upon a remarkable diversity of interneurons primarily determined during embryogenesis by genetic restriction of neuronal potential at the progenitor stage. Hippocampal interneurons arise from medial and caudal ganglionic eminence (MGE and CGE) precursors. Using a combination of molecular, anatomical, and electrophysiological analysis of MGE/CGE-derived interneurons we have shown that the MGE produces parvalbumin-, somatostatin-, and nitric oxide synthase-expressing interneurons including fast-spiking basket, bistratified, axo-axonic, oriens-lacunosum moleculare, neurogliaform, and ivy cells. In contrast, CGE-derived interneurons contain cholecystokinin, calretinin, vasoactive intestinal peptide, and reelin including non-fast-spiking basket, Schaffer collateral-associated, mossy fiber-associated, trilaminar, and additional neurogliaform cells.

During development excitatory synapses onto these inhibitory interneurons undergo cell-type specific alterations in the molecular and biophysical properties of their glutamate receptor subunit composition. Importantly, subunit expression, and developmental- and activity dependent-plasticity of both

interneuron AMPA and NMDA receptors is tightly controlled and determined by the ganglionic eminence of origin. This presentation will highlight the rules utilized by developing hippocampal inhibitory interneurons in establishing the precise spatiotemporal patterning of glutamate receptor expression and plasticity within well-defined cortical networks.

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SA18

Inefficient functional sympatholysis is an overlooked cause of malperfusion in contracting skeletal muscle

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In 1962 Remensnyder¹ and associates described how the sympathetic vasoconstrictor activity elicited by muscle contractions was overcome in the active muscles, allowing for a blood supply that matched the metabolic demands. NO and ATP have been discussed as possible blockers of the norepinephrine (NE) mediated vasoconstriction. The site has been proposed to be either the nerve terminal or the alpha receptor. The latest research data support an action at the alpha receptor level and the possibility that plasma ATP via a P2Y activation may explain the sympatholysis. Recently an experimental tool has become available to elevate the sympathetic nerve activity. It is named tyramine and it can be used in humans. This opens for studies of the efficiency of the functional sympatholysis during exercise in the active limbs at various healthy and diseased stages as well as the impact of the active muscles' training status. In ageing sedentary men, functional sympatholysis and muscle blood flow is impaired compared to young men. However, regular physical activity can prevent this age related impairment in muscle blood flow and functional sympatholysis. Even in young subjects, a two-week inactivation period causes a reduced ability for sympatholysis. Patients with well controlled type 2 diabetes or essential hypertension also exhibit impaired functional sympatholysis. In the latter group, two months of elevated physical activity normalized the muscle perfusion and functional sympatholysis during exercise. Thus, the role of regular exercise for a maintained sympatholysis capacity is clear, but it is too early to draw a conclusion as to whether loss of functional sympatholysis is a primary event or secondary to a systemic capacity that is too low to produce an ample blood flow.

Remensnyder J et al (1962). *Circ Res* 11, 370-80.

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SA19

Vasodilatation at maximal exercise in humans

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Muscle blood flow increases almost linearly with exercise and reaches peak values at maximal exercise. This is achieved by the combination of a small increase in perfusion pressure and massive vasodilatation. The greatest levels of muscle perfusion have been reported in the quadriceps muscle during knee extension exercise ($250\text{--}450\text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$) (Andersen & Saltin, 1985; Blomstrand *et al.*, 2011). A singularity of this exercise model is that the amount of muscle activated (about 2.5–3 kg) is small and, therefore, the pumping capacity of the heart is not taxed. Endurance training and high-intensity intermittent knee extension training increases maximal exercise vasodilatation by 20–30% mainly due to enhanced vasodilatory capacity, inasmuch as maximal exercise perfusion pressure changes little with training. However, part of the increase in maximal exercise oxygen conductance is explained by muscle hypertrophy (Blomstrand *et al.*, 2011). Maximal exercise vasodilatation results from the balance between vasoconstricting and vasodilating signals combined with the vascular reactivity to these signals. In young healthy humans the muscles that are activated are also fully vasodilated during small muscle mass exercise (Ray & Dudley, 1998). The infusion of potent vasodilators fails to increase total limb perfusion but alters blood flow distribution and $\text{O}_{2\text{M}}$ extraction at the microvascular level. The muscle areas less metabolically active are less vasodilated and, hence, are more sensitive to an intra-arterially infused vasodilator. Consequently, some blood flow is deviated to areas with lower oxygen demand and the $\text{O}_{2\text{M}}$ extraction is diminished (Heinonen *et al.*, 2010). Maximal exercise vasodilatory capacity is reduced or blunted with ageing, as well as in chronic heart failure patients and chronically hypoxic humans; reduced vasodilatory responsiveness and increased sympathetic activity are potential mechanisms accounting for this effect. During maximal whole body exercise maximal vasodilatation is restrained by the sympathetic system. This is necessary to avoid hypotension since the maximal vascular conductance of the musculature exceeds the maximal pumping capacity of the heart (Calbet *et al.*, 2004). Pharmacological counteraction of the sympathetic restraint may result in lower perfusion pressure and reduced oxygen extraction by the exercising muscles during whole body exercise (Calbet *et al.*, 2006; Lundby *et al.*, 2008). However, fast inhibition of the chemoreflex in maximally exercising humans may result in increased vasodilation (Stickland *et al.*, 2011), further confirming a restraining role of the sympathetic nervous system on maximal exercise-induced vasodilatation. This vasoconstriction by the sympathetic system is likely critical for the maintenance of blood pressure in exercising patients with a limited heart pump.

Andersen P & Saltin B. (1985). Maximal perfusion of skeletal muscle in man. *J Physiol* 366, 233–249.

Blomstrand E, Krstrup P, Sondergaard H, Radegran G, Calbet JA & Saltin B. (2011). Exercise training induces similar elevations in the activity of oxoglutarate dehydrogenase and peak oxygen uptake in the human quadriceps muscle. *Pflügers Arch* 462, 257–265.

Calbet JA, Jensen-Ustad M, Van Hall G, Holmberg HC, Rosdahl H & Saltin B. (2004). Maximal muscular vascular conductances during whole body upright exercise in humans. *J Physiol* 558, 319–331.

Calbet JA, Lundby C, Sander M, Robach P, Saltin B & Boushel R. (2006). Effects of ATP-induced leg vasodilation on $\text{VO}_{2\text{peak}}$ and leg O_2 extraction during maximal exercise in humans. *Am J Physiol Regul Integr Comp Physiol* 291, R447–453.

Heinonen I, Kemppainen J, Kaskinoro K, Peltonen JE, Borra R, Lindroos MM, Oikonen V, Nuutila P, Knuuti J, Hellsten Y, Boushel R & Kalliokoski KK. (2010). Comparison of exogenous adenosine and voluntary exercise on human skeletal muscle perfusion and perfusion heterogeneity. *J Appl Physiol* 108, 378–386.

Lundby C, Boushel R, Robach P, Moller K, Saltin B & Calbet JA. (2008). During hypoxic exercise some vasoconstriction is needed to match O_2 delivery with O_2 demand at the microcirculatory level. *J Physiol* 586, 123–130.

Ray CA & Dudley GA. (1998). Muscle use during dynamic knee extension: implication for perfusion and metabolism. *J Appl Physiol* 85, 1194–1197.

Reference 8

Stickland MK, Fuhr DP, Haykowsky MJ, Jones KE, Paterson DI, Ezekowitz JA & McMurtry MS. (2011).

Carotid chemoreceptor modulation of blood flow during exercise in healthy humans. *J Physiol* 589, 6219–6230.

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SA20

Vasodilator interactions in skeletal muscle blood flow regulationY. Hellsten¹, M. Nyberg¹ and S.P. Mortensen²

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Blood flow to skeletal muscle is regulated by a balance between, on one hand, sympathetic activity and locally formed vasoconstrictors and, on the other hand, locally formed vasodilators and sympatholytic compounds. During exercise, a number of vasodilators have been shown to contribute to the marked increase in blood flow to the active muscle and, importantly, interactions between these compounds appear to be essential for the precise regulation of blood flow. We and others have studied interactions between compounds including nitric oxide (NO), adenosine, ATP, prostacyclin and endothelial derived hyperpolarizing factor (EDHF). These studies have revealed that the potent vasodilating effects of both ATP and adenosine are mediated by NO and prostacyclin whereas the role for EDHF remains more obscure. It furthermore appears that prostacyclin can either promote or inhibit NO formation, depending on the conditions. Another observation of interest is that adenosine infused arterially also leads to an increased formation of NO and prostaglandins in the skeletal muscle interstitium, suggesting a bidirectional release of these vasodilators. Main questions that remain to be elucidated in this area are why so many different activators of NO synthesis are required and whether the different activators have separate roles depending on the actual physiological circumstances.

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SA21

The roles of nitric oxide and adenosine in relation to hypoxia dependent vasodilation

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Hypoxia can have profound influences on the circulation. In humans, acute exposure to moderate hypoxia has been demonstrated to result in vasodilation in the coronary, cerebral, splanchnic and skeletal muscle vascular beds. The combination of submaximal exercise and hypoxia produces a "compensatory" vasodilation and augmented blood flow in contracting muscles relative to the same level of exercise under normoxic conditions. This augmented vasodilation exceeds that predicted by a simple sum of the individual dilator responses to hypoxia alone and normoxic exercise. Additionally, this enhanced hypoxic exercise hyperemia is proportional to the hypoxia-induced fall in arterial oxygen (O₂) content, thus preserving muscle O₂ delivery and ensuring it is matched to demand. Several vasodilator pathways have been proposed and examined as likely regulators of skeletal muscle blood flow in response to changes in arterial oxygen content. The purpose of this talk is to put into context the present evidence regarding mechanisms responsible for the local control of blood flow during acute systemic hypoxia in contracting muscle. Specifically this talk will highlight the roles of nitric oxide (NO) and adenosine as potential vasodilator signals in hypoxia dependent skeletal muscle vasodilation.

In general NO has been implicated as a key vasodilator signal in resting skeletal muscle in response to acute hypoxia in humans and animals. Studies have demonstrated that NO synthase (NOS) inhibition attenuates the hypoxic vasodilator response in the human forearm and arterioles of rat skeletal muscle. Evidence in animals suggests that a portion of the NO-mediated hypoxic vasodilation is due to adenosine receptor activation. However, available data related to the contribution of adenosine in the hypoxic vasodilator response in human studies are conflicting. In this context, interstitial levels of adenosine are elevated in response to acute hypoxia, whereas adenosine receptor antagonism does not appear to attenuate hypoxia mediated vasodilation. Similar roles for NO and adenosine have been reported during hypoxic exercise in humans. Along these lines, there is a significant blunting of the compensatory vasodilation in the forearm during mild to moderate hypoxic exercise after NOS inhibition. Conversely, adenosine receptor antagonism does not alter skeletal muscle blood flow and vasodilation in the contracting human forearm or leg during hypoxic exercise. Taken together, the data to be discussed during this talk suggests that NO contributes to hypoxia mediated vasodilation in resting and contracting skeletal muscle. By contrast, the role of adenosine in hypoxic dependent vasodilation is not completely clear. Adenosine appears to contribute to the hypoxia mediated vasodilation in animals at rest but hasn't been shown to be obligatory in humans during acute hypoxia at rest or during exercise.

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SA22

Contribution of non-endothelium derived factors to exercise hyperaemia

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At rest, systemic hypoxia induces vasodilatation in skeletal muscle that is largely attributable to adenosine. Our *in vivo* and *in vitro* studies showed that in hypoxia, adenosine acts on A₁ receptors on endothelial cells, generating PGI₂ as an intermediate. PGI₂ acts back on endothelial cells to generate cAMP, increase nitric oxide synthase (NOS) activity and release NO, which causes vasodilatation (Ray et al, 2002). The hypoxia-induced release of adenosine is also NO-dependent. Thus, providing a tonic level of NO is present, endothelial cells *in vivo* or *in vitro*, release adenosine in response to NO donor or hypoxia. We deduced this reflects the competitive interaction between NO and O₂ at their binding site on cytochrome oxidase (cyta₃). Tonic NO raises the sensitivity of cyta₃ to O₂ such that even O₂ levels reached in systemic hypoxia, decrease endothelial ATP synthesis leading to adenosine release (see Edmunds et al, 2003; Ray & Marshall, 2005).

The question arises as to whether these same mechanisms operate in exercise, for adenosine, prostaglandins (PG) and NO are implicated in exercise hyperaemia. Our results suggest not. In anesthetized rats, hyperaemia associated with tetanic or twitch contractions for 5 minutes was partly mediated by adenosine, but acting via A_{2A} receptors, not A₁ receptors. Further, the contribution of adenosine to exercise hyperaemia persisted after NOS inhibition: neither the release nor action of adenosine is NO-dependent. Since systemic hypoxia increases adenosine in plasma whereas exercise increases adenosine in interstitium, the simplest explanation is that in exercise, adenosine mainly acts on extraluminal A_{2A} receptors on vascular smooth muscle to directly cause dilatation (Ray & Marshall, 2009a,b).

Nevertheless, our findings indicate that exercise is at least partially dependent on a fall in O₂ levels, but in the vicinity of skeletal muscle fibres rather than in plasma. The hyperaemia following static forearm contraction at 50% MVC for 3 minutes was similarly decreased by breathing 40% O₂ before, during and after contraction, or by blockade of cyclooxygenase (COX) to inhibit PG synthesis. Further, supplementary O₂ and COX applied together had no greater effect than either independently (Win & Marshall, 2005). We recently showed that breathing 40% O₂ attenuated post-contraction hyperaemia when given during static contraction at 100%MVC, but not when given throughout recovery. Also, supplementary O₂ during contraction decreased venous efflux of lactate and hydrogen ions. This indicates that increasing the level of O₂ in plasma increase the partial pressure of O₂ in muscle fibres and so affects their metabolism (Fordy & Marshall, 2012). In our most recent studies, 40% O₂ restricted to a 2 min period of static at 50% maximum voluntary contraction (MVC) or to rhythmic contraction at 50% MVC at 1sec intervals, reduced the post-contraction by ~30-40%; COX inhibition or both applied together had similar effects. Thus, the contribution of PGs to the hyperaemia associated with even modest levels of contraction, that are not generally expected to involve impaired O₂ delivery, is apparently dependent in some way on a fall in O₂ levels in muscle.

We have followed this up in studies on the rat, in which highly selective adenosine receptor antagonists can be used: theo-

phylline that is available for use in humans is a relatively weak competitive antagonist of adenosine receptors. So far, we have found that breathing 40% O₂ or 8-suphophenyltheophylline (8-SPT) similarly decreased hyperaemia associated with tetanic contraction and that 40% O₂ and 8-SPT applied together had no greater effect.

Drawing these results together, we propose that during exercise, adenosine is released from skeletal muscle into interstitium by an O₂-dependent process and that it acts on extraluminal A_{2A} receptors on arterioles to cause exercise hyperaemia. A contribution is also made by PGs that are released from skeletal muscle fibres by an O₂-dependent process. Whether these O₂-dependent influences are interdependent remains to be investigated, but it seems unlikely that adenosine acts on A₁ receptors to generate PGI₂ via the mechanisms we implicated in systemic hypoxia.

Ray, CJ, Abbas, MR, Coney, AM & Marshall, JM (2002). *J Physiol* 544, 195-209.

Edmunds, NJ, Moncada S & Marshall, JM (2003). *J Physiol* 546, 521-527

Win TS & Marshall JM (2005). *J Appl. Physiol.* 99, 45-52.

Ray CJ & Marshall JM (2005) *J Physiol* 568, 967-978.

Ray CJ & Marshall JM. (2009) *J Physiol* 587, 1565-1578

Ray CJ & Marshall JM. (2009) *J Physiol* 587, 1565-1578

Fordy GR & Marshall JM (2011). *Exp Physiol* 97, 362-374.

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SA23

Spreading the signal for vasodilation: Implications for blood flow control

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Resistance vessels controlling skeletal muscle blood flow are arranged in series and in parallel. Thus vasodilation must be coordinated among parent and daughter branches of vascular resistance networks to effectively control the magnitude and distribution of muscle blood flow during exercise. Dilation of terminal arterioles controlling capillary perfusion has little effect on local blood flow if upstream branches remain constricted. Further, at bifurcations, dilation of one daughter branch can 'steal' flow from its sister branch if the parent vessel does not dilate in concert. Because the fibers of individual motor units are dispersed within a muscle, vasoactive signals arising from discrete locations must be integrated by arteriolar networks to effectively increase oxygen delivery according to local metabolic demand. As contractile activity increases, vasodilation originating in downstream arterioles 'ascends' to encompass upstream arterioles and feed arteries, thereby increasing local perfusion. As we have observed with intravital microscopy and substantiated using intracellular recording with fluorescent markers, the ability of parent vessels to respond in such a coordinated manner reflects cell-to-cell electrical signaling through gap junction channels (GJCs). These protein complexes form intercellular channels that electrically couple neighboring endothelial cells (ECs) to each other along the intima. Further, myoendothelial GJCs couple the endothelium with surrounding smooth muscle cells (SMCs). Thus electrical signals (e.g., hyperpolarization) initiated at distinct sites

can spread rapidly along the endothelium to coordinate vasodilation along and among branches of vascular resistance networks.

The regulation of spreading vasodilation has centered on the role and modification of GJCs. We questioned whether an alternative mechanism could govern electrical signaling along the vessel wall. Small- and intermediate conductance Ca²⁺-activated K⁺ channels (SK_{Ca}/IK_{Ca}; K_{Ca}2.3/K_{Ca}3.1) are highly expressed in the plasma membrane of ECs. We therefore tested whether activation of these voltage-insensitive ion channels could inhibit electrical conduction by 'leaking' current through EC membranes. Intact endothelial tubes were freshly isolated from mouse skeletal muscle feed arteries for Ca²⁺ imaging and for intracellular recording during current microinjection. Remarkably, activating SK_{Ca}/IK_{Ca} channels (selectively with NS309 or indirectly with acetylcholine via intracellular Ca²⁺) inhibited electrical conduction. At the same time dye transfer between ECs confirmed that GJCs remained intact. Thus loss of current through open SK_{Ca}/IK_{Ca} channels effectively dissipated electrical signals along the endothelium independent of GJCs. In a reciprocal manner, inhibiting SK_{Ca}/IK_{Ca} channels (with apamin + charybdotoxin) enhanced intercellular electrical conduction by reducing current loss through EC membranes.

Aging is associated with elevated sympathetic nerve activity (SNA) and oxidative stress. In the gluteus maximus muscle of Old (~2 years) versus Young (~4 months) male C57BL/6 mice, enhanced activation of α -adrenoreceptors on SMCs effectively restricted muscle blood flow at rest and during contractile activity. Independent of SMCs, electrical conduction along endothelial tubes from Old (vs. Young) mice is impaired. Our recent findings explain this difference through revealing enhanced SK_{Ca}/IK_{Ca} channel activation by reactive oxygen species (ROS). Thus, with aging, the actions of SNA on vascular smooth muscle and of ROS on the endothelium work together to impair spreading vasodilation, restrict muscle blood flow and limit skeletal muscle function. Vascular disease and obesity are commonly associated with elevated SNA, oxidative stress and impaired muscle blood flow along with diminished physical activity. Our studies of the microcirculation focus on providing mechanistic insight towards the development of appropriate and effective therapeutic interventions to improve tissue perfusion and maintain the quality of life for aging populations and for individuals affected by vascular disease.

Bearden SE, Payne GW, Chisty A & Segal SS. (2004). Arteriolar network architecture and vasomotor function with ageing in mouse gluteus maximus muscle. *J Physiol* 561, 535-545.

Behringer EJ, Socha MJ, Polo-Parada L & Segal SS. (2012). Electrical conduction along endothelial cell tubes from mouse feed arteries: Confining actions of glycyrrhetic acid derivatives. *Br J Pharmacol* doi: 10.1111/j.1476-5381.2011.01814.x

Folkow B, Sonnenschein RR & Wright DL. (1971). Loci of neurogenic and metabolic effects on precapillary vessels of skeletal muscle. *Acta Physiol Scand* 81, 459-471.

Haug SJ & Segal SS. (2005). Sympathetic neural inhibition of conducted vasodilation along hamster feed arteries: complementary effects of α_1 - and α_2 -adrenoreceptor activation. *J Physiol* 563, 541-555.

Hilton SM. (1959). A peripheral arterial conducting mechanism underlying dilatation of the femoral artery and concerned in functional vasodilatation in skeletal muscle. *J Physiol* 149, 93-111.

Jackson DN, Moore AW & Segal SS. (2010). Blunting of rapid onset vasodilatation and blood flow restriction in arterioles of exercising skeletal muscle with ageing in male mice. *J Physiol* 588, 2269-2282.

Segal SS. (2005). Regulation of blood flow in the microcirculation. *Microcirculation* 12, 33-45.

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SA24

Bi-directional communication in the neurovascular unit in health and disease

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Brain activity is tightly correlated with blood flow changes, a process defined as neurovascular coupling (NVC). An understanding of the signaling events underlying NVC is crucial given the vast number of cerebrovascular-associated disorders showing compromised functional hyperemia in the brain. We hypothesize that hemodynamic changes are rapidly sensed by perivascular astrocytes and transduced to neighboring neurons adjusting, in this way, activity to blood flow supply. Disruption to this delicate form of communication is a major contributor of cerebrovascular-associated disorders such as hypertension. We thus evaluated if hemodynamic changes within parenchymal arterioles altered glial and/or neuronal activity in cortical brain slices from rats. We used a novel *in vitro* approach, which incorporates flow/pressure into cannulated parenchymal arterioles, while simultaneously measuring Ca^{2+} activity (confocal imaging) in perivascular astrocytes and firing activity (patch-clamp electrophysiology) from cortical pyramidal neurons. Values represent means $\pm$ SEM; comparisons include paired t-test and unpaired t-test for comparisons between WKY vs. SHR. At a steady flow rate ($0.5 \mu\text{l}/\text{min}$), parenchymal arterioles developed myogenic tone ($17 \pm 4\%$ constriction). In healthy rats, increased arteriolar flow rate altered Ca^{2+} oscillation frequency in cortical astrocytes. Responses revealed the presence of two types of astrocyte Ca^{2+} patterns: excitatory responses (54%) with a corresponding increase in Ca^{2+} oscillation frequency from 0.026 ± 0.003 to 0.044 ± 0.0003 Hz, $P < 0.0001$ and inhibitory responses (46%) with a decrease in Ca^{2+} oscillation frequency from 0.05 ± 0.003 to 0.03 ± 0.0003 Hz, $P < 0.0001$. Moreover, increased flow/pressure was associated with a reduction in pyramidal neuron firing rate (0.33 ± 0.10 to 0.05 ± 0.03 Hz, $P < 0.01$) and membrane hyperpolarization (-41.50 ± 3.13 to -44.10 ± 2.97 , $P < 0.005$). In the presence of the gliotoxin L-aminoadipic acid, no changes in flow-induced neuronal hyperpolarization were observed ($P = 0.31$) suggesting the participation of astrocytes in vascular-to-neuronal signaling. Next, we compared vascular reactivity in parenchymal arterioles from normotensive (WKY) vs. hypertensive (SHR) rats. Bath applied U46619 (150 nM), a thromboxane agonist, significantly increased vascular tone in SHR vs. WKY (42.06% vs. 29.78% ; $P < 0.05$). To determine if higher levels of tone compromised NVC mechanisms, arterioles were exposed to 10 mM K^+ , a potent vasodilatory signal involved in NVC. Responses from SHR were significantly higher when compared to WKY (26.63% vs. 12.43% ; $P < 0.05$). The present study supports reverse flow of communication at the neurovascular unit and suggests that hemodynamic changes are encoded into distinct astrocyte/neuronal responses. Moreover, we show that vascular tone is increased in SHR rats with no apparent changes in K^+ signaling.

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SA25

Dysregulated neural-vascular interactions in neurogenic hypertension

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Despite significant advances in pharmacotherapy in the last several decades, control and management of hypertension and associated cardiovascular disease pathophysiology remains a challenge in 30-40% of hypertensive patients. These drug resistant patients present chronic neural overactivity and neurogenic derivation of hypertension. Thus, we believe that innovative concepts must be considered in an attempt to develop therapeutic strategies for the treatment of these neurogenic hypertensive patients. We have proposed a novel concept that a dysregulated neural-peripheral communication between the brain and bone marrow (BM) could be a key in neurogenic hypertension and its associated vascular pathophysiology. Our hypothesis suggests that vascular health is maintained by integrated signals from the autonomic brain regions to the BM which upholds a balance between angiogenic vascular reparative cells and inflammatory cells. An altered neural-BM communication as a result of an increased sympathetic drive in hypertension causes decreases in vascular reparative cells and increases inflammatory cells. This shift in balance would not only provoke inflammation-induced vascular damage, but also compromise its repairability. Evidence will be presented in support of this hypothesis.

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SA26

Interaction between oxidative stress and neurovascular dysfunction in Alzheimer's disease

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Cerebral blood flow (CBF) is reduced in patients with Alzheimer's disease (AD), anticipates the development of dementia and correlates inversely with cognitive performance.¹ Multiple processes probably contribute to the reduced CBF. $\text{A}\beta_{40}$ -mediated up-regulation of reactive oxygen species production by NADPH oxidase has been shown to cause cerebral vasoconstriction,² and we have shown an intracerebral increase in the potent vasoconstrictor ET-1,³ associated with $\text{A}\beta_{42}$ -mediated upregulation of endothelial converting enzyme (ECE)-2.⁴ We have now examined the relationship between $\text{A}\beta$ exposure, oxidative stress and ET-1 release in the cerebral vasculature. We measured ECE-1 activity and ET-1 level in leptomeningeal vessels that had been dissected from the occipital lobe of 20 AD, 17 vascular dementia (VaD) and 20 age-matched normal human control brains provided by the South West Dementia Brain Bank, University of Bristol. We also measured ET-1 release by human cerebrovascular endothelial

cells in response to 24h exposure to A β or the nitric oxide donor DETA NONOate. In further experiments ET-1 release was measured in response to a combination of the antioxidant superoxide dismutase (SOD) and A β 40, or SOD alone. ECE-1 activity and ET-1 level correlated closely, and both were significantly elevated in leptomeningeal blood vessels from AD but not VaD brains. Exposure of endothelial cells to 10 μ M A β 40 or 5mM DETA NONOate significantly increased the amount of ET-1 in the culture medium. A β 40-induced release of ET-1 by endothelial cells was prevented by the addition of 1KU SOD. The findings from this and our previous studies indicate that A β stimulates ET-1 production in AD: A β 40 increases microvascular ECE-1 activity in AD, an increase at least partly mediated by oxidative stress, and A β 42 up-regulates neuronal ECE-2. Both processes lead to the production and release of ET-1. Through its vasoconstrictive action, the elevated level of ET-1 is likely to contribute to the abnormal reduction in CBF in AD. This may be amenable to pharmacological reversal through the use of drugs that block ET-A receptors.

Chao LL, Buckley ST, Kornak J, Schuff N, Madison C, Yaffe K, et al. ASL perfusion MRI predicts cognitive decline and conversion from MCI to dementia. *Alzheimer Dis Assoc Disord* 2010;24:19-27.

Park L, Anrather J, Forster C, Kazama K, Carlson GA, Iadecola C. A β -induced vascular oxidative stress and attenuation of functional hyperemia in mouse somatosensory cortex. *J Cereb Blood Flow Metab* 2004;24:334-42.

Palmer JC, Barker R, Kehoe PG, Love S. Endothelin-1 is elevated in Alzheimer's disease and upregulated by A β . *J Alz Dis* 2012;28:1-9.

Palmer JC, Baig S, Kehoe PG, Love S. Endothelin-converting enzyme-2 is increased in Alzheimer's disease and up-regulated by A β . *Am J Pathol* 2009;175:262-70.

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SA27

Signalling to CNS pericytes in health and disease

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Neural computation is highly energy demanding: the brain consumes 20% of the body's resting energy production despite being only 2% of its mass. To use the available energy efficiently the brain has evolved mechanisms, termed neurovascular coupling, which increase the blood flow, and hence the energy supply, specifically to regions where there are active neurons. Control of blood flow occurs at the arteriole level mediated by smooth muscle, but may also occur at the capillary level mediated by isolated contractile cells called pericytes. I will report experiments on rat cerebellar slices investigating the signalling pathways by which neurons and astrocytes control blood flow at the capillary level, and studying how pericytes malfunction in diseases such as stroke.

Application of noradrenaline constricts cerebellar capillaries at pericyte locations, and superimposing glutamate (to mimic neuronal activity) causes a dilation. The dilation evoked by glutamate is inhibited by blocking nitric oxide synthase (with L-nitroarginine) but unaffected by blocking guanylate cyclase (with ODQ) implying that glutamate and NO do not dilate via the cGMP pathway. The effect of NO synthase block was abolished by inhibiting production of 20-HETE (using HET0016), and glutamate-evoked dilation was still seen with NO and 20-HETE production blocked. Thus, NO acts by suppressing production of 20-HETE. Blocking EET production (with MSPPOH)

had no effect on the glutamate-evoked dilation while blocking prostaglandin EP4 receptors (with L-161,982) abolished it. These data suggest that glutamate dilates capillaries via a prostaglandin pathway, but also requires NO release to suppress release of vasoconstricting 20-HETE.

Patch-clamping cerebellar molecular layer pericytes revealed that glutamate or parallel fibre stimulation evoked an outward current that would be expected to hyperpolarize the cell and thus evoke dilation. Parallel fibre stimulation (12 Hz for 10 sec) evoked a capillary dilation with an onset (in 20% O₂) after 4.5 sec and a maximum after 22 sec.

Some pericytes constrict in ischaemia. We found that an hour's simulated ischaemia of cerebellar slices led to ~90% of pericytes dying. Blocking either NMDA receptors with 50 μ M D-AP5, MK-801 and 5-chlorokynurenate, or AMPA/kainate receptors with NBQX, reduced the death by half. These data suggest that death of pericytes, while they are in rigor after an ischaemia-evoked [Ca²⁺]_i rise, may contribute to the long-lasting decrease of cerebral blood flow that occurs after ischaemia even when an occluded vessel is made patent again.

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SA28

The effects of angiotensin II receptor antagonism on fear memory and immune cell modulation

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Pharmacological inhibition of the renin-angiotensin system (RAS) is commonly used to treat hypertension and more recently been suggested as a treatment of emotional stress (Saavedra et al.). In a clinical study we have recently shown a role for the RAS in the regulation of the stress response in patients with post-traumatic stress disorder (PTSD) (Khoury et al.). Psychological stress is also known to perturb the homeostasis of the immune system. The purpose of this study was to further examine the role of RAS in an animal model of PTSD and its impact on the immune system. We performed Pavlovian fear conditioning pairing auditory cues with foot shocks to examine the effects of the angiotensin II receptor antagonist Losartan (1mg/kg and 10mg/kg) on fear memory extinction (Choi et al.). 24 h after fear conditioning, Losartan was administered 1h prior to fear memory extinction training. While no effect of Losartan was observed on extinction training, there was a significant decrease in fear as measured by freezing behavior to the auditory cue when tested the following day (14.9% vs 27.3%; $p < 0.05$). Immune cells of the spleen were quantified using flow cytometry and Losartan treated mice exhibited significantly fewer CD45+CD11b+ (macrophage-like) cells ($p < 0.05$). These data suggest that the RAS may contribute to the retention of extinction fear memory and alter the effectiveness of the immune system to respond to a challenge.

Khoury et al., (2012) The Renin-Angiotensin Pathway in PTSD: ACE inhibitor and ARB medications are associated with fewer traumatic stress symptoms. *J Clin Psychiatry*, in press.

Saavedra et al., (2011) Blockade of brain angiotensin II AT1 receptors ameliorates stress, anxiety, brain inflammation and ischemia: Therapeutic implications. *Psychoneuroendocrinology* 36 (1) 1-18.

Choi DC, et al., (2010) Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. *Proc Natl Acad Sci U S A*. Feb 9;107(6):2675-80.

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SA29

Skeletal muscle lipid metabolism in exercise and insulin sensitivity

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Long-chain fatty acids (LCFA) derived from lipolysis in adipose tissue, from lipolysis of circulating VL DL-triacylglycerol and from lipolysis of intramyocellular triacylglycerol (IMTG) serve important functions in skeletal muscle. Apart from the fundamental role as a fuel for energy production, LCFA serve as important components of biological membranes and in selected signal transduction pathways to alter gene expression. LCFA from the circulation are either immediately oxidized or esterified to triacylglycerol (TG), which is stored in lipid droplets (LDs), recognized as functional organelles consisting of a core of TG and cholesteryl esters surrounded by a phospholipid monolayer and with several regulatory proteins associated with the LD surface.

The mobilization of fatty acids from the IMTG pool are catalyzed by three lipases:

adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), and monoacylglycerol lipase (MAGL) which sequentially degrade TG, diacylglycerol (DAG) and monoacylglycerol (MAG), respectively. The specific activity of ATGL for TG is 10 times higher than that for DAG, while HSL preferentially hydrolyses DAG with a 10-times higher lipolytic rate compared to TG.

In skeletal muscle HSL is considered the primary lipase activated by contractions. This notion is based on in vitro activity measurements, where the contraction induced increase in TG-lipase activity was completely blocked when adding an HSL-antibody to the assay medium. However, the in vitro activity assay does not include changes in important regulatory events such as translocation of lipases to the lipid droplets and interaction with lipid droplet associated proteins, and therefore may not entirely reflect the acute activation of muscle TG-lipases in vivo. In addition, in several human studies dissociations between in vitro HSL activity and net change in IMTG content during exercise have been observed. This may reflect that lipases other than HSL are at play. Knowledge about ATGL in skeletal muscle is limited, but ATGL protein expression and activity have been demonstrated in both rodent and human skeletal muscle. The functional importance of ATGL for basal TG-hydrolysis in skeletal muscle is highlighted by the finding of massive IMTG-accumulation in ATGL-KO mice. These data strongly suggest that ATGL has an important role for skeletal muscle TG-hydrolysis. However, it is not known under which physiological conditions ATGL is activated in skeletal muscle. Dysregulation of lipid metabolism in skeletal muscle is related to several pathological disorders, among these insulin resistance. Lipid intermediates or metabolic by-products derived from esterification of fatty acids into IMTG, from lipolysis of IMTG or from β -oxidation are all candidates involved in insulin resistance. Several molecular mechanisms behind lipid induced

insulin resistance in skeletal muscle have been suggested, however this topic is still unresolved.

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SA30

Skeletal muscle carnitine availability in exercise and insulin resistance

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More than 95% of the body's total carnitine store resides within skeletal muscle, where it plays two essential roles (for review see Stephens et al. 2007). Firstly, during intense exercise, when the rate of pyruvate dehydrogenase complex (PDC) flux exceeds the rate of acetyl group utilisation by the tricarboxylic acid cycle, free carnitine buffers acetyl group production to maintain the mitochondrial acetyl-CoA/CoASH ratio. Secondly, free carnitine is essential for the translocation of long-chain fatty-acids into the mitochondrial matrix for subsequent beta-oxidation. This presentation will highlight research demonstrating the significance of muscle carnitine availability to the regulation of muscle fuel metabolism in vivo at rest in normal and insulin resistant states. It will also draw attention to evidence published over the past decade that high muscle PDC flux and thereby acetylcarnitine accumulation during exercise can reduce muscle free carnitine availability to an extent that it will limit mitochondrial fat oxidation in vivo (van Loon et al. 2001).

Oral L-carnitine feeding has been advocated as an ergogenic aid, however L-carnitine feeding per se has no impact on muscle total carnitine content, fuel metabolism or exercise performance. We have demonstrated that intra-venous L-carnitine infusion (steady-state plasma [550-600] $\mu\text{mol.l}^{-1}$) under insulin clamp conditions acutely increased muscle total carnitine (by ~15%) in healthy male volunteers when serum insulin concentration was increased above 50 mU.l^{-1} . Furthermore, this reduced muscle glycolysis, blunted PDC activation (under conditions of fixed glucose delivery), increased glycogen storage, and increased muscle long-chain acyl-CoA content at rest (for review see Stephens et al. 2007). More recently, we have shown this increase in muscle total carnitine content can be achieved by combined carbohydrate and L-carnitine feeding over a 6 month period in healthy, male volunteers. Moreover, this increase in TC content reduced PDC activation and muscle glycogen utilisation during low intensity exercise (50% $\text{VO}_{2\text{max}}$), and maintained PDC flux and improved exercise performance during more intense exercise (80% $\text{VO}_{2\text{max}}$; Wall et al. 2011). Conversely, we have recently found that muscle total carnitine content can be markedly depleted by blocking renal carnitine retention using mildronate, which had the effect of reducing whole body fat oxidation and increasing muscle glycogen utilisation in a rodent model of obesity (Porter et al. unpublished observation).

Collectively these findings point to a central role for carnitine in the regulation of muscle fuel selection at rest and during exercise in obese and non-obese states. The demonstration that muscle carnitine availability can be readily manipulated

in humans, and significantly impacts on physiological function under a variety of conditions, will result in renewed interest in this compound.

van Loon LJ, Greenhaff PL, Constantin-Teodosiu D, Saris WH & Wagenmakers AJ (2001). The effects of increasing exercise intensity on muscle fuel utilisation in humans. *J Physiol* 536, 295-304.

Stephens FB, Constantin-Teodosiu D and Greenhaff PL (2007) New insights concerning the role of carnitine in the regulation of fuel metabolism in skeletal muscle. *J Physiol* 581:431-444.

Wall B, Stephens F, Constantin-Teodosiu D, Marimuthu K, Macdonald I and

Greenhaff P (2011) Chronic oral ingestion of L-carnitine and carbohydrate increases muscle carnitine content and alters muscle fuel metabolism during exercise in humans: the dual role of muscle carnitine in exercise metabolism. *J Physiol* 589:963-973.

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Regulation of skeletal muscle carbohydrate oxidation at the level of pyruvate dehydrogenase complex

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The pyruvate dehydrogenase complex (PDC) controls the rate-limiting step in carbohydrate (CHO) oxidation, the oxidative decarboxylation of pyruvate to acetyl-CoA, and occupies a central role in skeletal muscle intermediary metabolism. The activation of PDC is controlled by a phosphatase (PDP), which dephosphorylates and hence activates PDC and a kinase (PDK), which reduces PDC activity by phosphorylating the E1 component of the complex. The identification of 4 different PDK isoforms (PDK1-4) has intensified the research on their role as potential molecular regulators of glucose oxidation under conditions of altered glucose homeostasis. Two of those isoforms (PDK2 and PDK4) are abundant in human skeletal muscle, whereas PDK1 and 3 isoforms are expressed at very low levels.

Physiological hyperinsulinaemia stimulates skeletal muscle PDC activity, and hence glucose oxidation, but this effect is impaired in metabolic states characterised by elevated lipid availability and resistance to insulin's actions, such as starvation (Tsintzas et al. 2006), high fat diet (Chokkalingam et al. 2007) and lipid infusion (Tsintzas et al. 2007). An increase in muscle PDK4, but not PDK2, protein content is responsible for the long-term control of muscle PDC activity and the consequent reduction in glucose oxidation in response to starvation and high fat diet. Interestingly, this shift in substrate utilization from CHO to fat appears to precede changes in overall glucose uptake, which highlights a key role for PDK4 through its regulation of PDC in substrate metabolism and insulin action in human skeletal muscle (Chokkalingam et al. 2007). Acute lipid overload mediates both allosteric (via acetyl-CoA accumulation) and transcriptional modulations of skeletal muscle PDC activity (via changes in PDK4 content). In healthy humans, insulin infusion can rapidly suppress PDK4, but not PDK2, gene expression in skeletal muscle, whereas elevated levels of plasma free fatty acids (FFA) are positive regulators of PDK4 expression. Increasing the circulating FFA levels through lipid infusion

during short-term physiological hyperinsulinaemia attenuates the insulin-stimulated suppression of PDK4 expression and results in accumulation of intracellular long-chain Acyl-CoA, suggesting that intramuscular lipids may play an important role in the regulation of PDK4 expression in a manner independent from the Akt/FOXO3 pathway (Tsintzas et al. 2007). Although PDK4 is a target gene for a number of lipid-regulated transcription factors including PPAR α and PPAR δ , studies using primary human muscle culture revealed that the stimulatory effect of FFAs on PDK4 is largely mediated through activation of PPAR δ . However, transient changes in intramuscular substrate flux might not be important for the transcriptional regulation of PDK4. Indeed, in healthy humans short-term infusions of lipid and adrenaline can induce skeletal muscle PDK4 gene expression to a similar degree despite differential effects on metabolic fuel utilisation.

Hyperglycaemia increases the activity of PDC in human skeletal muscle and recent evidence suggests that it results in rapid downregulation of PDK4 gene expression in that tissue. Although the mechanism underlying the suppressive effect of hyperglycaemia on PDK4 is unknown, it appears that factors other than activation of the IRS1/Akt axis mediate this effect in skeletal muscle. The activity of PDC also increases during exercise in a calcium-dependent manner resulting in an increase in pyruvate flux, the formation of acetyl-CoA and a concomitant increase in CHO oxidation. Carbohydrate ingestion immediately before exercise (resulting in increased blood glucose and insulin concentrations) augments the exercise-induced activation of PDC in human skeletal muscle, which facilitates the increase in insulin-stimulated glucose oxidation under those conditions (Tsintzas et al. 2000). However, neither PDK4 protein content nor basal or insulin-stimulated PDC activity are affected by a single bout of exercise performed the day before (Stephens et al. 2010). These findings do not support the notion that a PDK4-mediated inhibition of PDC activation, and thus glucose oxidation, may facilitate the increase in insulin-mediated skeletal muscle glycogen synthesis during recovery from exercise.

In summary, in healthy humans, the impairment of insulin action in metabolic states characterised by elevated lipid metabolism is associated with, and often preceded by, impaired basal and insulin-stimulated activation of skeletal muscle PDC, and hence glucose oxidation, as a result of a selective upregulation of PDK4 protein content. Therefore, by virtue of its key role in the regulation of glucose oxidation, PDK4 is a potential molecular target for future strategies aiming to maintain optimum insulin action.

Tsintzas et al. 2000. *Exp Physiol* 85(5):581-586

Tsintzas et al. 2006. *J Physiol* 575:291-303

Chokkalingam et al. 2007. *J Clin Endocrinol Metab* 92:284-292

Tsintzas et al. 2007. *J Clin Endocrinol Metab* 92:3967-3972

Stephens et al. 2010. *Exp Physiol* 95(7):808-18

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SA32

Carnitine acetyltransferase as a critical metabolic node for fuel selection, glucose tolerance and exercise performance

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The concept of “metabolic inflexibility” was first introduced to describe the failure of insulin resistant human subjects to appropriately adjust mitochondrial fuel selection in response to nutritional cues. This phenomenon has since gained increasing recognition as a core component of the metabolic syndrome, but its molecular basis has remained elusive. Recent work in our laboratory suggests that metabolic inflexibility in the obese state might be partly attributable to a decline in muscle activity of carnitine acetyltransferase (CrAT), a mitochondrial matrix enzyme that catalyzes the conversion of acetyl-CoA and other short chain acyl-CoAs to their membrane permeant acylcarnitine esters. By doing so, CrAT regulates intracellular and inter-tissue trafficking of carbon intermediates. Studies in muscle-specific CrAT knockout mice, primary human skeletal myocytes and human subjects undergoing L-carnitine supplementation revealed an essential role for CrAT in controlling substrate switching, glucose tolerance and exercise performance. The results support a model wherein CrAT promotes metabolic flexibility during feeding and exercise by permitting mitochondrial efflux of excess acetyl moieties that otherwise inhibit key regulatory enzymes, such as pyruvate dehydrogenase. These findings along with ongoing studies seeking to further delineate the role of CrAT in combatting nutrient-induced mitochondrial stress will be discussed.

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Skeletal muscle mitochondrial respiration in obesity and type 2 diabetes

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The mechanism(s) behind the decreased ability of insulin to facilitate glucose uptake in insulin sensitive tissues as seen in type 2 diabetes is not resolved. With the rapidly increasing prevalence of this disease world-wide, and the many complications that follow the disease, large resources are used in the attempt to resolve the mechanisms of insulin resistance. In this context, a dysfunction of mitochondria in the skeletal muscle has been suggested to play a pivotal role. It has been postulated that a decrease in the content of mitochondria in the skeletal muscle can explain the insulin resistance. Complementary to this also specific defects of components in the respiratory chain in the mitochondria have been suggested to play a role in insulin resistance. A key element in these mechanistic suggestions is inability to handle substrate fluxes and subsequently an accumulation of ectopic intramyocellular lipids, interfering with insulin signaling. In the presentation I will present arguments for the unlikelihood of this scenario being instrumental in human insulin resistance.

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Fuel utilisation, exercise training and skeletal muscle insulin resistance

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Regular endurance training enhances fat oxidation during exercise at a given exercise load and decreases muscle glycogen utilisation. In addition to training status the daily dietary macronutrient intake as well as the consumption of carbohydrate containing beverages and/or food during exercise will influence fuel utilisation during exercise. After ultra endurance exercise where different exercise modalities are performed more or less continuously for several days fat oxidation during exercise is increased both at rest and during submaximal exercise (1). However, the increased fat oxidation may well be due to decreased glycogen stores and inability to maintain energy balance, both of which will increase fat utilization during exercise. To further elucidate the effect of excessive prolonged exercise on fat oxidation during exercise 14 days of very prolonged submaximal exercise was studied in 6 elderly male subjects. During a 14 day period the subjects performed approximately 10 hours of cycle exercise per day. During the 14 days the subjects consumed an ad lib high carbohydrate diet and were able to maintain body weight despite of the massive exercise load. Before and approx. 30-34 hours after completion of the last of the 14 days maximal fat oxidation was measured during a graded cycle exercise protocol in the overnight fasted condition. Interestingly a marked decrease in maximal fat oxidation and a decreased maximal oxygen uptake was observed after regular excessive prolonged exercise (Authors unpublished findings). The first part of this talk will focus on the result of this study and further discuss the possible mechanisms explaining this observation.

In diabetes and obesity a common trait is the occurrence of excess fat accumulation in skeletal muscle and insulin resistance. Although muscle triacylglycerol is often correlated to insulin resistance, a causal link remains to be demonstrated. However, increased intracellular concentrations of long chain Acyl CoA are probably present when muscle triacylglycerol content is high, and this may well lead to increased concentrations of skeletal muscle bioactive lipid intermediates, such as ceramide and diacylglycerol (2). Ceramide and/or diacylglycerol can attenuate insulin signalling in muscle and may be the link between muscle triacylglycerol and skeletal muscle insulin resistance. In skeletal muscle ceramides are generated primarily through de novo synthesis from palmitate and serine or through breakdown of sphingomyelin in the cell membranes (2). In the second part of this talk focus will be on the muscle ceramide content in man and the available evidence for a role in human skeletal muscle insulin resistance. In particular focus will be on the role of manipulation of plasma fatty acid content achieved either by lipid heparin infusion during a euglycemic hyperinsulinemic clamp or by prolonged intake of fat rich diet. Furthermore the focus will be on the effect of training and/or inactivity on muscle ceramide content studied with a longitudinal approach.

Helge JW, Rehner NJ, Pilegaard H, Manning P, Lucas SJ, Gerrard DF, Cotter JD. Increased fat oxidation and regulation of metabolic genes with ultraendurance exercise. *Acta Physiol (Oxf)* 2007;191:77-86.

Summers SA. Ceramides in insulin resistance and lipotoxicity. *Prog Lipid Res* 2006;45:42-72.

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SA35

Gene therapy for Duchenne muscular dystrophy

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Muscular dystrophies refer to a group of inherited disorders characterized by progressive muscle weakness, wasting and degeneration. So far, there are no strongly effective treatments but new gene-based therapies are currently being developed with particular advances in using conventional gene replacement strategies, RNA-based approaches, or cell-based gene therapy and with a main focus on Duchenne muscular dystrophy (DMD). DMD is the most common and severe form of muscular dystrophy and current treatments are far from adequate. However, genetic and cell-based therapies, in particular exon skipping induced by antisense strategies, and corrective gene therapy via functionally engineered dystrophin genes hold great promise, with several clinical trials ongoing. Proof-of-concept of exon skipping has been obtained in animal models, and most recently in clinical trials; this approach represents a promising therapy for a subset of patients. In addition, gene-delivery-based strategies exist both for antisense-induced reading frame restoration, and for highly efficient delivery of functional dystrophin mini- and micro-genes to muscle fibres *in vivo* and muscle stem cells *ex-vivo*. In particular, AAV-based vectors show efficient systemic gene delivery to skeletal muscle directly *in vivo*, and lentivirus-based vectors show promise of combining *ex vivo* gene modification strategies with cell-mediated therapies. This research lecture will discuss:

Global significance of neuromuscular disease.

Advances and clinical trials in antisense and RNA directed therapies.

Advances in AAV vector and microdystrophin gene therapy for muscle diseases.

Manipulating myostatin to counter muscle atrophy.

Gene editing technology for targeted correction of the Duchenne muscular dystrophy gene.

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Modifying vein grafts by gene therapy

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Patency rates for coronary artery bypass grafting (CABG) procedures using autologous saphenous vein (SV) remain poor. The vast majority of failures are due to neointima formation and superimposed atherosclerosis. There remains a requirement to develop a novel therapy through which to improve patency rates. Since many of the molecular and cellular mechanisms that lead to neointima formation have been identified, a number of strategies have emerged. These include modulation of smooth muscle cell migration, proliferation and/or apoptosis, acceleration of endothelial regeneration and improvement in endothelial function. Vein grafting is highly suited for human gene therapy since it allows *ex vivo* manipulation of the vein prior to grafting into the coronary circulation of the patient. This has clear safety advantages over *in vivo* gene therapeutic applications but, due to the short clinical window through which gene transfer can be administered, efficient vector systems are required. Adenoviral vectors have proven efficient for gene delivery in this context although expression of transgenes using first-generation vectors is transient in nature and associated with inflammation. However, this may be advantageous since a number of candidate therapeutic transgenes have been targeted to either block vascular smooth muscle cell migration and/or proliferation or to promote apoptosis. Our research has shown that overexpression of certain genes, including TIMP-3, p53 and NogoB has a beneficial effect by reducing neointima formation. This represents a valid therapeutic strategy to prevent vein graft failure in patients. In addition, we have developed a number of engineering strategies to manipulate the tropism of the adenovirus capsid to enhance gene delivery to the smooth muscle cell compartment.

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Oncolytic viruses as cancer therapeutics

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Gene therapy for cancer has evolved over the last 20 years from its initial proof of principle strategies involving plasmid vectors and non-replicating genetically modified viruses to the current era to the extensive evaluation of live replicating oncolytic viruses (OV). Importantly clinical application has progressed from the phase I 'proof of principle' type studies to the randomised phase III clinical trial arena.

Remarkably OV appear in the medical literature on a number of occasions over the last 100 years including prospective clinical studies in the 60's and 70's [1]. The finding from these studies have been regarded more as medical curiosities but evolving understanding of both molecular pathology of common malignancies as well as basic viral biology has brought the two fields back together again. Around 20 viruses have been

or are been tested preclinically and a dozen or so viruses have been used in clinical trials over the last century. There are currently 7 viruses in clinical trial at present.

Broadly there is a natural division between wild type and engineered viruses and for the engineered agents, a variety of transgenes ranging from immunostimulatory molecules through to tumour-associated antigens. However, opinion is still divided as to whether the anti-tumour efficacy of OV is due to direct cellular cytotoxicity or whether indeed oncolytic viruses are effectively a sophisticated immunotherapy where tumour cell lysis by OV primes an anti-tumour response which is then sustained by further rounds of oncolysis.

The prototype OV were adenoviruses which evolved from their replication defective versions which in turn were problematic due to inadequate transduction efficiency. The ONYX 015 was the first administered replication competent engineered OV in humans and was evaluated in patients with malignancy in the pancreas, brain and head and neck, both as single agent and in combination with chemotherapy [2]. A similar agents has been licensed in China for the treatment of head and neck cancer.

A number of other viruses are being tested through systemic and local delivery in humans. Reoviruses are naturally occurring and ubiquitous non-pathogenic virus. Cancer cells are susceptible to reovirus cytotoxicity partially as cancer cells are unable to activate PKR in response to viral double stranded RNA unlike normal cells. Clinical evaluation of reovirus alone or in combination with chemotherapeutics and radiation therapy is the largest OV programme currently ongoing worldwide. Evidence of anti-tumour activity has been documented radiologically and in tumour biopsies post treatment [3]. Four strains of engineered herpes simplex virus type 1 (HSV-1) have progressed to clinical assessment. These viruses have deletions in the gene including ICP 34.5 to support tumour selectivity and diminish neurovirulence. The most advanced programme is using the Oncovex agent; the ICP 34.5 gene replaced by a sequence encoding GM-CSF to enhance anti tumour responses. The results of the current Oncovex programme are impressive with evidence of distance immune responses after local injection of patients of recurrent malignant melanoma. Newcastle Disease Virus is an apathogenic virus in humans and has been evaluated in over 100 patients with documented objective responses. Finally there has been a large clinical programme evaluating oncolytic vaccinia The Wyeth strain has been modified by deletion of thymidine kinase and incorporation of GM-CSF the recent reports are successful phase I study [4].

There are a number of issues that are currently challenging the development of OV therapy. A robust regulatory framework now exists in the UK so many of the problems are focused on practical issues such as handling of genetically modified agents within the Health Service, necessity for specialised pharmacy facilities, and defining where the agents are administered and any containment required. The other hurdles are factors limiting the anti-cancer efficacy. These include the access of the virus to tumours after injection which is limited by a profound and early neutralising immune response against the virus [5]. Other issues are how to measure the anti-tumour responses secondary to viral treatment. Convention uses traditional RECIST response criteria it may be less appropriate to these agents bearing in mind their tropism for tumour vasculature and propensity for tumours to necrose.

Finally it is unlikely that OVs will have sufficient efficacy as single agents. Combination with cytotoxic and more targeted therapies, are the realistic route to adoption as a new anti-cancer modality. To date it is clear that current co-administration

of cytotoxic agents does not damage the viruses and does not lead to enhanced toxicity to the patients.

Liu TC, Galanis E, Kirn D. Clinical trial results with oncolytic virotherapy: a century of promise, a decade of progress. *Nat Clin Pract Oncol*. 2007 ; 4(2):101-117.

Nemunaitis J, Senzer N, et al. A phase I trial of intravenous infusion of ONYX-015 and enbrel in solid tumor patients. *Cancer Gene Ther*. 2007 ; 14(11):885-893.

Vidal, L., H. S. Pandha, et al. A phase I study of intravenous oncolytic reovirus type 3 Dearing in patients with advanced cancer." *Clinical cancer research* 2008;14(21): 7127-7137.

Breitbart CJ, Burke J, et al Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans. *Nature*. 2011; 31:477(7362):99-102

White, C. L., K. R. Twigger, et al. Characterization of the adaptive and innate immune response to intravenous oncolytic reovirus (Dearing type 3) during a phase I clinical trial. *Gene therapy* 2008; ;15(12): 911-920

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SA38

The clonal distribution of viral integration sites in clinical gene therapy

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Monogenetic diseases have already been successfully treated by gene therapy using retroviral vectors. By now, several diseases like X-linked severe combined immunodeficiency (X-SCID), adenosine deaminase (ADA)-deficient SCID, X-linked chronic granulomatous disease (X-CGD) and X-linked Wiskott Aldrich Syndrome (X-WAS) have been treated successfully. However, with the enhancement of retroviral gene transfer efficiency the occurrence of side effects increased. It was shown that the insertion of functional retrovirus LTRs can lead to insertional mutagenesis. By now, severe side effects have exclusively been observed in clinical trials using gamma-retroviral vectors as gene delivery vehicles. Individual patients enrolled in clinical gene therapy trials for X-SCID, X-CGD and X-WAS suffered from leukemia or myelodysplastic syndrome. For all reported cases of severe side effects, it was shown that vector-induced overexpression of nearby oncogenes (LMO2, CCND2, BMI1, MDS1-EV11), was contributing as one step toward the malign transformation of individual cell clones. The occurrence of severe side effects highlighted the need for safer vectors systems and lead to the development HIV-1 based lentiviral vectors with self-inactivating (SIN) configuration. This vectors are a promising alternative to LTR-driven gamma-retroviral vectors, showing a reduced genotoxic risk in preclinical testing's and so far no severe adverse events have been reported from clinical trials.

We have performed an in depth integration site analysis of patient samples from the first clinical trial to treat a monogenetic cerebral disease using autologous hematopoietic stem cell transplantation with a HIV-1 based lentiviral SIN-vector. The cerebral disease has been stabilized in 2 out of 4 patients, the follow-up being too short in the 4th patient to draw any conclusion of clinical efficacy. So far, the correction of hematopoietic stem cell has not been accompanied by signs of clonal dominance or even premalignant disproportional distribution of cellular contributions in the 4 treated patients. Our large

scale vector integration sites (IS) analysis performed on ex vivo transduced cells prior to reinfusion and on engrafted cells by LAM-PCR and subsequent 454 pyrosequencing showed a polyclonal hematopoietic reconstitution for samples from the first four patients treated by now. Downstream bioinformatics analysis of retrieved raw sequence data revealed the characteristic insertion profile reported for lentiviral vectors, showing gene coding regions as preferred targets for lentiviral vector integration (P1: 74%; P2: 74%; P3: 72%; P4: 71%), in line with a favored integration on chromosomes harboring gene dense regions. A successful ex vivo transduction of early hematopoietic progenitors is indicated by the presence of identical IS identified in myeloid and lymphoid lineages in P1, P2 and P3 (analysis is ongoing for P4). Interestingly, a preference of lentiviral vector insertion for particular genes has been observed by the identification of lentiviral vector integration sites as common integration sites in the same genes or genomic regions, observed in all four patients. Among others, the following genes were targeted by the vector: KDM2A, HLA and PACS1. High throughput distribution analysis of the IS repertoire indicates that lentivirus vectors offer great promise for safe and effective correction of human stem and progenitor cells.

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Clinical evaluation of gene therapy for cystic fibrosis: safety and expression of a single dose of lipid-mediated CFTR gene therapy to the upper and lower airways of patients

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Cystic Fibrosis (CF) is life threatening single gene disorder that affects the lungs, pancreas, intestines and reproductive tract. All cases of CF can be ascribed to the inheritance from both parents of a mutant allele of the cystic fibrosis transmembrane conductance regulator gene, CFTR. The CFTR protein regulates the flow of water and ions across secretory epithelia. Whereas the effects of CFTR deficiency in the pancreas and intestines are relatively well managed, the repeated lung infections and progressive loss of lung function are not so. Gene therapy has the potential to halt this progression if safely and effectively delivered to the lung. Pilot studies in the mid 90's suggested that this might be possible, but expression was transient. In a ten year collaborative programme, the UK CF Gene Therapy Consortium has made significant progress on several fronts to improve the safety and efficacy of these original formulations. Here, I will describe that progress towards the clinical, the results from a Phase 1 dose-ranging study and the status of a follow-on multi-dose Phase 2 study.

Results - In a Phase 1 dose-ranging trial to assess safety, a single dose of pGM169/GL67A was administered to CF patients by spray to the nasal epithelium and by nebulisation to lower airways. pGM169 is a CpG-free plasmid encoding a human CFTR cDNA driven by a CMV enhancer and human elongation factor 1 alpha (hCEFI) promoter. GL67A is a lipid gene trans-

fer agent produced by Genzyme. The gene therapy formulation is prepared simply by mixing the two components allowing the lipid to complex with the plasmid DNA.

CFTR transgene expression in the airways was measured using QRT-PCR on epithelial cells harvested by brushing. Nasal and bronchial potential differences (PD) were also measured. Thirty-five adult CF patients were recruited with a baseline FEV1 (forced expiratory volume in 1 sec; a standard measure of lung function) of at least 60% predicted.

Each was nebulised with 20 mL (n=17), 10 mL (n=10) or 5 mL (n=8) of pGM169/GL67A. A transient drop in FEV1 was observed over the next 6 h (mean [SD]: 20 mL 25.7[10.2]%; 10 mL 17.7[9.9]%; 5 mL 13.0[4.4]%; this was dose-related, well-tolerated, not accompanied by desaturation, showed a restrictive pattern and did not respond to bronchodilators. A similarly dose-related systemic inflammatory response was also noted, the symptoms of which responded well to standard anti-pyretics. Generally this was confined to the first 24-48 hours after dosing. A rise in white blood cell count was observed which correlated significantly with the magnitude of the drop in FEV1, implying that the acute lung function effect may be an inflammatory phenomenon. Interestingly, none of a subset of 6 patients (4 from the 10 mL dose and 2 from the 5 mL dose) given 4 g paracetamol over an 18 hour period after dosing developed a fever: these subjects also displayed less marked systemic inflammatory responses. This phenomenon may be related to the pulmonary inflammation attenuation effects of paracetamol observed in influenza-infected mice and reported by Lauder et al [Thorax (2011) 66:368].

Pre- and post-dosing (day 6 or day 14) bronchoscopies were conducted on 10 patients. QRT-PCR detected CFTR transgene expression in samples from some of this cohort. In bronchial PD measurements, the majority of the patients' zero chloride responses to isoproterenol increased towards non-CF values after dosing. Eleven of the 19 patients receiving a 2 mL nasal dose responded when assessed by nasal PD measurement: a positive response was defined as a chloride secretory response outside the range of the subject's pre-dose measurements. Intriguingly, PD was noticeably more often positive when measured at later time points after dosing. In the two best responders, maximal zero chloride responses exceeded 10 mV (clearly within the normal non-CF range) and persisted to days 63 and 91 respectively. This is concordant with the plasmid's engineered ability to build up transgene expression slowly, but maintain peak expression for an extended period.

Transgene expression was seen in nasal and lower airways, and restoration of CFTR function to the non-CF range was observed by PD measurement out to 91 days following a single nasal dose.

Conclusions/Implications - These data, together with our supportive toxicology results, encourage us to embark on a repeated dosing clinical trial.

We regard the pulmonary and systemic side effects observed after the 20 mL nebulised dose as undesirable in a repeated administration context. Those seen with 10 mL were more acceptable: 5 mL produced only small effects. Simple antipyretic treatment moderates the symptoms, and the repeated dosing clinical trial will use 5 mL.

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Use of microbial photoreceptors to light-manipulate cells and organisms

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Photoreceptors from archaea, bacteria, and green algae were molecularly identified in recent years. We could show that some of them are ideal tools to manipulate animal cells by illumination. The Channelrhodopsins from the unicellular green alga *C. reinhardtii* are Light-gated cation channels which allow fast light-induced depolarization (1,2) of the plasma membrane. Mutations led to a slower photocycle and therefore to Channelrhodopsins with higher light sensitivity. Neuronal expression of Channelrhodopsin-2 (ChR2) yields Light-induced action potentials and Light-manipulated behaviour in *C. elegans* (3). The Light-activated chloride pump halorhodopsin (HR) from the archaeum *Natronomonas pharaonis* hyperpolarizes the plasma membrane and therefore allows Light-induced silencing of neurons (4). These two antagonistic rhodopsins may even be expressed in the same cell and still specifically be light-activated with 460 nm for ChR2 and 580 nm for HR.

Photoactivated Adenylyl Cyclases (PAC) from *Euglena gracilis* (5,6) or *Beggiatoa spec.* (7) are flavoproteins which quickly elevate cytoplasmic cyclic AMP by illumination with blue light in cultured cells and living animals or plants.

Nagel G., D. Ollig, M. Fuhrmann, S. Kateriya, A.M. Musti, E. Bamberg, P. Hegemann (2002). Channelrhodopsin-1: a light-gated proton channel in green algae. *Science* 296: 2395-2398

Nagel, G, T. Szellas, W. Huhn, S. Kateriya, N. Adeishvili, P. Berthold, D. Ollig, P. Hegemann, E. Bamberg. (2003) Channelrhodopsin-2, a directly Light-gated Cation-selective Membrane Channel. *Proc Natl Acad Sci U.S.A.* 100:13940-13945

Nagel, G., M. Brauner, J.F. Liewald, N. Adeishvili, E. Bamberg, A. Gottschalk (2005) Light-activation of Channelrhodopsin-2 in excitable cells of *Caenorhabditis elegans* triggers rapid behavioral responses. *Current Biology* 15(24):2279-84.

Zhang F., L.-P. Wang, M. Brauner, J.F. Liewald, K. Kay, N. Watzke, P.G. Wood, E. Bamberg, G. Nagel, A. Gottschalk, and K. Deisseroth (2007) Multimodal fast optical interrogation of neural circuits. *Nature* 446:633-639

Schröder-Lang, S., M. Schwärzel, R. Seifert, T. Strünker, S. Kateriya, J. Looser, M. Watanabe, U. B. Kaupp, P. Hegemann, G. Nagel (2007) Fast manipulation of cellular cAMP level by light in vivo. *Nat Methods* 4(1):39-42

Looser J, Schröder-Lang S, Hegemann P, Nagel G. (2009) Mechanistic insights in light-induced cAMP production by photoactivated adenylyl cyclase alpha (PACalpha). *Biol Chem.* 390(11):1105-11

Stierl M, Stumpf P, Udvari D, Gueta R, Hagedorn R, Losi A, Gärtner W, Petereit L, Efetova M, Schwarzel M, Oertner TG, Nagel G, Hegemann P. (2011) Light-modulation of cellular cAMP by a small bacterial photoactivated adenylyl cyclase, bPAC, of the soil bacterium *Beggiatoa*. *J Biol Chem.* 286(2):1181-8

Funded by the German Research Society (DFG). I am indebted to my collaborators and coworkers, especially Alexander Gottschalk (Univ. Frankfurt/M.), Ernst Bamberg (MPI Biophysik Frankfurt/M.), Karl Deisseroth (Stanford Univ.), Saskia Schröder-Lang (MPI Biophysik Frankfurt/M.), Jens Looser (Univ. Würzburg), Ronnie Gueta (Univ. Würzburg), and Peter Hegemann (Humboldt Univ. Berlin).

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SA41

Light-sensitive G-protein-coupled receptors

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The tractability of light activated receptors makes them attractive tools by which to study the brain. They allow for the non-invasive and specific control of neuronal signaling and could allow for the study of receptor pathways that occur faster than the rate of diffusion. Furthermore, with the aid of implantable light devices light activated receptors have the potential for use in live animals and later in humans to control and cure GPCR pathways involved in diseases. We demonstrate here the use of vertebrate rhodopsin to control ion channel modulation, spinal cord, cerebellar and serotonergic signaling via activation of the pertussis toxin sensitive Gi/o pathway by light.

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SA42

Optogenetic tools: Genomic mining and molecular engineering

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Optogenetic tools enable the optical perturbation of complex cellular physiology in intact tissue, by sensitizing genetically targeted cell populations via the heterologous expression proteins with known light-activated physiological effect. For example, arbitrary trains of action potentials can be optically elicited with millisecond resolution by depolarizing excitable cells expressing light-gated cation channels. The optogenetic toolbox is an incredibly rich one, enabling panoply of inducible physiological perturbations with spatio-temporal precision: depolarizing channels, hyperpolarizing pumps, GPCR's, nucleotide cyclases, allosteric modulators of protein-protein interactions, among others. Ultimately, since they are commonly derived from lower organisms and microbes, innovation in optogenetic tool development has been greatly accelerated by genomic technologies, both in next-generation sequencing and de novo synthesis. This talk will cover the use of such genomic technologies as well as automated phenotyping systems for the discovery and molecular engineering of novel optogenetic tools, and their potential translational application toward a next-generation of neuromodulation devices to correct aberrant activity in neural circuits implicated in nervous system disorders and abnormal pathologies.

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SA43

Optogenetic control of astrocytesA.G. Teschemacher¹, S. Lane¹, F. Tang¹, N. Marina², A.V. Gourine² and S. Kasparov¹¹*Physiology and Pharmacology, Univ Bristol, Bristol, UK and*²*Neuroscience, Physiology & Pharmacology, University College London, London, UK*

Over the past decade astroglia has come to be recognised as an active player in information processing in the central nervous system. It has been implicated in a variety of vital functions such the regulation of breathing as we have previously reported (Gourine et al., 2010). In order to elucidate the varied and important roles of astrocytes in different functional contexts, optogenetic approaches are proving invaluable for selective astrocyte stimulation. Whilst not electrically excitable, astrocytes display excitability based on $[Ca^{2+}]_i$ increases following metabotropic receptor activation and Ca^{2+} mobilisation from stores and/or Ca^{2+} entry from the extracellular space. Our initial experiments employing light sensitive ion channels made use of the Ca^{2+} permeability of the channelrhodopsin variant ChR2(H134R) to excite astrocytes and initiate glial $[Ca^{2+}]_i$ waves which self-propagate through release of ATP. Experiments in nil external Ca^{2+} and using thapsigargin (1 μ M) showed that ChR2(H143R)-induced Ca^{2+} excitation of astrocytes involves Ca^{2+} release from intracellular stores. Currently we are exploring other opsins such as CatCh as tools for control of astrocytic $[Ca^{2+}]_i$ (Kleinlogel et al., 2011). An approach more closely simulating astrocytic cell physiology employs light-activated G-protein-coupled receptors. We adapted the rhodopsin-adrenoceptor (AR) chimaeras Opto- α 1-AR and Opto- β 2-AR (Airan et al., 2009) for selective expression in astrocytes using lenti- and adenoviral vectors and a transcriptionally enhanced, shortened glial fibrillary acidic protein promoter (Liu et al., 2008).

We are specifically interested in the communication between astrocytes and central noradrenergic neurones. To study this interaction, confocal $[Ca^{2+}]_i$ measurements and whole cell patch clamp recordings in organotypic rat brainstem slices were carried out and revealed that optogenetic excitation of astrocytes leads to powerful excitation of locus coeruleus (LC) or C1 neurones (a ventral group of pre-sympathetic catecholaminergic neurones). This response was delayed (~60-100 sec) and could be prevented by the glycogen metabolism blocker 1,4-dideoxy-1,4-imino-D-arabinitol (DAB; 500 μ M), indicating that it may be triggered by lactate released by activated astrocytes. We then investigated the effects of optogenetic stimulation of astrocytes on noradrenaline (NA) release measured by fast scan cyclic voltammetry. Opto-X-AR expressed in astrocytes in organotypic brain slices containing LC were activated with blue light flashes (15 – 25 Hz). Stimulation of astrocytes evoked robust NA release from neurones which was blocked by the P2Y purinoceptor antagonist MRS2179 (10 μ M) and by DAB, in line with the hypothesis that astrocytes in the LC signal via ATP and lactate secretion to evoke neuronal NA release. To further confirm this, ATP (400 μ M) or L-lactate (0.4 mM) were bath applied and mimicked the effect of astrocytic light stimulation on NA release. The effect of ATP was inhibited by co-application of MRS2179 or DAB. This suggests that activation of purinergic receptors on astrocytes may trigger lactate production and release, and that extracellular lactate may be the principle messenger leading to neuronal NA release. Consistent with this idea, activation of opto-AR in cultured dissociated astrocytes led to intracellular acidification as revealed

by confocal measurement of the ratiometric pH indicator SNARF-5, and this acidification could be prevented by DAB. The signalling pathway between astrocytes and noradrenergic neurones may be important in various brain areas as *in vivo* optogenetic stimulation of astrocytes in ventral brainstem areas containing noradrenergic and adrenergic neurones activates sympathetic outflow. Molecular mechanisms of this signalling pathway are currently under investigation.

Airan RD, Thompson KR, Fenno LE, Bernstein H, & Deisseroth K (2009). Temporally precise *in vivo* control of intracellular signalling. *Nature* 458, 1025-1029.

Gourine AV, Kasymov V, Marina N, Tang F, Figueiredo MF, Lane S, Teschemacher AG, Spyer KM, Deisseroth K, & Kasparov S (2010). Astrocytes control breathing through pH-dependent release of ATP. *Science* 329, 571-575.

Kleinlogel S, Feldbauer K, Dempski RE, Fotis H, Wood PG, Bamann C, & Bamberg E (2011). Ultra light-sensitive and fast neuronal activation with the $Ca(2)^+$ -permeable channelrhodopsin CatCh. *Nat Neurosci* 14, 513-518.

Liu B, Paton JF, & Kasparov S (2008). Viral vectors based on bidirectional cell-specific mammalian promoters and transcriptional amplification strategy for use *in vitro* and *in vivo*. *BMC Biotechnology* 8, 49.

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SA44

Optical reporters of synaptic activity

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Synapses are perhaps the most numerous computational elements within neural circuits. The process of chemical transmission can transform neural signals and, because synapses are plastic, these transformations can be altered over different time-scales to adjust the input-output relation of the circuit as a whole. We therefore need to assess the activity of large populations of synapses if we want to understand how neural circuits function.

I will describe some experimental strategies that allow the synaptic basis of circuit function to be studied *in vivo* by imaging of genetically-encoded reporters (Dreosti and Lagnado, 2011). The two most promising approaches available currently are pHluorin-based reporters of synaptic vesicle fusion (Miesenböck et al., 1998; Granseth et al., 2006) and genetically-encoded calcium indicators localized to presynaptic terminals (Dreosti et al., 2009). I will illustrate how these reporters can be used to analyze circuit function with examples drawn from our work on the synaptic processing of visual information in the retina (Dreosti et al., 2011; Baden et al., 2011; Odermatt et al., 2012).

Baden, T., F. Esposti, A. Nikolaev, and L. Lagnado. (2011). Spikes in retinal bipolar cells phase-lock to visual stimuli with millisecond precision. *Current Biology*, 21: 1859-1869.

Dreosti, E., B. Odermatt, M.M. Dorostkar, and L. Lagnado. (2009). A genetically encoded reporter of synaptic activity *in vivo*. *Nature Methods*, 6: 883-889.

Dreosti E. and L. Lagnado (2011). Optical reporters of synaptic activity in neural circuits. *Experimental Physiology* 96: 4-12.

Dreosti, E., F. Esposti, T. Baden, and L. Lagnado. (2011). In vivo evidence that retinal bipolar cells generate spikes modulated by light. *Nature Neuroscience*, 14: 951-952.

Granseth, B., B. Odermatt, S.J. Royle, and L. Lagnado. (2006). Clathrin-mediated endocytosis is the dominant mechanism of vesicle retrieval at hippocampal synapses. *Neuron*, 51: 773-786.

Miesenböck G, De Angelis DA, Rothman JE (1998). Visualizing secretion and synaptic

transmission with pH-sensitive green fluorescent proteins. *Nature*, 394:192-5.

Odermatt, B., A. Nikolaev, and L. Lagnado. (2012). Encoding of luminance and contrast by linear and non-linear synapses in the retina. *Neuron*, 73: 758-773.

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SA45

Optogenetic monitoring of neuronal circuit dynamics

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Protein-based fluorescent probes of neuronal activity are at the core of emerging approaches to study the dynamics of neuronal circuits that are composed of heterologous cell types. The rationale behind our large effort to develop genetically encoded voltage indicators lies in the fact that these probes allow us and others to move beyond the electrophysiological analysis of individual or small numbers of cells without neglecting cellular diversity or compromising temporal resolution. Work in our and other laboratories during the last 15 years resulted in a new generation of voltage-sensitive fluorescent proteins (VSFPs) based on the voltage-sensing domain (VSD) of *Ciona intestinalis* voltage sensor-containing phosphatase (Ci-VSP). To this end, our laboratory explored different design principle families for these engineered proteins and characterized numerous mutational variants for each facility. We demonstrated that recent versions of VSFPs can report membrane voltage signals in isolated neurons, brain slices and living mice. The most advanced probes enable the optical recording of action potentials from individual neurons in single sweeps and voltage imaging of population activity, including synchronized activities in the gamma frequency band, from defined cell populations in acute brain slices. In living mice, VSFPs afford sufficient SNR for probing sensory-evoked responses and enables univocal detection of spontaneous electrical population activity in somato-sensory cortex during light anesthesia or quiet alertness.

Along with the ability to target specific genetically-defined cell populations, VSFPs open a new experimental window for the study of the interaction dynamics of neuronal assemblies, facilitate the investigation of information processing mechanisms of the brain, such as the circuit operations involved in sensing our environment and generation of body movements, but will also be applicable to directly visualize cognitive functions.

AKEMANN, W., MUTOH, H., PERRON, A., ROSSIER, J., KNÖPFEL, T. (2010) Imaging brain electric signals with genetically-targeted voltage-sensitive fluorescent proteins. *Nature Methods* 7(8): 643-649.

KNÖPFEL, T. & BOYDEN, ES (2012) Editors. Optogenetics: tools for controlling and monitoring neuronal activity. *Progress in Brain Research*. Vol 196.

KNÖPFEL, T., DIEZ-GARCIA, J., AKEMANN, W. (2006) Optical probing of neuronal circuit dynamics: Genetically encoded versus classical fluorescent sensors. *Trends Neurosci*. 29: 160-166.

MUTOH H., PERRON A., IWAKOTO Y., AKEMANN W., KNÖPFEL, T. (2011) Optogenetic monitoring of membrane potentials. *Exp Physiol*. 96: 13-18.

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SA46

Hypoxic pulmonary hypertension: A clinical perspective

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PULMONARY HYPERTENSION AND HYPOXIA IN THE NORMAL PULMONARY CIRCULATION

Hypoxic vasoconstriction involves mostly small arterioles with an internal diameter of 200 – 600 μ m (1) and is predominantly caused by alveolar hypoxia rather than hypoxemia (2) Hypoxic pulmonary vasoconstriction is a disadvantageous physiological response at high altitude. When the whole lung is exposed to hypoxia, hypoxic pulmonary vasoconstriction results in a substantial increase in pulmonary vascular resistance and pulmonary arterial pressure.

In lowlanders a vigorous vasoconstrictor response to hypoxia may result in two conditions which may be lethal: high altitude pulmonary oedema (HAPE) and subacute mountain sickness. First described in highlanders in Peru who descended to the seaboard for several days before returning home above 4000 m (3), HAPE is a consequence of a pulmonary capillary leak into the interstitial and alveolar spaces (4). Since the pulmonary capillaries lie downstream of the small pulmonary arteries which vasoconstrict, high pressure in the capillaries could not occur were it not for uneven vasoconstriction resulting in patchy high pressure overperfusion of areas of lung tissue. The condition is rapidly reversible on descent.

Subacute mountain sickness is a condition in which chronic hypoxic pulmonary vasoconstriction leads to right heart failure in children in Tibet (5). It has also been reported in soldiers who engaged in strenuous exercise at 5800 – 6700 m for several months (6).

PULMONARY HYPERTENSION: PATHOPHYSIOLOGY IN THE DISEASED PULMONARY CIRCULATION

PH exists when the mean pulmonary arterial pressure is greater than 25 mm Hg (7). This may as a consequence of increased resistance to blood flow in the pulmonary circulation, increased downstream pressures in the left side of the heart requiring an increased pulmonary arterial pressure to maintain forward flow in the pulmonary circulation, a high cardiac output or a combination of these haemodynamic situations. The clinical consequence of maintaining a high pulmonary arterial pressure is to increase the afterload on the right ventricle resulting in uncoupling of the right ventricle and pulmonary circulation. The right ventricle progressively dilates and undergoes hypertrophy. Ultimately myocardial failure supervenes with a progressive fall in cardiac output resulting in death. For many of its causes pulmonary hypertension has a poor prognosis. Increased vascular resistance in the pulmonary circulation may be a consequence of hypoxic pulmonary vasoconstriction, pulmonary arterial hypertension, parenchymal lung disease,

myocardial and / or valve diseases of the left side of the heart, chronic thromboembolic disease as well as a variety of unknown mechanisms.

Chronic hypoxic pulmonary vasoconstriction results in medial hypertrophy of the pulmonary arteries. In contrast pulmonary arterial hypertension may result in vasoconstriction not associated with hypoxia, intimal proliferation into the vessel lumen causing obstruction to blood flow as well as the formation of vascular plexiform lesions and thrombosis. Progression of pulmonary arterial hypertension is associated with the development of hypoxaemia as a consequence of ventilation perfusion mismatch and low cardiac output.

Parenchymal lung disease such as chronic obstructive pulmonary disease (COPD) may cause pulmonary hypertension as a consequence of hypoxic pulmonary vasoconstriction but as lung parenchyma is lost as in emphysema or becomes fibrosed so too do its blood vessels, and the overall cross-sectional area of the pulmonary circulation is reduced. Other proposed mechanisms of pulmonary hypertension in lung disease include inflammation of blood vessels, and vasoconstriction caused by mechanical hyperinflation.

DRUG THERAPY FOR PULMONARY HYPERTENSION: WHO BENEFITS AND WHO DOES NOT?

It is clear that long-term oxygen therapy is the treatment of choice for patients with chronic hypoxic pulmonary vasoconstriction. In particular long-term oxygen may reduce progression of pulmonary hypertension in COPD although structural changes in the blood vessels remain unchanged.

Disease specific targeted drug therapies for pulmonary arterial hypertension fall into three groups: prostacyclin and its analogues, endothelin receptor antagonists and phosphodiesterase 5 inhibitors. Drug therapies used in pulmonary arterial hypertension may worsen gas exchange in patients with chronic parenchymal lung disease due to inhibition of pulmonary vasoconstriction. Vasodilators have not been shown to improve long-term outcomes and no trials of drug therapies for pulmonary arterial hypertension have shown these drugs to be effective.

Shirai M, Sada K, Ninomiya I: Effects of regional alveolar hypoxia and hypercapnia on small pulmonary vessels in cats. *J Appl Physiol* 1986;61:440-448.

Marshall C, Marshall BE: Influence of perfusate PO₂ on hypoxic pulmonary vasoconstriction in rats. *Circ Res* 1983;52:691-696.

Hultgren HN, Marticorena EA: High altitude pulmonary edema. Epidemiologic observations in Peru. *Chest* 1978;74:372-376.

Swenson ER, Maggiorini M, Mongovin S, Gibbs JSR, Greve I, Mairbäurl H, Bärtsch

P: Pathogenesis of high-altitude pulmonary edema: Inflammation is not an etiologic

factor. *JAMA* 2002;287:2228-2235

Sui GJ, Liu YH, Cheng XS, Anand IS, Harris E, Harris P, Heath D: Subacute infantile

mountain sickness. *J Pathol* 1988;155:161-170

Anand IS, Malhotra RM, Chandrashekar Y, Bali HK, Chauhan SS, Jindal SK,

Bhandari RK, Wahi PL: Adult subacute mountain sickness - A syndrome of

congestive heart failure in man at very high altitude. *Lancet* 1990;335:561-565.

. Galie N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, Beghetti M, Corris P, Gaine S, Gibbs JS, Gomez-Sanchez MA, Jondeau G, Klepetko W, Opitz C, Peacock A, Rubin L, Zellweger M, Simonneau G. Guidelines for the diagnosis and treatment of pulmonary hypertension: The Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). *Eur Heart J*. 2009;30:2493-2537.

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SA47

Oxygen stress and control of breathing during development

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The carotid body is critical in regulating ventilation in response to changes in oxygen tension, and it provides essential sensory input during early development to stabilize and maintain breathing throughout life. Exposure to chronic sustained or intermittent hypoxia or sustained hyperoxia during early postnatal development differentially modifies breathing responses to acute hypoxic challenges which may be persistent or reversible with maturation. Premature infants are particularly vulnerable to early environmental exposures that modify neuronal circuits that control ventilation; these circuits continue to develop during early postnatal development. Furthermore, premature infants have less antioxidant defenses to counterbalance free radical production that is often associated with oxygen stress. In my presentation, I will compare and contrast the physiological consequences of different paradigms of oxygen stress, the molecular and cellular effects that lead to changes in structure and function of the carotid body and central networks that control breathing during development. Understanding mechanisms that disrupt respiratory reflexes is critical to evaluation and treatment of premature infants who are at high risk for persistent apnea and bradycardic events, reduced arousal responses to an asphyxial event during sleep, and sudden death. All of these adverse physiological events occur because of perturbations of the respiratory network of which the exposure to the extremes of oxygen tension may be operative

Chavez-Valdez R, Mason A, Nunes AR, Northington FJ, Tankersley C, Ahlawat R, Johnson SM, Gauda EB. Effect of hyperoxic exposure during early development on neurotrophin expression in the carotid body and nucleus tractus solitarius. *J Appl Physiol*. 2012 Mar 15.

Prabhakar NR, Kumar GK, Nanduri J. Intermittent hypoxia augments acute hypoxic sensing via HIF-mediated ROS. *Respir Physiol Neurobiol*. 2010 Dec 31;174(3):230-4

Pawar A, Nanduri J, Yuan G, Khan SA, Wang N, Kumar GK, Prabhakar NR. Reactive oxygen species-dependent endothelin signaling is required for augmented hypoxic sensory response of the neonatal carotid body by intermittent hypoxia. *Am J Physiol Regul Integr Comp Physiol*. 2009 Mar;296(3):R735-42.

Bisgard GE, Olson EB Jr, Wang ZY, Bavis RW, Fuller DD, Mitchell GS. Adult carotid chemoafferent responses to hypoxia after 1, 2, and 4 wk of postnatal hyperoxia. *J Appl Physiol*. 2003 Sep;95(3):946-52.

Bavis RW. Developmental plasticity of the hypoxic ventilatory response after perinatal hyperoxia and hypoxia. *Respir Physiol Neurobiol*. 2005 Nov 15;149(1-3):287-99

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SA48

Hypoxia and beyond - NADPH oxidases in the pulmonary circulation

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Alveolar hypoxia has two effects in the pulmonary circulation – vasoconstriction and pulmonary vascular remodelling. Hypoxic pulmonary vasoconstriction (HPV) is essential for adaptation of blood perfusion to regional ventilation. Under conditions of regional alveolar hypoxia it reduces blood flow to hypoxic areas, thereby increasing the perfusion of better ventilated regions. Perturbations to HPV, which may occur in pneumonia, the adult respiratory distress syndrome, and liver failure, can result in severe arterial hypoxemia. Under conditions of generalized hypoxia, however, HPV increases pulmonary vascular resistance and thus causes acute pulmonary hypertension. If alveolar hypoxia becomes chronic, in addition to vasoconstriction a vascular remodelling process is induced which morphologically narrows the vascular lumen and fixes pulmonary hypertension. Moreover, effects of hypoxia can be relevant during ischemia and reperfusion. Despite intensive research, the underlying mechanisms of HPV, pulmonary vascular remodelling and ischemia-reperfusion have not been fully elucidated yet. On the level of the oxygen sensors reactive oxygen species (ROS) producing systems have been proposed to be the initial part of a reaction chain leading to these physiological and pathophysiological effects. However, with regard to ROS there is still discussion whether an increase or a decrease of ROS under hypoxia induces HPV and vascular remodelling. With regard to its sources mitochondria as well as NAD(P)H-oxidases have been suggested. Against this background we provided evidence that NADPH oxidases as well as mitochondria contribute to the regulation of HPV by an increase of ROS and that the pulmonary vascular remodelling may be dependent on the NADPH oxidase isoform NOX4. In contrast, the phagocytic NADPH oxidase isoform NOX2 in endothelial cells is a critical protein complex for the ischemia-reperfusion induced lung injury. Thus, mitochondria and NADPH oxidases maybe targeted to improve deteriorated gas exchange, treat hypoxia-induced pulmonary hypertension and prevent ischemia-reperfusion induced oedema during lung transplantation.

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Regulation of carotid body functions by ion channels and neurotransmitters

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The carotid bodies (CB) are paired secondary sensory receptors whose natural stimuli are decreases in arterial PO₂ and increases in arterial PCO₂ and [H⁺]. Chemoreceptor cells detect the stimuli and transduce them into specific patterns of release of neurotransmitters. Upon their release, neurotransmitters activate the sensory nerve endings of the carotid sinus nerve (CSN) to set a level of action potential frequency that conveys information on the nature, intensity and time course of the stimuli to brain stem nuclei. Brainstem respiratory and vasomotor control centres integrate CB-CSN incoming information to generate reflex ventilatory and cardiovascular responses aimed to maintain blood gases and pH the closest possible to normality. At the cellular level, probably the most relevant question physiologically deals with the molecular mechanisms operating in the detection-sensing of natural stimuli in chemoreceptor cells. Intimately related to the sensing of natural stimuli is the definition of the mechanisms which couple the sensing devices to the chemoreceptor cells effectors, including the exocytotic machinery responsible for the release of neurotransmitters, as well as their regulation by second messengers. Finally, it is also of prime interest the knowledge of the relative importance of each neurotransmitter in setting the CSN activity and its relation with the intensity of stimulation. We will present some recent information generated in our laboratory related to these fundamental aspects of CB cellular physiology. Animal protocols were approved by the University of Valladolid Institutional Committee for Animal Care and Use following international laws and policies (Guide for the Care and Use of Laboratory Animals, National Institutes of Health, 85–23, 1985). In every instance animals were anaesthetised by intraperitoneal injection of sodium pentobarbital, 40 mg/Kg for guinea pigs and 60 mg/Kg for rats. Since our initial description of the O₂-sensitive K⁺ currents in chemoreceptor cells, they have been considered key pieces in the O₂-sensing and transduction machinery in these cells. However, pharmacological data and particularly information gathered from animals genetically deficient in different K⁺ channels would appear to drive the attention to alternative sensor-effector mechanisms. In this front we have chosen guinea pigs, species which have naturally ablated their ventilatory response to hypoxia, and found that their chemoreceptor cells, which are responsive to depolarizing stimuli (by increasing their intracellular Ca²⁺ and increasing their release of neurotransmitters), do not respond to hypoxia, and more importantly, their voltage-dependent K⁺ currents lack O₂-sensitive components. Hydrogen sulfide (H₂S) has recently been recognized as a regulator of the CB hypoxic responses. We have observed that H₂S donors (NaHS and GYY4137) activate rat chemoreceptor cells increasing their intracellular Ca²⁺ and promoting their release of neurotransmitters. Hydroxycobalamin, which reacts with H₂S to form sulfocobalamin, abolishes

the effects of sulfide donors, does not affect the Ca^{2+} and release responses elicited by high external K^{+} , and inhibits by about 60% the response elicited by hypoxia. These findings locate H_2S actions in the transduction cascade upstream of depolarization. At the neurotransmission level, we have found that rat chemoreceptor cells release adenosine and ATP in a manner dependent on the intensity of hypoxic stimuli: adenosine is released preferentially in response to hypoxia of low and moderate intensity, while ATP release increases in direct proportion to the intensity of hypoxia. Consistent with the release pattern, selective antagonists of adenosine receptors are maximally effective to inhibit CSN discharges elicited by moderate hypoxia while ATP receptor antagonists inhibit by a greater percentage the CSN activity elicited by intense hypoxia.

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Carbon monoxide (CO) and Hydrogen sulfide (H_2S) in hypoxia-response coupling

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Carotid bodies are the sensory organs for detecting O_2 levels in arterial blood. Hypoxemia increases the sensory nerve activity of the carotid body leading to reflex stimulation of breathing and elevation of blood pressure. This presentation focuses on the roles of endogenous carbon monoxide (CO) and hydrogen sulfide (H_2S) in hypoxic sensing by the carotid body. In mammalian cells, CO is generated during oxidative cleavage of heme by heme oxygenases (HO) and molecular oxygen is essential for this reaction. Two forms of HO have been identified including an inducible HO1, which resembles stress-inducible protein HSP-32, and a constitutively expressed HO2. Glomus cells, the site of O_2 sensing in the carotid body, express HO2. Hypoxia decreases CO generation in a stimulus-dependent manner. Rats treated with HO-inhibitor and mice lacking HO2 (HO2 knockout mice) exhibit increased baseline carotid body activity and augmented sensory response to hypoxia, suggesting that endogenous CO is physiological inhibitor to the carotid body activity. Cystathionine β -synthase (CBS) and cystathionine- γ -lyase (CSE) are the major enzymes responsible for generation of endogenous H_2S . Hypoxia increases H_2S generation in the carotid body in a stimulus-dependent manner (Peng et al. 2010). Although glomus cells express both CBS and CSE, genetic deletion of CSE alone is sufficient to prevent hypoxia-evoked H_2S generation as well as hypoxic sensing by the carotid body (Peng et al. 2010). Exogenous application of H_2S donor stimulates the carotid body activity with a time course and magnitude similar to hypoxia. These observations suggest that endogenous H_2S is a physiological mediator of carotid body sensory response to hypoxia. $[\text{Ru}(\text{CO})_3\text{Cl}_2]_2$, a CO donor inhibited, whereas CrIIIMP, a HO inhibitor, stimulated H_2S generation in the carotid body. The enhanced H_2S generation by HO inhibitor was absent in CSE knockout mice suggesting that hypoxia-evoked H_2S generation in the carotid body requires the interaction of HO2 with CSE. Based on these studies it is proposed that hypoxic sensing by the carotid body requires protein-protein interactions between HO2 and CSE

working in concert as a "chemosome". Supported by NIH-HL-76537, HL-90554, and HL-86493.

Peng YJ, Nanduri J, Raghuraman G, Souvannakitti D, Gadalla MM, Kumar GK, Snyder SH, Prabhakar NR. H_2S mediates O_2 sensing in the carotid body. *Proc Natl Acad Sci U S A*. 107:10719-24, 2010.

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SA51

The Lkb1-AMPK signalling pathway is required for regulation of breathing by hypoxia and thereby energy supply to the whole body

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Our investigations continue to provide support for the proposal that, consequent to inhibition of mitochondrial oxidative phosphorylation, AMP-activated protein kinase (AMPK) mediates hypoxia-response coupling in all oxygen-sensing cells (Evans, 2006). Consistent with this view, we have demonstrated that physiological levels of hypoxia precipitate an increase in the ADP:ATP and AMP:ATP ratio in pulmonary arterial smooth muscle, and that this is accompanied by activation of AMPK and phosphorylation of acetyl CoA carboxylase; a well-established marker for AMPK action. Most significantly, we then showed that pharmacological activation of AMPK elicits constriction of pulmonary arteries, and in a manner that mimics precisely the mechanisms of that underpin hypoxic pulmonary vasoconstriction (Evans et al., 2005). These studies therefore suggested that activation of AMPK by hypoxia may aid ventilation-perfusion matching in the lung.

The proposal that AMPK may be of general importance to hypoxia-response coupling in all oxygen-sensing cells then gained further support from our studies on the carotid body. Consistent with the effects of hypoxia, intracellular dialysis from a patch pipette of an active (thiophosphorylated) recombinant AMPK heterotrimer ($\alpha 2\beta 2\gamma 1$) or application of the AMPK activators AICAR and A769662: (1) Inhibited BKCa currents and TASK potassium currents in rat carotid body type I cells (Wyatt et al., 2007; Ross et al., 2011); (2) Inhibited whole-cell currents carried by KCa1.1 (Wyatt et al., 2007; Ross et al., 2011) and TASK3, but not TASK1 channels expressed in HEK293 cells (unpublished); (3) Triggered carotid body activation (Evans et al., 2005; Wyatt et al., 2007). We therefore sought to determine whether or not Lkb1, the upstream kinase required for activation of the AMP-activated protein kinase by energetic stress, is necessary for oxygen-sensing by the carotid body. Conditional deletion of the Lkb1 gene in mouse type I cells abolished the increase in intracellular calcium by hypoxia. Lkb1 deletion also ablated the hypoxia-evoked increase in afferent discharge in the carotid sinus nerve, and prevented the increase in breathing frequency by hypoxia in these mice (unpublished). Thus, the Lkb1-AMPK signalling pathway mediates carotid body activation by, and the ventilatory response to, hypoxia. The Lkb1-AMPK signalling pathway is therefore required for hypoxia-response coupling, and serves to regulate oxygen and thereby energy supply at the whole body level.

Evans AM (2006). *J. Physiol.* **574**, 113-123.

Evans AM, Mustard KJ, Wyatt CN, Peers C, Dipp M, Kumar P, Kinnear NP, Hardie DG (2005). *J. Biol. Chem.* **280**, 41504-41511.

Wyatt CN, Mustard KJ, Pearson SA, Dallas ML, Atkinson L, Kumar P, Peers C, Hardie DG, Evans AM (2007). *J. Biol. Chem.* **282**, 8092-8098.

Ross FA, Rafferty JN, Dallas ML, Ogunbayo O, Ikematsu N, McClafferty H, Tian L, Widmer H, Rowe IC, Wyatt CN, Shipston MJ, Peers C, Hardie DG, Evans AM (2011). *J. Biol. Chem.* **286**, 11929-11936.

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SA52

Anabolic resistance to nutrients and exercise: insights from protein turnover studies

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Proteostasis describes the control of, and balance between tissues rates of protein synthesis and breakdown. The two main external factors governing skeletal muscle proteostasis are nutrition and loading patterns, as can be illustrated by the muscle catabolism that occurs with starvation and unloading (atrophy), or in contrast, the anabolic effects of loading (hypertrophy). The principal means by which nutrition and loading regulate proteostasis is through controlling both arms of protein turnover i.e. protein synthesis (PS) and protein breakdown (PB). For instance, in periods of inactivity and nutritional deficiency- such as when sleeping- there exists a disequilibrium (PB>PS) which is reversible only upon restoration of activity and nutritional supply in the waking hours (PS>PB). It follows that maintenance of this dynamic equilibrium (and thus muscle mass) requires 'regular' physical activity and nutrition. Of all nutrients, it is solely the essential amino acids (EAA) (1) which cause the large (~3-fold) albeit brief (~1.5 h saturable at ~10 g EAA (2, 3)) rise in MPS after feeding. The related increase in insulin orchestrates a more minor role, suppressing PB ~50% but having no functional role in EAA-induced increases in MPS (4). Loading of muscle (i.e. exercise) stimulates MPS to a similar amplitude to that of EAA supply, with the difference being that raised MPS persists for longer durations (4-24 h) depending upon the strength of the exercise stimulus and nutrient status (5). Intracellular signalling pathways governing increases in PS with nutrition and exercise are complex and in the latter case involve integration of extracellular cues (e.g. growth factors), ions (e.g. Ca²⁺), mechanotransduction and synergy with nutrient signalling. Nonetheless, to a large extent, nutrient and exercise-induced signalling converge on the mammalian-target of rapamycin (mTOR). It has been long known that ageing is associated with declines in muscle mass and function; termed sarcopenia and dynapenia, respectively. Age-related loss of muscle mass and strength are incipient processes leading to frailty, increased risk of metabolic disease, loss of mobility and increased mortality; often precipitated principally by falls (an estimated 25% to 39% of adults >65 y fall each year with a 20% <2 y mortality rate). However, until recently there had been no explanation available in terms of the underlying dysregulation of proteostasis underpinning sarcopenia since there exists no depressions in PS, or increases in PB, at least where comparisons have been between younger and older humans under postabsorptive, rested conditions (3). On this basis it is of great interest that the concept of "anabolic resistance" has been an emerging feature

of metabolic dysregulation in ageing muscles (3, 6-8). In a nutshell, anabolic resistance manifests as an acute blunting in PS and PB responses to the anabolic cues of physical activity and nutrition. This points toward an age-related nutrient and exercise resistance in protein metabolism (e.g. analogous to insulin resistance in glucose metabolism in type II Diabetes) and likely represents a key facet in the failure of ageing muscle to sustain a dynamic equilibrium in proteostasis such that over-time, sarcopenia develops. Consolidating the role of anabolic resistance as a causative and pervasive feature of muscle atrophy are reports of: (i) age-related blunting of hypertrophy after exercise-training (9), (ii) pre-clinical models reporting its existence and identifying an underlying mechanism as inflammation on the premise that cyclooxygenase inhibitors ameliorate sarcopenia commensurate with the reversal of anabolic resistance (10), and (iii) its extension beyond ageing into other conditions of muscle atrophy (e.g. immobilisation, type II Diabetes, Cancer cachexia, chronic heart failure (11-13). Nevertheless, work is needed to define the mechanistic basis of anabolic resistance in humans, the contribution of physical inactivity, and to find effective interventions with which to "overcome" it.

P. J. Atherton, K. Smith, T. Etheridge, D. Rankin, M. J. Rennie, *Amino Acids* 38, 1533 (2010)

P. J. Atherton et al., *Am. J. Clin. Nutr.* (2010)

D. Cuthbertson et al., *FASEB J.* 19, 422 (2005)

P. L. Greenhaff et al., *Am. J. Physiol Endocrinol. Metab* 295, E595 (2008)

N. A. Burd et al., *J. Nutr.* 141, 568 (2011)

C. S. Fry et al., *Skelet. Muscle* 1, 11 (2011)

E. A. Wilkes et al., *Am. J. Clin. Nutr.* 90, 1343 (2009).

Reference 8 V. Kumar et al., *J. Physiol* 587, 211 (2009).

Reference 9 C. A. Greig et al., *Exp. Gerontol.* 46, 884 (2011).

Reference 10 I. Rieu et al., *J. Physiol* 587, 5483 (2009).

Reference 11 M. J. Toth, M. M. LeWinter, P. A. Ades, D. E. Matthews, *Clin. Sci. (Lond)* 119, 467 (2010).

Reference 12 N. E. Deutz et al., *Clin. Nutr.* (2011).

Reference 13 E. I. Glover et al., *J. Physiol* 586, 6049 (2008).

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SA53

Mechanisms of ageing related loss of skeletal muscle: Potential role of mitochondria/redox signalling

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Chronic loss of skeletal muscle mass and function is a major contributor to frailty and weakness in the elderly. The major cause of age-related loss of muscle mass is a decrease in the number of skeletal muscle fibres associated with atrophy and weakness of the remaining fibres. The fundamental causes of loss of tissue function with increasing age are the subject of considerable research activity, but remain unclear. A large number of studies have reported a dysregulation of reactive oxygen species (ROS) homeostasis during ageing that may potentially lead to increased oxidative damage to tissues and/or to defective redox signalling. Skeletal muscle tissue from aged organisms contains increased amounts of oxidative damage, but whether this is the cause of age-related deficits in muscle

or a consequence of ageing has been the subject of controversy. Studies from our group have examined the changes in ROS generation that occur with ageing in man and experimental models and have also utilised a transgenic approach to modify ROS generation in model organisms. Data from these experiments support the hypothesis that aberrant ROS regulation plays a role in the deficits in skeletal muscle and motor neurons that occur during ageing, but also indicate this does not simply occur through increased oxidative damage to tissues. A number of studies have focussed on the role played by mitochondria in aberrant generation of ROS during ageing. There is little evidence that ageing is associated with gross changes in mitochondrial function in skeletal muscle, but most studies have reported increased generation of ROS by mitochondria isolated from muscle tissue of old animals and man although interpretation of such data is complicated by the potential presence of a small number of denervated fibres in biopsy samples since these fibres may have gross elevations in mitochondrial ROS generation.

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SA54

Satellite cells and regenerative capacity of older muscle

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Muscle satellite cells are responsible for the regenerative events in response to daily activity-related injuries or disease-induced muscle fibre insults. Because of their central role in the regenerative capacity of skeletal muscle, it is currently hypothesized that satellite cells might be involved in the age-related loss of muscle mass. Evidence supporting the role of intrinsic and extrinsic factors in the defective satellite cell function in aged skeletal muscle has been proposed. Arguments against this hypothesis have also been suggested. It is important to delineate the exact role played by satellite cells during aging in order to deliver adequate pharmacological or non-pharmacological treatment. Currently, there are important critical issues such as the species studied and the characterisation of the population included that need to be carefully considered in order to reach reliable conclusions and the question of whether the proliferation and differentiation capacity of human satellite cells is important in the dynamic regulation of myofibre size deserves further attention.

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SA55

Inflammation in sarcopenia: does it imply therapeutic potential?

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Skeletal muscle and the immune system are intimately related: this close connection facilitates muscle's ability to respond to environmental stresses rapidly. Immune cells and inflammatory cytokines are important in the promotion of muscle protein turnover, both in response to direct muscle injury and to enable amino acid delivery to other tissues. In aging, there is evidence of increased inflammatory signaling systemically, and of disordered cytokine responses to injury. In chronic inflammatory diseases and animal models of muscle wasting, however, cytokine blockade has not consistently reversed muscle wasting. Recent evidence suggests that key skeletal muscle atrophy and differentiation pathways are downstream of the NFkB/IL-1/TNF inflammatory cascade, and act via the activin receptor to inhibit myotube differentiation and the regenerative response to injury. Taken together, these data suggest that direct cytokine inhibition may not be sufficient to repair muscle atrophy or sarcopenia, and that additional anti-catabolic or anabolic treatments will be needed.

Trendelenburg et al. *Skeletal Muscle* 2012, 2:3 (<http://www.skeletal-musclejournal.com/content/2/1/3>)

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SA56

Sexual dimorphism and maintenance of muscle mass in old age

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It is well known that there is sexual dimorphism with regards to body composition. Healthy adult women have less lean body and muscle mass and more fat than men and the age-associated decrease in muscle mass is slower in women than in men. Several studies, however, indicate that there is no difference in the basal rate of muscle protein synthesis or muscle protein breakdown or the anabolic responses to nutritional stimuli and resistance exercise in young and middle-aged adult men and women. Nevertheless, recent evidence suggests that aging affects muscle protein turnover differently in men and women. The basal rate of muscle protein synthesis is greater in old women than in old men; in addition, both old women and old men are resistant to the anabolic effects of exercise and nutrition and old women appear to be more resistant than old men. This suggests that differences in muscle protein turnover between men and women might be most apparent when muscle mass is changing (i.e., during aging vs. young and middle-adulthood when muscle mass is steady). Men and women

therefore need to be considered separately when evaluating muscle protein synthesis rates in older adults.

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SA57

Efficacy of interventions for maintenance of muscle mass in old people

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Ageing is associated with a loss of muscle mass (muscle atrophy), and a decline in strength measured at the level of the whole joint system (joint torque). In relative terms, the decline in strength is much greater than the loss of muscle mass, as other factors such as muscle architecture, the level of neural activation and antagonist muscle coactivation all play a role in contributing to this strength decline with ageing. Declines in strength and muscle mass with ageing have important consequences for daily functional tasks, which can impinge upon independent living and quality of life. The ageing-related decline in muscle mass and strength can be at least partially mitigated by interventions that increase the level of loading on skeletal muscle above that to which it is habitually accustomed. Typically these interventions have used resistance training, which involves lifting and lowering known loads (concentric and eccentric muscle contractions, respectively) in a single plane to load specific major muscle groups. The extensor muscle groups at the knee and ankle appear to be most severely affected by ageing-related declines in strength and size and therefore, have typically been the focus of resistance training programmes for older adults. Most resistance training programmes utilise relatively high loads, based upon the premise of activating as high a proportion of the muscle's motor unit pool as possible. Typically training loads are prescribed as a percentage of a 'repetition maximum', defined as the maximum load that can be lifted and lowered under control and repeated a given number of times. Typically training loads are recommended between 60 and 100% of the one-repetition maximum, however, effective muscular adaptations have also been seen in older adults with slightly lower training loads (e.g., Vincent et al., 2002). Increases in strength (measured as isometric torque) following resistance training programmes in older people can vary considerably depending upon a number of factors, but for the knee extensors for example, have ranged between 7 and 57% (Tracy et al., 1999; Hakkinen). Concomitant increases in muscle size in response to resistance training have ranged between 2 and 12% (Tracy et al., 1999; Roth et al., 2001). The large variation in the reported muscle size and strength adaptations to training interventions is due to many factors. For example, aspects such as the resistance training load, frequency and total duration of training all vary between studies. An important point in relation to the degree of strength adaptation reported between studies is the mode of testing. 'True' strength gains will be overestimated if expressed as the repetition maximum on the training device, due to learning and coordination effects. A more objective measure of training-induced strength gains can be gained by measuring isometric torque. The plasticity of the muscular system in older adults is highlighted by the fact that strength gains in older adults can be comparable to those reported in younger adults following similar resistance training durations

(Reeves et al., 2006). Muscle mass is typically assessed by measuring a muscle's cross-sectional area or total volume. Although these measures are a useful indication of muscle size and any changes with training interventions, they fail to take into account information on the internal muscle architecture and any subsequent modifications with training. Architectural parameters within a muscle include the length of muscle fascicles and the angle of fascicular insertion into the tendon aponeurosis, known as the pennation angle. These parameters affect factors such as the transmission of force through to the tendon and both fascicle lengths and pennation angles have been modified positively after resistance training interventions in older adults (e.g., Reeves et al., 2004). Recently, we have shown how eccentric-only training can cause specific adaptations in muscle architecture, different to those changes seen with 'conventional' resistance training in older adults (Reeves et al., 2009). This type of training (eccentric-only) also enabled older adults to train using much higher loads, but with far lower ratings of perceived exertion, which may be an important factor for adherence to training programmes. In summary, resistance training interventions have been shown to be effective for increasing muscle size and strength in older adults, which can at least partially reverse some of the detrimental consequences on skeletal muscle observed with ageing.

Hakkinen K et al. (1998). *J Appl Physiol* 84, 1341–1349.

Reeves ND et al., (2009). *Exp Physiol* 94, 825–833.

Reeves ND et al., (2004). *Exp Physiol* 89, 675–689.

Reeves ND et al., (2006). *Exp Physiol* 91, 483–498.

Roth SM et al. (2001). *J Am Geriatr Soc* 49, 1428–1433.

Tracy BL et al. (1999). *J Appl Physiol* 86, 195–201.

Vincent KR et al. (2002). *J Am Geriatr Soc* 50, 1100–1107.

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SA58

Signaling networks between endothelial and vascular smooth muscle cells: role of reactive oxygen species

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Interactions between endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) are fundamental in maintaining vascular integrity, structure and function. ECs and VSMCs act as a coupled system for transmission of signals from the endothelium to the underlying vascular media and vice versa. Such interactions are fundamental in the regulation of vascular integrity, structure and function. Communications between ECs and VSMCs occur through synthesis and release of mediators or through direct cell-to-cell contact. ECs regulate vascular tone through vasorelaxing molecules such as nitric oxide (NO), hydroxyeicosatetraenoic acid (HETE), and prostacyclin (PGI) and vasoconstricting molecules such as endothelin-1 and thromboxane. Recent studies demonstrate that ECs regulate VSMC proliferation, migration, differentiation, and gene expression through reactive oxygen species (ROS). All vascular cell types, including ECs, VSMCs, and adventitial fibroblasts produce superoxide hydrogen peroxide (H_2O_2), and NO, primarily through NADPH oxidases (Nox2,4,5). Physiologically ROS are produced in a controlled manner at low concentrations and function as inter- and intracellular signaling mole-

cules. In pathological conditions increased activity/expression of ROS generating enzymes or decreased defenses by antioxidants in the vasculature result in increased bioavailability of ROS, leading to oxidative stress and vascular damage. In basal conditions ECs protect VSMCs from oxidative injury, in part through upregulation of VSMC antioxidant systems, whereas in pro-inflammatory conditions this effect is blunted, resulting in oxidative stress and vascular injury. VSMC-derived superoxide has been implicated in the quenching of NO in ECs to form injurious ONOO⁻, which further promotes vascular inflammation and injury. Emerging evidence indicates that cross talk between oxidant systems occurs at multiple levels within the vessel wall. Not only can ROS produced within ECs influence ROS formation in VSMCs, and vice versa, but ROS produced by one cell type can influence signaling in adjacent cells. This presentation focuses on the role of ROS in the cross-talk between ECs and SMCs and the molecular processes that modulate vascular function and structure.

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SA59

Molecular regulation of vascular smooth muscle cell calcification

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Vascular calcification is a ubiquitous feature in the ageing population and is associated with increased mortality, morbidity and surgical intervention. It occurs at two anatomical sites in the vessel wall; the intima in association with atherosclerosis while medial calcification is prevalent in diabetes and renal failure. These calcifications were once considered to be due to passive degenerative processes however, accumulating evidence suggests that calcification is driven by cell-mediated processes similar to bone formation. Vascular smooth muscle cells (VSMCs) uniquely orchestrate the calcification process. Unlike other contractile muscle cell types VSMCs are not terminally differentiated. In response to injury they can undergo phenotypic modulation to become proliferative and migratory and deposit a modified extracellular matrix (ECM); with this phenotypic transition essential to repair the vessel wall and stabilize atherosclerotic plaques. The factors that regulate VSMC phenotypic transition are still poorly understood however it is clear that prolonged exposure to stress and ageing perturb these repair processes. The ultimate outcome of this failed repair is calcification and a number of VSMC-mediated processes are required for calcification to occur. An immediate and consistent feature of the VSMC phenotypic transition is the induction of osteo/chondrocytic differentiation. This phenotype is characterized by upregulation of a number of mineralization-regulating proteins and ECM components normally expressed in bone. The most important of these is the master bone regulatory transcription factor (TF) Runx2 which activates its putative targets alkaline phosphatase (ALP), bone sialoprotein (BSP) and osteocalcin (OCN). The role of these osteo/chondrocytic proteins in VSMC function is not clear but their expression in vivo co-localizes with sites of calcification. Other key events in the induction of calcification are loss of expression/function of local (Matrix Gla protein (MGP)) and circulating (fetuin-A) inhibitors of calcification. In addition, apoptosis as well as membrane bound vesicles released from phenotypically modified VSMCs have an essential role in promot-

ing calcification by forming the first nidus for mineralization. Taken together, our studies suggest that vascular calcification is a clinically quantifiable 'read-out' of vascular damage, induced by a multitude of factors including mineral overload and oxidative, mechanical or genotoxic stress. Studies focussing on the specific factors that induce a 'calcific milieu' in patients as well as factors that may be targeted for intervention such as (1) increasing inhibitor function and (2) blocking maladaptive signalling that promotes osteogenic differentiation, are now key to ameliorating these disease processes.

British Heart Foundation

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SA60

Considering obesity as a chronic brain disease

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It is an inescapable fact that the underlying cause of obesity is a result of consuming more energy than you burn. The question that is more complex to answer is why some people eat more than others. Over the past 15 years, insights from human and mouse genetics have illuminated multiple pathways within the hypothalamus, brainstem and higher brain regions that play a key role in the control of food intake. We now know for example, that the brain leptin-melanocortin signalling pathway is central to the control of mammalian food intake. Intriguingly, it is becoming clear that in addition to engaging classical "neuropeptide/receptor" systems within the brain, leptin also rapidly modifies synaptic connections between neurons. There is also evidence for neurotrophins, which are critical in the development and maintenance of neuronal connections, playing a role in the control of energy homeostasis. However, although monogenic alterations in these pathways result in extreme Mendelian obesity, these remain incredibly rare. The major burden of disease is carried by those of us with "common obesity," which to date has resisted yielding meaningful biological insights. Progress however, has been made with genomewide association studies. For example, sequence variants in the first intron of FTO (Fat mass and Obesity related) are strongly associated with human obesity and carriers of the risk alleles show evidence for increased appetite and food intake. Although global FTO null mice display decreased fat and lean body mass, increased metabolic rate and food intake, this is seen against a complex phenotype of postnatal growth retardation and mortality. In contrast, when we modulated FTO levels discretely in the hypothalamic arcuate nuclei of adult animals, we were able to influence food intake, suggesting tissue specific functions for FTO. FTO's physiological role and how it influences bodyweight is yet to be determined. Using a variety of in vivo, in vitro and biochemical methods, we are currently characterising the molecular mechanism by which FTO controls of energy balance. Further GWAS have now revealed more than 30 different candidate genes, most of which are highly expressed or known to act in the CNS, emphasizing, as in rare monogenic forms of obesity, the role of the brain in predisposition to obesity.

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Potential mechanisms that link diabetes with obesity

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Obesity and diabetes are rapidly developing into epidemics in both the developed and developing countries and are major health problems that place a huge economic burden on society. The parallel increases in prevalence of these pathophysiological conditions suggest there are potential mechanisms that link the pathophysiology of obesity with diabetes. Obesity is characterized by an excess adipose tissue mass and is usually associated with elevated levels of circulating leptin - an adipose-derived hormone - that fails to suppress appetite due to leptin resistance. It is also known that obese individuals and rodent models are only insulin resistant in their early stages and do not develop diabetes because the pancreatic beta cells are capable of compensating for the insulin resistance by increasing their mass and appropriately increasing insulin secretion. A failure of the pancreatic beta cells to compensate leads to the development of overt diabetes. While the precise mechanisms that trigger the failure of the pancreatic beta cells in the obese state are not fully understood, it has been suggested to be linked to the effects of adipokines secreted by the adipocyte. Several adipokines are secreted by adipocytes including leptin, adiponectin, free fatty acids, interleukin-6, tumor necrosis factor- α , C-reactive protein and potentially others. Of relevance to this presentation are the effects of leptin on the endocrine pancreas. Indeed, in addition to its effects on the hypothalamus numerous studies indicate that leptin acts on multiple peripheral tissues including the endocrine pancreas. Exogenous leptin is able to modulate insulin secretion *ex vivo* in isolated islets from rodents and humans. Mice with a knockout of the leptin receptor in the pancreas manifest alterations in both beta cell mass and glucose-stimulated insulin secretion. Other studies suggest that lack of leptin signaling in the pancreas enhances the ability of the incretin hormone and sulphonylureas to promote insulin secretion. Intriguingly, the effects of leptin on insulin secretion are altered *in vivo* when mice are fed a high fat diet. Similarly, leptin effects on beta cells are altered when islets are chronically treated with free fatty acids. These studies suggest cross talk in the molecular pathways that link the actions of leptin with those of glucose, GLP-1, free fatty acids and sulphonylureas in the overall regulation of islet cell function and proliferation. These data will be reviewed in the presentation. There will be a discussion regarding the role of leptin in modulating islet biology independent of its effects on the hypothalamus with consequences for the development of diabetes in the obese state.

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SA62

RFamides and metabolic regulation

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Treatment options for obesity and type 2 diabetes include efforts to avoid the original weight gain: reducing energy input either by life-style changes or pharmacological manipulation. However, success is proving difficult, and it is therefore important to provide a better understanding of how other aspects of energy regulation might prevent the development of metabolic disease. We and others working on satiety signalling have gone some way to understanding brain communication with the gut to control meal size. However, it is equally important to regulate other aspects of energy homeostasis through central regulation of other peripheral organs. For example, clinical interest has highlighted the importance of post-prandial regulation of hepatic gluconeogenesis to avoid development of diabetes, and the potential for brown adipose-dependent thermogenesis in adult humans to avoid obesity. We will discuss animal models we are using to elucidate the role of RFamide peptides in satiety, thermogenesis and glucose tolerance.

The RFamides are so called because they all have common C-terminal arginine and phenylalanine residues. Through evolution, from nematodes and gastropods to birds and mammals, the RFamides have had a role in feeding behaviour. In rodents, brainstem neurones containing the RFamide, prolactin-releasing peptide (PrRP), mediate the effects of the gut-brain-gut satiety signal, cholecystokinin. Thus, PrRP can mimic satiation and the reduction in gastric emptying, whilst the actions of cholecystokinin are absent in mice lacking either PrRP or its receptor (GPR10). More recently, cholecystokinin has been shown to help protect against post-prandial hyperglycaemia by activating a gut-brain-liver reflex which reduces hepatic glucose production. Although GPR10 knock-out mice have normal fasting glucose levels, they demonstrate a large excursion in blood glucose following oral gavage (2 g glucose/kg body weight; glucometer measurements from tail vein blood samples). Conversely, pre-administration of PrRP (4 nmol; *icv*) into normal mice reduces the glucose excursion during a similar oral glucose tolerance test. We have demonstrated also that PrRP neurones in the hypothalamus, are responsible for brown adipose tissue-mediated, non-shivering thermogenesis in response to either leptin (6 mg/kg; *ip*) or an acute cold stimulus (4°C for 2 hours).

The GPR10 knock-out mouse is obese. Agonistic targeting of the "catabolic peptide" PrRP and its receptor may provide a route to controlling different aspects of metabolic dysfunction. By contrast, another RFamide, QRFP, is anabolic and has many opposing effects to PrRP. Therefore, it may be open to antagonistic targeting. For example, QRFP (2 nmol; *icv*) increases food intake, reduces glucose tolerance and increases lipogenesis. Perhaps unexpectedly, the peptide does not have an obvious direct effect on energy expenditure. The role of QRFP on feeding appears to be a central action, while its other effects may be mediated through peripheral tissues.

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Bariatric surgery and appetite

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A good model to investigate appetite reduction in humans and rodents with associated major weight loss is bariatric surgery. Gastric bypass, but not gastric banding caused increased post-prandial PYY and GLP-1 favouring enhanced satiety. An early and exaggerated insulin response mediates improved glycaemic control. The rodent model of bypass showed elevated PYY, GLP-1 and gut hypertrophy compared with sham-operated rats. Moreover, exogenous PYY reduced food intake while blockade of endogenous PYY increased food intake. A prospective follow-up human study of gastric bypass showed progressively increasing PYY, enteroglucagon, and GLP-1 responses associated with enhanced satiety. Blocking these responses in animal and human models leads to increased food intake. Thus, following gastric bypass, a pleiotrophic endocrine response may contribute to improved glycaemic control, appetite reduction, and long-term lowering of body weight. Energy expenditure in gastric bypass rats is also increased after gastric bypass surgery with major contributions coming from enhanced basal metabolic rate and diet induced thermogenesis, while no changes were noted in physical activity, body temperature or activity of brown adipose tissue. The sustained nature of weight loss and reduced appetite may be explained by gut adaptation and chronic hormone elevation.

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SA64

Ghrelin and food reward

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Accumulating evidence suggests that ghrelin's physiological role extends beyond appetite and energy balance to include reward-seeking behavior, involving a direct action at the level of the midbrain dopamine system. Indeed, central ghrelin signaling is important for animals to receive reward from addictive drugs including alcohol (1). Recently, we explored the role of the central ghrelin signaling system in food preference, food reward and food motivation. Direct injection of ghrelin into the brain ventricles or the VTA increased the intake of palatable/rewarding food. Preference for palatable food was suppressed by 7 days peripheral treatment with a GHS-R1A antagonist. The antagonist also suppressed the ability of rewarding food to condition a place preference (2). Finally, we explored motivated behavior for a food reward in an operant conditioning model (ie lever pressing for food in a progressive ratio schedule). Ghrelin increased motivated behavior for a sucrose reward when injected peripherally, icv (3) or intra-VTA but not intra-NAcc (4). By contrast, ghrelin administration to both the VTA and NAcc increased the free feeding of chow. In a state of overnight food restriction, where endogenous levels of ghrelin are increased, GHS-R1A blockade in the VTA was sufficient

to decrease the motivation to work for a sugar reward. Blockade of GHS-R1A in VTA or NAcc was not sufficient, however, to reduce fasting-induced chow hyperphagia (4). Thus, the VTA but not the NAcc appears to be a direct target site for ghrelin's effects on food motivation. In conclusion, the central ghrelin signaling system is important for food preference, food reward and food-motivated behavior.

Jerlhag E et al (2009). PNAS 106, 11318-11323.

Egicioglu E. et al. (2010) Addict Biol 15, 304-311.

Skibicka KP et al (2012) Addict Biol 17, 95-107.

Skibicka KP et al (2011) Neuroscience 180, 129-137.

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SA65

The use of gut hormones as anti-obesity drugs

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Obesity is a major worldwide health problem. Current anti-obesity drugs are ineffective. Bariatric surgery is highly effective, but impractical to apply to the growing numbers of obese patients. These limited treatment options have fuelled tremendous interest in novel anti-obesity agents, but their development has been impaired by efficacy and safety issues.

This talk will review the current stage of anti-obesity drugs in development, focusing on gut hormone-based therapies.

The gastrointestinal tract is the body's largest endocrine organ and releases a number of peptide hormones that can reduce appetite, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), oxyntomodulin and peptide YY (PYY). These hormones are sensitive to gut nutrient content and are released following a meal. They act on the appetite centres of the brain in the hypothalamus and brain stem to regulate food intake. Coordinated changes in gut hormone release are thought to modulate feelings of satiation and satiety.

Anorectic gut hormones offer a potentially safe and viable option for the treatment of obesity. The potential utility of gut hormones has been dramatically improved by the development of gut hormone analogues with improved pharmacokinetics and efficacy. Additionally, specific combinations of gut hormones have been demonstrated to have additive anorectic effects. However, commandeering such peptidergic systems still poses a number of problems which need addressing. Recent research investigating the mechanisms by which the gut senses nutrients to stimulate gut hormone release may also reveal new targets for functional food ingredients to promote satiation and satiety.

Gut hormones or the systems stimulating gut hormone release may prove effective targets for obesity therapies. Further work is required to elucidate the physiological mechanisms underpinning the effects of these hormones on appetite in order to optimise the design of such therapeutic agents.

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SA66

The molecular basis of aldosterone regulation of sodium transport in the collecting duct

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Aldosterone effects on Na⁺ transport in the kidney tubules require new gene transcription, none-the-less, they occur fairly rapidly, with increased Na⁺ current detectable in < 30 min. SGK1 is one of the most rapidly responding aldosterone-regulated genes, with changes in mRNA level within 10 min, and changes in protein within 20 min. Furthermore, SGK1 is under dual regulation: its protein levels are controlled through aldosterone-dependent changes in transcription, and its activity is controlled by rapid kinase cascades. Recently, significant progress has been made in elucidating these kinase cascades, which alter SGK1 activity in seconds-to-minutes. Notably, SGK1 has two phosphorylation sites—termed the “activation loop” and “hydrophobic motif”, which undergo regulated phosphorylation by PI3-kinase and mTOR, respectively. Our recent work indicates that mTOR-mediated phosphorylation is essential for specific activation of SGK1 under conditions in which other similar kinases are not activated. mTOR is found in two major complexes, mTORC1 and mTORC2. SGK1 is recruited specifically to mTORC2 by an mTORC2 component, mSin1. This interaction is necessary for SGK1 activation, but not activation of other kinases that are activated by mTOR; notably Akt, which is also phosphorylated by mTORC2 does not interact with mSin1. This regulatory cascade is essential for activation of the epithelial sodium channel (ENaC). Thus, SGK1 serves as a key integrator of multiple signals, which feed into aldosterone-dependent regulation of sodium transport in the kidney tubules.

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SA67

Rapid aldosterone actions on epithelial sodium channel (ENaC) trafficking and cell proliferation via protein kinase D

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Aldosterone induces rapid signalling events independent of de novo protein synthesis and these effects play a crucial role in the fine-tuning of latent aldosterone-induced genomic responses. Aldosterone (10nM) rapidly activates Protein kinase D1 (PKD1) within 2 minutes, in M1 cortical collecting duct cells (M1-CCD) - this activation occurs via the mineralocorticoid

receptor, transactivation of EGFR and the activation of protein kinase C δ and ϵ (1, 2). PKD1 is a serine/threonine kinase with multiple functions including the regulation of vesicle fission from the trans golgi network (TGN), and thereby the control of the rate of protein trafficking to the cell surface. Aldosterone controls sodium homeostasis by regulating the epithelial sodium channel (ENaC) on multiple levels. In M1 cells, PKD1 is essential for the efficient aldosterone-mediated apical trafficking of heterologously expressed ENaC-eCFP subunits on a short time-scale (2min) (1) as well as endogenously expressed ENaC after 24h 10nM aldosterone (3).

Aldosterone promoted the translocation of PKD1 from cytosol to the TGN in M1 cells after 30 min, as shown by co-localization with a specific marker of the TGN (TGN38), visualized by immunocytochemistry and laser scanning confocal microscopy. Phosphatidylinositol-4-kinase III β (PI4KIII β) is a substrate of PKD1 and is localized specifically to the TGN in M1 cells. Aldosterone induced a significant increase in the interaction between PKD1 and PI4KIII β as shown by co-immunoprecipitation and Western blotting (n = 6, p < 0.01, Student's t-test). It was shown previously that PKD1 phosphorylates PI4KIII β to promote the fission of vesicles from the TGN. Using a vesicular stomatitis virus glycoprotein (VSVG) protein transport assay as a measure of the rate of vesicle fission, we did not observe a significant increase in the percentage of cells showing VSVG-GFP localization at the plasma membrane, when pre-treated with 10nM aldosterone for 10min (45.12 $\pm$ 5.95), 30min (49.00 $\pm$ 5.48) or 60min (66.57 $\pm$ 2.40) as compared to vehicle-treated controls 10min (33.97 $\pm$ 7.91), 30min (41.99 $\pm$ 6.52), 60min (52.72 $\pm$ 9.97), values shown as mean $\pm$ SEM. These results indicate that the regulation of ENaC trafficking via the rapid activation of PKD1 is not a consequence of a general upregulation in the rate of TGN vesicle fission, but rather due to a specific regulation of the trafficking of ENaC-containing vesicles, via rapid PKD1-PI4KIII β signalling.

Aside from the well-known effects of aldosterone on the regulation of sodium and water homeostasis, aldosterone can also produce deleterious structural changes in tissues by inducing hypertrophy and the dysregulation of proliferation and apoptosis, leading to fibrosis and tissue remodelling (4). In M1-CCD cells, 10nM aldosterone induced an increase in proliferation after 48h, an effect that was PKD1-, PKC δ - and ERK1/2-dependent (5). Overall, rapid aldosterone-induced PKD1 signalling events underlie diverse physiological responses; the specificity of signalling being defined by the subcellular localization of PKD1, its proximity to various substrates and as yet unknown scaffolding proteins, as well as the specific kinetics of activation of the kinase.

McEneaney V, Harvey BJ, Thomas W. Aldosterone regulates rapid trafficking of epithelial sodium channel subunits in renal cortical collecting duct cells via protein kinase D activation. *Mol Endocrinol*. 2008 Apr;22(4):881-92.

McEneaney V, Harvey BJ, Thomas W. Aldosterone rapidly activates protein kinase D via a mineralocorticoid receptor/EGFR trans-activation pathway in the M1 kidney CCD cell line. *J Steroid Biochem Mol Biol*. 2007 Nov-Dec;107(3-5):180-90.

McEneaney V, Dooley R, Yusef YR, Keating N, Quinn U, Harvey BJ, et al. Protein kinase D1 modulates aldosterone-induced ENaC activity in a renal cortical collecting duct cell line. *Mol Cell Endocrinol*. 2010 Aug 30;325(1-2):8-17.

Dooley R, Harvey BJ, Thomas W. The regulation of cell growth and survival by aldosterone. *Front Biosci*. 2011;16:440-57.

McEneaney V, Dooley R, Harvey BJ, Thomas W. Protein kinase D stabilizes aldosterone-induced ERK1/2 MAP kinase activation in M1 renal cortical collecting duct cells to promote cell proliferation. *J Steroid Biochem Mol Biol*. 2010 Jan;118(1-2):18-28.

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SA68

Interaction between mineralocorticoid receptor and epidermal growth factor receptor signaling

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The mineralocorticoid receptor (MR) belongs to the steroid receptor superfamily and acts as a ligand-dependent transcription factor with aldosterone as endogenous ligand. Physiologically, aldosterone and MR are involved in salt and water homeostasis and thereby long-term blood pressure regulation. However, activated MR can also induce pathophysiological effects in the renocardiovascular system leading to inflammation, endothelial dysfunction and remodeling processes. In clinical trials, MR inhibitors significantly improve mortality and morbidity in patients with heart failure or after myocardial infarction. The underlying mechanism for these pathophysiological MR effects is not clear, especially since the MR shares a common hormone-response-element with its closest relative, the glucocorticoid receptor (GR) which elicits different effects and shows anti-inflammatory properties.

One possible mechanistical explanation for pathophysiological MR effects is a cross-talk between MR and other signaling pathways. One promising interaction candidate with potential to mediate pathophysiological MR effects is the epidermal growth factor receptor (EGFR). The EGFR is a membrane receptor with tyrosine kinase activity that leads to proliferation, vasoconstriction and migration of cells. Additionally, many vasoactive substances like angiotensin II, endothelin-1 and phenylephrine also induce vascular remodeling and mediate at least part of their pathophysiological effects by transactivating the EGFR. We could show that a cross-talk between MR and EGFR signaling takes place both on the functional and transcriptional level. On the one hand, MR can enhance EGFR signaling by transactivating the EGFR, which then leads to enhanced phosphorylation of ERK. A key player involved in this interaction is the kinase c-src. Overall consequence of this cross-talk is facilitation of nuclear shuttling of MR and thereby induction of classical genomic MR signaling and modification of extracellular matrix composition. On the other hand, activated MR can induce EGFR expression by enhancing EGFR promoter activity. Characterization of the interaction partners identified a 65 bp mineralocorticoid response element (MRE) on the EGFR promoter. Furthermore, SP1 was discovered as a cofactor binding to MRE and necessary for binding of MR to EGFR promoter. MR mutants lacking the N-terminal A/B domain were not able to induce promoter activity, suggesting that the N-terminus of the MR interacts with SP1 at the mineralocorticoid response element to induce EGFR expression. Importantly, no change in EGFR promoter activity was detectable with activated GR, thus suggesting an MR specific effect. Overall, activated MR is able to transactivate and induce the expression of EGFR, which provides possible mechanisms underlying MR-specific pathophysiological effects in the renocardiovascular system.

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SA69

The regulation of microRNAs by aldosterone and their impact on ENaC

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microRNAs (miRs) are short (~22 nucleotide) lengths of non-coding RNA that have been implicated in the regulation of post-transcriptional gene expression. Mature miRs incorporate into an inhibitory complex, and bind to recognition sequences on the 3'-untranslated region (UTR) of target mRNA, to prevent protein translation or degrade target mRNA. An upregulation of miR expression is therefore associated with a decrease in target protein levels. The role of miRs in the physiological regulation of sodium transport, and the potential for aldosterone to regulate miR expression has yet to be fully appreciated. There are no reports of miR regulation of the epithelial sodium channel (ENaC).

To determine the impact of aldosterone on miR expression in principal cells of the cortical collecting duct (CCD), a microarray analysis was carried out on small RNAs collected from mouse CCD cells treated with 50nM aldosterone for 24 hours. In comparison to non-aldosterone treated controls, the expression levels of 21 miRs were significantly altered (up and down) by aldosterone (n=5). We investigated the three most significantly downregulated miRs, namely mmu-miR-290-5p (0.85 +/- 0.04 fold), mmu-miR-335-3p (0.79 +/- 0.06 fold) and mmu-miR-1983 (0.64 +/- 0.04 fold), as miR downregulation would result in the upregulation of target proteins. The timecourse of miR regulation by aldosterone was monitored by qPCR for these miRs and a reduction in miR expression was observed by 6 hours with maximal inhibition at 12hrs post aldosterone stimulation (n=4).

By artificially reducing the expression of these three miRs using transfected antisense locked nucleic acids (LNAs) we were able to increase amiloride-sensitive sodium transport in the mCCD cells by 70.2 +/- 4.4% (n=52) without aldosterone stimulation. Overexpression of the three miRs blunted an aldosterone response suggesting that at least part of the aldosterone stimulation of sodium transport occurred through miR signaling. To determine which proteins could be altered by these miRs to regulate sodium transport, in silico predicted protein targets were screened in mCCD cells to test for their involvement in ENaC regulation. One of the candidate miR targets was ankyrin 3 (ank3). Ank3 expression increased in mCCD cells stimulated with 50nM aldosterone for 24hrs. Direct inhibition of miR by LNAs increased ank3 protein levels without aldosterone stimulation indicating that Ank3 was a miR target. To demonstrate the regulation of Ank3 by miRs we transiently transfected the 3'-UTR of ank3 linked to a dual luciferase reporter into mCCD cells. Both aldosterone and changes in miRs themselves altered luciferase expression confirming the UTR as a miR target. Knockdown of ank3 reduced ENaC short-circuit currents compared to control transfected cells (53.9 +/- 5.13%, n=22) and overexpression of ank3 raised amiloride-sensitive short-circuit currents (140.6 +/- 9.75%, n=12), independent of aldosterone or miR regulation.

These studies directly linked expression of Ank3 to sodium transport. Ank3 therefore represents a novel aldosterone-

induced and miR-regulated protein involved in regulating sodium reabsorption across the CCD.

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SA70

Apical BK channels conduct colonic K⁺ secretion

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Distal colonic K⁺ excretion is determined by the balance of K⁺ absorption and K⁺ secretion by the enterocytes. This presentation addresses the current knowledge of the cellular mechanism for colonic K⁺ secretion, with focus on the luminal secretory K⁺ channel. Several recent observations highlight the unique role of the large conductance, Ca²⁺-activated K_{Ca}1.1 (BK, KCNMA) channel as the functionally relevant luminal K⁺ efflux pathway in mouse distal colonic crypts. This conclusion is based on functional studies in BK channel α -subunit knock-out mice. Several relevant issues will be presented: 1. BK channels mediate the resting distal colonic K⁺ secretion, 2. they are acutely stimulated by luminal nucleotide receptor activation and an intracellular Ca²⁺ increase, 3. BK channels are up-regulated by aldosterone, 4. Also the cAMP-stimulated distal colonic K⁺ secretion is mediated via BK channels, 5. and finally aldosterone was found to specifically up-regulate the ZERO (e.g. cAMP activated) C-terminal splice variant of the BK channel. We propose the BK channel as the sole exit pathway for transcellular K⁺ secretion in mammalian distal colon, which is the target for short term intracellular Ca²⁺ and cAMP activation and long term aldosterone regulation.

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SA71

Aldosterone regulated acid-base transport in the collecting duct

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The renal collecting duct serves the fine-tuning of renal acid-base excretion and regeneration of bicarbonate. Type A intercalated cells excrete protons and ammonium thereby generating new bicarbonate replenishing bicarbonate lost during metabolism. In contrast, type B intercalated cells secrete bicarbonate into urine, a process coupled to reabsorption of chloride via pendrin. Both, acid excretion and as well as bicarbonate-dependent chloride reabsorption by type A intercalated cells or type B intercalated cells, respectively, are directly and indirectly regulated by the renin-angiotensin II – aldosterone system. Impaired aldosterone dependent regulation leads to hypotension as well as to type IV distal renal acidosis with hyperkalemia. The molecular basis of the aldosterone effects on acid-base transport in the collecting duct is only partially understood. We will discuss three mechanisms by which aldosterone affects the function of type A and B intercalated cells.

First, aldosterone exerts indirect effects on proton secretion by type A intercalated cells by enhancing the expression and activity of the epithelial sodium channel ENaC in neighboring principal cells. Sodium absorption generates a more lumen-negative potential that drives both potassium secretion by principal cells as well as proton secretion by type A intercalated cells. The connecting tubule is the main site of this interaction. Second, all subtypes of intercalated cells express the mineralocorticoid receptor but only low levels of the cortisone-metabolizing enzyme 11 β -hydroxy steroid dehydrogenase II. In these cells, aldosterone but not cortisone stimulates H⁺-ATPase-dependent proton secretion within 5 minutes. This rapid effect is most likely independent from the mineralocorticoid receptor, involves and requires an elevation of intracellular calcium and is mediated by a PKC dependent pathway leading to the remodeling of the luminal membrane with extensive extrusions and accumulation of proton pumps. However, the long-term effects of aldosterone most likely involving genomic regulation via the mineralocorticoid receptor are not very well characterized. Thirdly, aldosterone stimulates chloride reabsorption by type B intercalated cells through enhanced mRNA and protein expression of pendrin. Pendrin mediates chloride absorption in parallel to sodium absorption by ENaC and enhances also the expression of ENaC, possibly through affecting the local pH environment.

In summary, aldosterone exerts complex effects on the collecting duct linking electrolyte and acid-base handling. Some of the effects of aldosterone appear to be mediated by non-classic non-genomic pathways that require further characterization to elucidate the underlying molecular mechanisms and their relevance to overall aldosterone actions.

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SA72

What is standard setting?

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In many assessments, the establishment of an appropriate pass mark is key to the exam's reliability as a means of decision-making. But how do we establish the score that we expect a borderline student to achieve and thus be confident that the selection of a particular pass mark has been correct? That is, how do we set a 'standard'? Such questions become increasingly pertinent in an age of heightened student expectation and raised student fees coupled with increased time-pressures upon teaching staff in an HE sector concerned with the threat of litigation. However, these questions are self-standing as the cornerstones of academic teaching and student learning and thus warrant answering in their own right. A standard can be defined as a judgment made about performance against a social or educational construct and is thus an abstract borderline position that determines the difference between acceptable and unacceptable performance (Kane, 1994; Norcini 2003). Therefore, as a simple definition, a standard-set exam will not establish the position of this border via a fixed mark on an observed scale. A number of relative and absolute methods exist to determine this border and each has particular merits that range from the defensible to the feasible. As such decisions delineate the competency level or grad-

ing of a student, they can have substantial impact upon future careers and should not be treated lightly. Practices for setting a pass mark vary across the sector, but an absolute requirement by the General Medical Council for “valid and reliable methods to standard-setting” as outlined in their 2009 document ‘Tomorrow’s Doctors: outcomes and standards for undergraduate medical education’ has focused attention on this issue for many physiologists and life scientists engaged with teaching and assessing medical students. It thus appears timely to review the position of such formal standard setting practices in other, non-professional exams. This introductory talk will outline the range of such methods that can be applied to assessments and will aim to convince of their merits whilst pointing out potential limitations for their adoption and usage across all academic subjects.

Kane, M. (1994). Validating the performance standards associated with passing scores. *Review of Educational Research*, 64, 425-461.

Norcini, J. J. (2003). Setting standards on educational tests. *Medical Education*, 37, 464-469.

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SA73

Using the Ebel method for standard setting

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The Ebel standard-setting method involves rating an examination question on two dimensions. The first dimension relates to the importance of the assessed item – is it essential, important or acceptable (‘nice-to-know’) material for the students being assessed? The second dimension relates to whether the assessed item is easy, medium or hard. Each member of a panel of standard-setters completes a 3x3 grid, allocating every question to one of the nine boxes in the grid. The standard-set pass mark is determined by an algorithm in which, for example, ‘hard, acceptable’ questions push the pass mark down whereas ‘easy, essential’ questions push the pass mark up.

In this workshop session participants will work in groups to complete standard-setting grids for typical ‘single best answer’ multiple choice questions across a range of physiological topics that could be included in examinations for medical students or BSc students. This will enable us to compare the outcomes from different groups of participants, and also to see whether the standard-set pass mark might differ for medical vs BSc students.

Standard-setting grids that have been used in recent medical examinations in Bristol will be presented and the strengths and weaknesses of the Ebel method will be discussed.

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SA74

Can we use the modified Angoff for short answer questions?

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Whilst no method of standard setting is without limitations, the use of the modified Angoff method appears to be favoured by many medical courses when establishing the cut-off score (or pass mark) for clinical MCQs. This method requires a panel of judges to define a borderline group; that is, a group with as equal a chance of passing as of failing the specific assessment and to then determine, independently of each other, the percentage of the group that would be expected to pass each of the questions in the assessment. Following subsequent discussion between judges, the cut-off score is established as the totalled average of each judge’s final score for every question and thus an absolute standard is set. Whilst conceptually simple and relatively easy to perform, the test may be particularly suitable for MCQ exam formats and for those assessments that gauge a readily agreed-upon competency. In addition, an acceptable minimum number of judges of around 8-10 may also preclude full adoption of such methodology in many Departments where staff numbers and/or subject knowledge is relatively low. Might therefore such a method be unsuitable for short answer questions (SAQ) in physiology where both the format and the required competency(ies) be less amenable to simple judgment? Or are staff numbers a greater issue that might limit adoption? This short, interactive session will utilise past paper exam questions from physiology exams undertaken by students at the University of Birmingham to determine an Angoff score for each question prior to revealing and comparing against actual performance statistics. The session will conclude with a discussion of the reliability of the modified Angoff for standard setting SAQs and the relative merits of this and other methods in setting the standard of exams where competency may be less easily defined.

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SA75

Standard setting for core biomedical science assessments: Oxford’s experience with a new method

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In any examination, standard-setting should seek to separate out variance due to the difficulty of the examination (bearing in mind that year’s educational experience), from that due to the performance of the students, which is the aim of the assessment. For many years the Oxford preclinical school has separated the assessment of ‘core’ information and wider reading and understanding. The latter has always been done by essays in which students have a choice. The assessment of core was first done by short answers, but it proved very difficult to achieve consistency of examiners marking and standard setting.

From 2004 onward core assessment for each of the three major subjects each year has been done by twenty 5-part questions (either single best answer or extended match format), blue-printed across the range of the syllabus, to determine pass-fail rather than ranking. The questions are delivered on-line by the Question Mark Perception System which not only relieves examiners of tedious marking but also delivers both a numerical mark almost instantaneously, together with a detailed analysis of the performance of the cohort and the questions. The question then was, how to standard-set these questions. At first the practice with the short answers – namely that the students had to get 4 of the 5 parts, in 12 of 15 questions was adopted, but this produced considerable variance in the outcome both within subjects year by year and also between subjects. In the early years, the fact that many new questions were used contributed to this, though with judicious repeated use, the question bank has been upgraded by studying carefully questions that the top performing students were having problems with and removing ambiguities. Following our practice with the short answer core questions an absolute passmark of 70% was first adopted, but examiners often found it necessary to modify this in the light of the performance on particular questions, and between subjects.

Many assessments in UK are standard-set by one of the variety of 'relative' Angoff, Ebel, or Hofstee methods, but it can be difficult (and very costly) to get together sufficient staff with the appropriate expertise to do this and, in the author's experience, variance among those participating can be large. We have therefore turned to a method (Cohen-Schotanus & van der Vleuten, 2010, *Medical Teacher* 32:154-160) which takes as its fundamental premise that the standard of a top cohort of students is consistent from year to year and examination to examination. The absolute standard (70%) is then adjusted to allow for the performance of the top 5% of the cohort (who usually score between 94-100% on our core examination). Analysis of the scores of all students on questions used again from year to year strongly suggests that the fundamental premise of this method is sound. Importantly, we show that the distribution of marks does not follow a normal distribution (although examination data is frequently presented with a mean and standard error for the cohort) but deviates from normal significantly at either end of the distribution. However, we show by a Q-Q analysis that the 2-factor Weibull distribution linearises the Q-Q plot throughout the distribution and particularly at both ends. Using this distribution, one could therefore use any part of the cohort to determine the difficulty of the test. However, for the moment, we have continued to use the performance of the top 5%. Most importantly, understanding the distribution gives us greater confidence at the cut-off at the critical pass-fail level. The particular practical advantages of the use of this method are its ease and speed of use, particularly when combined with presentation and analysis of the test on line. New questions can be tested in summative examinations knowing that if they prove unexpectedly difficult, this is automatically compensated. Most importantly it has given us a rational and defensible way to determine pass-fail cut-offs.

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SA76

Do we need standard setting in physiology and related biomedical sciences degree programmes?

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This session will reflect on the lessons learnt from the application of standard setting within Medicine, with participants discussing whether we should consider utilising this approach within physiology and related biomedical sciences degree programmes.

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SA77

Olfaction from the periphery to the brain

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A major question in neuroscience is to understand how the brain processes social information. A predominant source of information necessary for social recognition is encoded by olfactory and pheromonal signals. The detection of these signals by neuronal populations in the nose and the formation of a memory based on the recognition of these signals play a key role in many social behaviors ranging from feeding, aggression and fear, to reproductive physiology. But, what are the mechanisms by which olfactory stimuli generate their effects on emotion and behavior? Advances in uncovering the molecular mechanism leading to the detection of socially relevant cues by the olfactory system, their influence on innate behavior and reproduction, and the dissection of neural circuits underlying chemosensory induced modulation of social behaviors have set the stage to begin to explore this question.

New clues regarding the general structural and functional organization of the mammalian olfactory system have been revealed using multidisciplinary approaches in which investigations at the genetic and molecular level have been combined with cellular and systems level analyses. We now know that the mammalian olfactory system is composed of a number of subsystems, which make distinct neural connections to regions in the olfactory bulb. They can be distinguished by the receptors they express and the signaling mechanisms they employ to detect and transduce chemosensory stimuli. Interrupting the information flow at the primary transduction pathway of odor detection will give rise to various behavioral deficits. Recently, we have used gene targeting to examine the role of the sodium channel Nav1.7 and the G protein $G_{\alpha o}$ in mouse olfactory function. Both tissue-specific gene deletions cause severe disruptions in olfactory sensing. In the absence of Nav1.7, olfactory neurons are still electrically active and generate odor-evoked action potentials but fail to initiate synaptic signaling causing a total inability to sense odors. More partial deficits, but not less dramatic, are induced in a mouse line carrying the tissue-specific disruption of the $G_{\alpha o}$ gene. This G protein is essential for the signal transduction in a subpopulation of vomeronasal sensory neurons (VSNs), leading to alterations in membrane potential and calcium entry in these neurons. The cellular $G_{\alpha o}$ phenotypes are accompanied

by striking alterations in aggressive behavior indicating that G α o is a critical requirement for the neural coding of chemosensory cues that promote aggressive interactions in both male and female mice. The disruptions in sensory detection may help us to decipher the effect of particular chemosensory cues on modulating social behaviors and neural circuits underlying olfactory processing in the brain.

As expected by the existence of the different olfactory subsystems, olfactory sensory signals follow distinct neural pathways in the brain. Whereas some signals are relayed through the primary olfactory cortex to higher cortical areas, other signals are transmitted via the amygdala to the hypothalamus and related regions. Currently, new genetic models are being developed with which neural circuits in the brain can be unequivocally identified and targeted. Socially important chemosensory cues have been shown to converge onto a small subset of neurons that produce gonadotropin-releasing hormone (GnRH). We therefore started to investigate the properties of the downstream target cells expressing the GnRH receptor (GnRHR) to ultimately help us in investigating neural circuits involved in olfactory-encoded behaviors.

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SA78

Olfactory processing in the medial amygdala

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The medial nucleus of the amygdala (MeA), referred to as the vomeronasal amygdala, plays a key role in defensive and reproductive behaviour. It receives processed information from the associational cortical amygdala (CoA) as well as direct olfactory inputs from the accessory olfactory bulb (AO). Little is known about the processing of olfactory sensory information in the MeA. In this study we examined the physiological properties CoA and AO inputs to neurons in the posteroventral nucleus of the MeA (MePV). Whole cell recordings were made from neurons in acute coronal brain slices made from adult male GAD67-eGFP knock-in mice. Synaptic responses were evoked by electrical stimulation of the afferents in the CoA and/or in the MeA molecular layer where the axon terminals of projections from the AO are located. Cells were filled with biocytin during recording and later visualised using immunohistochemistry. Stimulation of either CoA or AO inputs evoked excitatory synaptic inputs to MeA neurones. Both CoA and AO stimulation activated dual component (AMPA/NMDA) glutamatergic synapses. In voltage clamp, AMPA-receptor mediated excitatory synaptic currents (EPSCs) evoked by stimulation of AO axons showed significantly slower kinetics as compared to those evoked by CoA stimulation. The rise times were 2.04 ± 0.24 ms vs. 0.96 ± 0.08 ms; ($n=24$, $p<0.001$) and decay time constants were 10.1 ± 1.35 ms vs. 4.06 ± 0.27 ms ($n=24$, $p<0.001$). Upon reconstruction of neurones, we found their dendrites extend to the molecular layer forming distal dendritic tufts. Focal pressure application of TTX (1μ M) at the distal dendritic tuft that blocked the accessory olfactory synap-

tic input, leaving the CoA evoked responses intact (80 ± 0.6 % block vs. 6.2 ± 1.6 % block; $n=6$, $p<0.0001$). Calcium imaging of the dendritic tree while stimulating the AO afferents revealed synaptically evoked calcium transients that were restricted to dendrites that extend to the molecular layer. These results show that MePV neurons receive convergent AO and CoA inputs at separate locations on their dendritic tree, with the AO inputs synapsing at the distal dendrites and the associational afferents from CoA synapsing mainly at the proximal dendrites. We suggest that MeA neurones process olfactory and cortical inputs using distinct dendritic compartments.

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SA79

Novel vs familiar: dynamic odor representations revealed by chronic imaging in awake mice

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The ability to detect novel stimuli in the changing environment is critical for the survival of all animals throughout their lifetime. A central question, then, is how sensory systems in the adult brain represent novel sensory inputs and how these representations change as the stimuli become familiar. Here we address this question in the mouse olfactory bulb, where odors are represented by the activity of ensembles of mitral cells. Using chronic two-photon calcium imaging in awake mice (Komiyama et al., 2010), we show that odor familiarization by brief repeated exposure leads to a marked reduction of odor-evoked mitral cell activity without affecting sensory neuron input to the bulb. This mitral cell plasticity is odor-specific; individual mitral cells show a shift in their odor tuning to preferentially represent less-experienced odors. Experience-induced changes in odor representations recover gradually over many weeks and can be repeated with different odors. Thus, odor tuning is dynamically modified by recent experience such that unfamiliar odors strongly activate mitral cell ensembles. Ultimately, this experience-dependent plasticity provides a potential mechanism to encode novel stimuli with greater salience.

Komiyama T, Sato TR, O'Connor DH, Zhang YX, Huber D, Hooks BM, Gabitto M, Svoboda K. (2010). Learning-related fine-scale specificity imaged in motor cortex circuits of behaving mice. *Nature*, 464: 1182-6.

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SA80

Contribution of ventral prefrontal cortex to the regulation of amygdala-dependent negative and positive emotions

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Dysregulated emotions are a core feature of many neuropsychiatric disorders and are often associated with altered activity in limbic emotional circuitry that includes the amygdala, hippocampus and prefrontal cortex (PFC). Altered activity in serotonergic forebrain systems has also been implicated and currently, the front-line treatment of these disorders includes drugs that target the serotonin system. However, our understanding of the interaction between these brain structures, and their modulation by serotonin, in the control and regulation of emotion is only in its infancy. Much insight has been gained recently into the role of the medial PFC in the regulation of the amygdala-dependent freezing response to a fear conditioned stimulus, primarily from studies in rodents. However, the neuroimaging of patients with mood and anxiety disorders have revealed structural and activity changes not only in the medial but also the ventral PFC including orbitofrontal and ventrolateral PFC. These regions are at their most highly developed in primates and thus, to further our understanding of the regulation of amygdala dependent emotional learning and expression by the ventral PFC we have developed models of positive and negative emotional learning and expression in a new world primate, the common marmoset. Since emotional responses are composed of both physiological and behavioural components we use an automated telemetry system to allow the simultaneous measurement of behavioural and autonomic e.g. heart rate and blood pressure, emotional responses in freely moving marmosets. This also helps bridge the gap between current human and rodent studies in which the primary measures of emotional expression are autonomic activity and behaviour, respectively. So far we have identified the critical role of the orbitofrontal cortex in the regulation of both positive (Reekie et al, 2008) and negative (Agustin-Pavon et al, 2012) emotional responses, responses that we have already shown to be dependent upon the amygdala. Lesions of the OFC not only disrupt the contextual regulation of autonomic and behavioural emotional responses as contingencies in the environment change but also lead to their uncoupling, an effect that could have a major impact on overall levels of emotionality. A separate contribution is also made by the ventrolateral PFC. In addition, we have identified the critical role of serotonin in modulating the processing of positive and negative feedback within the amygdala and ventral PFC, dissociating its role from that of dopamine. More recently, we have begun to explore prefronto-amygdala circuits in the context of individual differences in both genes and behavioural traits. It has been hypothesised that high trait anxiety and the 5-HT transporter polymorphism may act as vulnerability factors for developing neuropsychiatric disorders and so, using structural MRI, microPET and microdialysis we have begun to identify alterations in the prefronto-amygdala network in marmosets that are related to these individual differences.

Reekie Y. L., Braesicke K., Man M., Roberts A.C. (2008) Uncoupling of behavioral and autonomic responses following lesions of the primate orbitofrontal cortex. *Proceedings of the National Academy of Sciences*. 105:9787-92.

Agustín-Pavón C., Braesicke K., Shiba Y., Santangelo A.M., Mikheenko Y., Cockroft G., Asma F., Clarke H., Man M., Roberts A.C. (2012) Lesions of ventrolateral prefrontal or anterior orbitofrontal cortex in primates heighten negative emotion. *Biological Psychiatry*, epub ahead of print: PMID 22502990.

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SA81

Mechanics of emotion processing in central amygdala microcircuits

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The amygdala integrates sensory stimuli, encodes emotional states and instructs other brain centers to initiate physiological and behavioral responses. We are using circuit genetic approaches to resolve how the networks of various amygdala neuronal populations orchestrate these processes. Pharmacology, optogenetics and viral tracing uncovered a local inhibitory network of two antagonistic neuronal populations in the lateral central amygdala (CEl) that gates amygdala output to control conditioned fear. In search for underlying circuit mechanisms, we conducted combined pharmacogenetics and *in vivo* electrophysiological recordings. Results from these experiments suggest that the antagonistic neuronal populations operate like a seesaw which alternates between two states: in the absence of a conditioned stimulus (CS), so called CE_l^{off} neurons, identified by the expression of PKC δ , are active, inhibiting their counterpart CE_l^{on} neurons and amygdala output; in the presence of the CS, CE_l^{on} neurons are active, inhibiting CE_l^{off} neurons, which disinhibits amygdala output and fear signals to the brain stem. In support of this model, initial pharmacogenetic experiments have shown that a reduction of CE_l^{off} neuronal activity results in significantly more amygdala output and conditioned freezing than usual. Current circuit genetic experiments aim at further investigating macrocircuit and pharmacological control of emotional gating in CE.

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SA82

Imaging fear systems using awake rodent fMRI

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Functional magnetic resonance imaging (fMRI) is a powerful method for exploring cognitive brain responses in humans, however much less work has been done using rodent fMRI in

awake animals. Here we have developed a novel awake rat fMRI fear conditioning paradigm which activates neural circuitry involved in learned fear conditioning. Using this method we show temporal activation of the amygdala and related fear circuitry in response to a conditioned stimulus and demonstrate that the magnitude of fear-circuitry activation is increased in a rodent model of affective disorders based on early life stress. This technique provides a new translatable method for testing environmental, genetic and pharmacological manipulations on amygdala activation and emotional brain circuitry in awake rodent models.

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SA83

Multi-modal imaging of the rodent heart

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Single Photon Emission Tomography (SPECT) and Positron Emission Tomography (PET) are uniquely able to investigate functional and molecular aspects of the heart. While SPECT and PET imaging modalities are successfully established for years in the clinics applying these technologies in small animals such as rodents is a huge challenge: it needs ultrahigh spatial and temporal resolution, small injection volumes, non-interfering drug concentrations and such.

This talk will review the current status and technologies as well as the potential and spectrum of SPECT and PET alone or in combination with CT or MRI (multi-modal) to non-invasively study the rodent heart. A special focus is put on applications in mice.

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SA84

Investigating cardiac energetics in heart failure

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The energetic requirements of the heart are weight-for-weight higher than for any other organ, providing non-stop function for a lifetime while maintaining reserve in response to increased demand. This is met by continuously recycling a relatively small pool of adenosine triphosphate (ATP), which when hydrolysed to ADP liberates 'free energy' available to perform work (ΔG_{ATP}). This process is aided by mitochondrial-creatine kinase (CK) located at the mitochondria which catalyses the transfer of a high-energy phosphoryl from ATP onto creatine to form ADP and phosphocreatine (PCr), and by CK coupled to ATPases which catalyses the reverse reaction. This system functions to maintain local ATP/ADP ratios, provide rapid transfer of chemical energy, and as an energy buffer, with PCr available for rapid regeneration of ATP when demand outstrips supply. In the failing heart, total creatine levels, PCr, and CK activity are all down-regulated, regardless of species or aetiology, and eventually even ATP levels are reduced. This has led to the concept of the failing heart as 'energy starved', but whether these

energetic changes are biomarkers or significantly contribute to the pathophysiology of heart failure is the subject of continued debate. Key methodologies are now becoming available in vivo to help address this question in rodent models. For example, ^{31}P magnetic resonance spectroscopy (MRS) can be used to detect ATP, PCr, and measure CK flux using saturation transfer protocols, while ^1H -MRS can detect total creatine. In addition, recent advances have drastically shortened scan times for anatomical MRI in rodents, enabling multi-parametric MR under the same general anaesthesia. A major challenge will be the combination of these approaches to calculate ΔG_{ATP} in vivo, which represents the ultimate integrated measure of energetic status. These techniques have been applied to genetic mouse models of impaired creatine biosynthesis and CK ablation, which have served to highlight remarkable plasticity in cardiac energy metabolism, and confirm the importance of the CK system under conditions of high workload and acute stress. Surgical models of rodent heart failure develop the same hallmark defects in cardiac energetics and have proven to be particularly informative. Key factors affecting interpretation of these models will be discussed, as will recent studies demonstrating beneficial effects from therapeutic targeting of the CK system. It is anticipated that these important new findings will stimulate interest in metabolic therapies and drive technological advances aimed at investigating cardiac energetics in vivo.

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SA85

Understanding the physiology of heart failure using cellular and in vivo techniques

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Heart failure commonly results in a progressive decline of electrical and hemodynamic cardiac function. Furthermore, activation of compensatory mechanisms affecting the surviving myocardium and cardiomyocytes significantly contributes to cardiac dysfunction. To address pathophysiological mechanisms in heart failure, we investigate multiple levels from cellular biophysics to in vivo physiology with a focus on early, potentially causal alterations. The following (i) disease models and (ii) strategies are considered. (i) Rapidly progressing disease models including myocardial infarction; monogenetic, late-onset disease models; monogenetic models requiring modifier mechanisms for disease expression. (ii) The following techniques are considered in the context of heart failure and cardiomyopathy: diagnostic in vivo phenotyping techniques; noninvasive imaging and quantitative electrophysiological evaluation of the multicellular substrate; isolated cell imaging and electrophysiology; and subcellular super-resolution imaging. Examples of a combination of strategies to elucidate multifactorial disease mechanisms of defective ion transport and abnormal membrane excitability are discussed. In conclusion, progressive tissue remodeling and arrhythmogenic substrate expression represent complex disease biology, improved understanding of which depends critically on reproducible pathophysiological models and integrative translational approaches.

This work received support through Deutsche Forschungsgemeinschaft (KFO 155 subproject LE 1313/2-2); a Halbach Foundation award; a DAAD person exchange program to the University of Maryland; and the German Center for Cardiovascular Research (DZHK). The research leading to these results has received funding from the European Community's Seventh Framework Program FP7/2007-2013 under grant agreement no HEALTH-F2-2009-241526, EUTrigTreat.

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SA86

High resolution echocardiography in the assessment of cardiac physiology and disease

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High resolution echocardiography has increasingly become a tool for the rapid assessment of cardiac physiology in small animals (Foster et al 2002;2009). Ultrasound scanning is performed with the animals lightly anaesthetised, hair is removed using a depilatory cream and an acoustic coupling gel is applied to the chest wall of the animals. Cardiac scanning for rat, mice and zebra-fish is performed using probes of 25, 30 and 55 MHz frequency respectively with higher frequencies giving better resolution (better than 100µm) but less depth penetration (limited to 1-2cm). The electrocardiogram (ECG) from rodents are obtained from electrodes on the heated plate on which the ultrasound scanning is undertaken. LV functional analysis can be undertaken enabling measurements of ejection fraction, LV volume, cardiac and stroke volume. Wall dynamics of infarcted and ischemic myocardial regions can be compared to those from normal myocardium. By focusing on one line within the ultrasound image, it is possible to obtain an M-mode trace which enables high temporal resolution measurements for measurement of wall thickening and in the assessment of valvular motion throughout the cardiac cycle. Using the Doppler imaging mode, blood flow patterns across valves and in associated vessels (pulmonary artery and aorta) can be measured indicating potential abnormalities of the valve leaflets and enabling quantification of diastolic and systolic blood flow. The Doppler technique can also be used to study tissue motion – which is of particular value in the assessment of ischemic and infarcted regions. Extending this technique to strain imaging allows segmental and trans-mural wall motion to be evaluated.

The techniques described above have also been applied to embryonic mice and rats (E10.5 or later). In such instances, since only the maternal ECG is obtained, assessment of embryonic heart-rate, is obtained from the trans-mitral Doppler or left ventricular M-mode embryonic scans. From such scans, parameters such as myocardial performance index (MPI) can be obtained – a measure of the combined systolic and diastolic haemodynamic function of a heart. MPI can also be assessed in a similar way from embryonic zebra-fish embedded in agar.

The administration of ultrasonic contrast agents, via tail-vein injections, enables vascularity within myocardial regions to be assessed. These gas-filled encapsulated microbubbles circulate freely within the blood and enhance the ultrasound signal. By monitoring the rate and magnitude of the increase in

the ultrasound signal, within different regions of the myocardium, relative perfusion indices can be obtained. Using tissue-mimicking phantoms developed in-house we have shown that spatial resolution better than 100 µm can be achieved using preclinical ultrasound (Moran et al 2011). Such resolutions, coupled with the high temporal resolution achieved enable preclinical ultrasound imaging to be a real-time, inexpensive, rapid-throughput screening tool for the assessment of cardiac physiology and disease.

Foster FS, Zhang MY, Zhou YQ, Liu G, Mehi J, Cherin E, Harasiewicz KA, Starkoski BG, Zan L, Knapik DA, Adamson SL. A new ultrasound instrument for in vivo microimaging of mice. *Ultrasound Med Biol* 2002;28:1165-1172.

Foster FS, Mehi J, Lukas M, Hirson D, White C, Chaggares C, Needles A. A new 15-50MHz array-based micro-ultrasound scanner for pre-clinical imaging. *Ultrasound Med Biol* 2009;35:1700-1708.

Moran CM, Pye SD, Ellis W, Janeczko A, Morris KD, McNeilly AD, Fraser HM. A comparison of the imaging performance of high resolution ultrasound scanners for preclinical imaging. *Ultrasound Med Biol* 2011;49:3501.

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SA87

Advancements in pressure-volume catheter technology – stress remodelling after infarction

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Micro conductance catheters have been successfully applied to measure left ventricular (LV) function in the mouse to assess cardiac or pharmacological interventions for a number of years. New complex admittance methodologies produce an estimate of the parallel admittance of cardiac muscle that can be used to correct the measurement in real-time. This contrasts with existing conductance technologies that require *in vivo* calibration using a bolus of hypertonic saline. Here we report the application of this emerging technology in the context of myocardial infarction and LV remodelling.

Using a combination of non-invasive imaging (MRI & high resolution ultrasound) and LV conductance catheters, we have compared measures of LV function using an admittance system (ADVantage system, Scisense, Ca) and a traditional conductance-derived pressure-volume (PV) system in models of cardiac dysfunction following myocardial infarction and pressure overload (Clark JE, et al. *J Pharmacol Toxicol Methods*. 2009, 59(2):94-9). We have also subjected mice to focal myocardial ischaemia-reperfusion injury while measuring cardiac function with different systems to determine the reliability and accuracy of these methods to distinguish between normal, and dysfunctional LV, contractile performance.

We have shown that the ADVantage system, in our hands, provides a straightforward solution for assessing LV function in mice. Using this technique in combination with other established methods we have demonstrated marked LV dysfunction following coronary artery occlusion and reperfusion which can be reversed using preconditioning agents, and found that functional readouts are representative of other methods. We have found that, especially in diseased tissue, LV pressure-volume

loops derived from complex admittance provide a reproducible and reliable method of determining LV function without the need for technically challenging calibration. Our data suggest that the ADVantage system records accurate/physiological LV cavity volumes when compared to other invasive methods in the same animal.

The ADVantage system is both effective and reproducible in measuring LV function and dysfunction in the mouse, without the need for complicated interventions to calibrate the measurements or training in a new technology. We have also demonstrated the application of this system to adverse remodeling following infarction and LV hypertrophy following pressure overload. This may mark the way toward a fast and accurate assessment of murine cardiac function in normal and disease models.

Scisence, Canada for providing Advantage PV system for these studies

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SA88

Imaging the healing murine myocardial infarct: ultrasound, magnetic resonance imaging and near-infrared fluorescence

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Survival following myocardial infarction (MI) is improving thanks to better early intervention to restore blood supply to the ischaemic myocardium. However, the myocardium is frequently damaged, increasing the longer-term risk for the development of heart failure. We¹ and others have shown that the extent of infarct related injury can be limited by intervening to prevent expansion during the healing process that follows MI, leading to improved long term function.

MI can be modelled in rodents, for example by ligation of the left anterior descending coronary artery. The infarct healing process that follows is increasingly well-defined² and the influence of interventions can be characterized by collecting tissue for *in vitro* analysis after induction of infarction. However, separate cohorts of animals must be used for analysis of e.g. infarct size, inflammatory cell recruitment, matrix metalloproteinase activation, vessel density, collagen content at relevant times during infarct healing and for long term functional assessment. Advanced *in vivo* imaging is now available to assess several of these processes non-invasively.^{3,4} Our aim is to incorporate this approach into our studies to permit improved correlation between infarct healing events and long-term functional outcome, and also to reduce animal use.

Imaging modalities currently available in Edinburgh Preclinical Imaging facility include high frequency ultrasound,⁵ magnetic resonance imaging (MRI)⁶ and optical (near infrared fluorescence and bioluminescence) imaging.³ The presentation will include examples of how these are used to image aspects of myocardial structure and function, infarct injury, inflammatory cell recruitment and perfusion; comparing them with other imaging options including microPET and microCT. The pros and cons of each approach will be considered, including resolution, imaging depth, suitability of imaging agents and practical aspects including cost, time required for imaging and the impact on animal welfare.

McSweeney SJ et al. (2010) Improved cardiac function follows enhanced inflammatory cell recruitment and angiogenesis in 11 β -hydroxysteroid dehydrogenase type 1 deficient mice post-MI. *Cardiovasc Res*, 88, 159-167

Frangiogiannis N (2012) Regulation of the inflammatory process in cardiac repair. *Circ Res*, 110:159-173

Leuschner F, Nahrendorf M. (2011) Molecular imaging of coronary atherosclerosis and myocardial infarction: considerations for the bench and perspectives for the clinic. *Circ Res*;108:593–606.

Lee WW et al. (2012) PET/MRI of inflammation in myocardial infarction. *J Am Coll Cardiol*, 10, 153-63

MA Denvir et al., (2005) Influence of scanning frequency and ultrasonic contrast agent on reproducibility of left ventricular measurements in the mouse. *J Am Soc Echo* 18, 155-162

Gilson et al., (2007) Borderzone contractile dysfunction is transiently attenuated following reperfused myocardial infarction in iNOS knock-out mice. *J Am Coll Cardiol*, 50, 1799-1807

In vivo imaging is conducted in collaboration with Edinburgh Preclinical Imaging (<http://www.cvs.med.ed.ac.uk/Research/Core%20facilities/Imagin>). Research in the GAG lab is supported by the Wellcome Trust and the British Heart Foundation. The Support of the BHF CoRE is acknowledged.

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SA89

Getting there and getting back: the physiology of sustained acceleration in space flight

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Since the earliest days of manned space flight, the acceleration required for a space vehicle to reach orbit and to return to Earth has presented a physiological challenge to the vehicle's occupants. Even modest levels of sustained acceleration may have profound effects on the cardiovascular and respiratory systems, resulting in reduced cerebral perfusion, pulmonary ventilation-perfusion mismatch and desaturation of arterial blood. Whilst improvements in rocketry resulted in a reduction in acceleration exposure during the days of the US Space Shuttle programme, the advent of innovative space vehicles associated with 'space tourism' may well promote a return to higher levels of acceleration. Furthermore, the passengers of future vehicles may comprise a population group far less tolerant to acceleration than the specially medically selected astronauts of the early space programme. In this presentation, the physiological challenges of sustained acceleration will be described, as well as the potential countermeasures. Acceleration levels in various space vehicles during launch and re-entry will be considered, together with the physiological burden of short or long duration microgravity exposure prior to re-entry acceleration.

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SA90

Microgravity as a tool to investigate perception of, interaction with, and navigation on Earth

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Humans are so successfully adapted to life on Earth and its gravity (1g), that we have devised ways to achieve escape velocity and leave that gravitational environment. The 'weak' force of gravity is omnipresent on Earth, modulating many biological processes and the physical-chemical interactions that underlie them. However, just how gravity affects cellular biological processes and organism behaviour is largely unknown. Transition into microgravity can elicit acute spatial disorientation and a range of autonomic symptoms, collectively defined as space adaptation syndrome (SAS). These symptoms, thought to arise from sensory-mismatching, whilst not life threatening, are unpleasant and can on occasions incapacitate. In the event of an emergency, this may place the afflicted and their colleagues in danger and prevents extra-vehicular activity (EVAs) being scheduled early in a mission. As a consequence, potential mechanisms and methods of mitigation have received much attention, despite the fact that SAS is usually transient - ameliorating over 2-3 days. SAS may however, be re-activated in novel situations such as an EVA, or when a crew member unexpectedly floats by.

This sensitivity may relate to the fact that visual cues become relatively more important for spatial orientation, a feature common with a range of sensory pathologies on Earth. Indeed microgravity leads to adaptation of vestibular, somatosensory, proprioceptive and other gravitoceptive (relating to fluid distribution/pressure) mechanisms. Adaptation to microgravity is rapidly reversible (with a similar temporal profile to adaptation) on return to a 1g environment. Such acute sensory-reweighting in otherwise healthy individuals may inform interventional strategies in those whom adaptation to sensory-loss (or hyper-excitability) is inhibited.

Microgravity has also been shown to retard pre-natal (e.g. vestibular projections), and post-natal development (e.g. righting behaviour and locomotion) in rodents. Intriguingly, the existence of a critical exposure g threshold or 'time window' remains to be determined. Exploration of these issues using analogues or the partial gravity environments of the Moon (~0.167g) and Mars (~0.38g) may inform understanding, providing insights for (developmental) rehabilitation and sensory augmentation strategies on Earth.

Production of a consistent 'artificial' 1g environment might mitigate sensori-motor changes, however significant engineering challenges exist. Imposition of hypergravity for brief periods has been proposed as a potential countermeasure. However, determination of the tolerability, relative efficacy, and the required 'effective dose' in terms of both magnitude and exposure (duration x frequency) remain to be determined, which again may have possible terrestrial applications.

In addition to vestibular changes, somatosensory and proprioceptive mechanisms including static and dynamic position sense demonstrate adaptation during prolonged microgravity exposure. These changes must be integrated in order to perform a range of tasks, from eating and personal hygiene, to complex docking manoeuvres (with or without visual contact). As a result accurate and appropriate prediction of exocentric motion and egocentric force production and/or motion are required. Intriguingly, there is recent evidence that Newtonian laws of motion are 'hard wired' evident even in microgravity, offering critical insights into Earth bound sensory-motor function.

Microgravity provides an opportunity to differentiate mechanisms that depend upon relative movement with, or without respect to a gravitational vector. On Earth, the effect of gravity is so ubiquitous that its effects are difficult to differentiate, or are simply overlooked. However, it may obscure or modulate other operative physiological mechanisms. Microgravity experiments were used to disprove Barany's (Nobel Prize winning) convection hypothesis as the operative mechanism for caloric stimulation, a routine vestibular clinical test that evokes perception of rotation without actual movement.

Gravity also appears to play a vital role in the perception of size, distance and inclination. Work is in its infancy but promises much, particularly as these processes may have been a key factor in humankind's success in inhabiting vast swathes of the globe, and one of the major reasons for astronaut, rather than robotic exploration beyond it.

Micro- and partial gravity experiments offer opportunities to understand the fundamental mechanisms that underlie the perception of, interaction with, and navigation on Earth. Critically, it also offer insights into approaches to ameliorate spatial disorientation and imbalance, thereby reducing falls risk, the UK's leading cause of injury-related deaths in older adults, and a major precipitator of socio-economic inactivity in an ageing population.

Micro- and partial gravity experiments offer opportunities to understand the fundamental mechanisms that underlie the perception of, interaction with, and navigation on Earth. Critically, it also offer insights into approaches to ameliorate spatial disorientation and imbalance, thereby reducing falls risk, the UK's leading cause of injury-related deaths in older adults, and a major precipitator of socio-economic inactivity in an ageing population.

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SA91

Effects of space flight on the cardiovascular system

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It is now more than 50 years since the first human space flight took place. Since then some 500 humans have experienced the weightlessness of space flights, mostly during orbital flights, but a few individuals have travelled to the moon and back. At present time astronauts and cosmonauts live and work at the International Space Station (ISS), and the normal duration of a stay at ISS is six months. The human body readapts to the new environment, and therefore is no longer adapted to the normal gravity when returning to earth. For the cardiovascular system this leads to orthostatic intolerance and impaired exercise capacity during the initial period after landing. These symptoms are observed already after space-flights of 1-2 week duration. During the US Space Shuttle program with flight durations up to 16 days some 40 per cent of the astronauts could not finish a 10 min stand test and had to interrupt earlier due to signs of impending vasovagal syncope. Several factors act together to cause orthostatic intolerance: Already after 24 h in space there is a manifest reduction of the plasma volume and a redistribution of interstitial fluid from the legs to the head and neck, the latter causing the classical signs of "chicken legs and puffy face". A headward redistribution of blood and fluid is perceived by volume-regulating mechanisms as an over-all hypervolemia, which is then corrected by a combination reduced fluid intake and increased fluid output. A condition similar to motion sickness during the first days of microgravity contributes to the reduced fluid intake. After a week or so there is a 2-3 kg reduction of body mass to which

the reduction of fluid volume has contributed. Upon return to normal gravity the relative hypovolemia manifests itself with reduced stroke volumes during rest and exercise. There is also a reduced sensitivity of the carotid-cardiac baroreflex, but the impact of that on orthostatic tolerance is likely to be small since there are marked tachycardic responses both to standing and exercise. Indirect evidence points to an inability to defend the arterial blood pressure by vasoconstriction in the lower extremities. The responsible mechanism here is not clear; a hypothetical over-expression of beta-2 receptors in resistance vessels has not been substantiated. Neither is the output of sympathetic nerves impaired since muscle-sympathetic neural activity is increased rather than decreased during and after space flight. The aerobic capacity is reduced by up to 25 per cent after space flight and appears proportional to the reduced stroke volume and plasma volume. Apart from the hypovolemia there are signs of moderate cardiac atrophy with a smaller left ventricular myocardial mass and a somewhat stiffer myocardium. As plasma volume is recovered after a week in normal gravity the aerobic capacity and the exercise stroke volume are recovered. With the perspective of human planetary exploration in the future, much present research work is focusing on how to preserve orthostatic tolerance and exercise capacity after extended periods of microgravity, so that explorers arriving to Mars can manage independently of the ground support they would have had if they landed on Earth. Clearly the present exercise prescription of 1-2 hours of daily work on a cycle ergometer or on a treadmill helps to prevent an even more marked relative hypovolemia than that described above. However, it has little effect on orthostatic intolerance. Moreover, exercise consumes precious resources such as food and oxygen, so there is a need for more time-effective countermeasures, which at the same time should prevent skeletal muscle atrophy and bone demineralization. Present studies in that area include a combination of strength training and artificial gravity.

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A review of the effects of acute and sustained microgravity exposure on lung function

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Gravity has shaped the anatomy and physiology of human beings over millions of years. Exposure to microgravity has been shown to affect every single body system. These physiological changes may lead to undesirable health consequences. This paper aims to present and discuss the effects of short and long-term microgravity exposure on lung volumes, capacities and function, along with a final consideration regarding a new method to evaluate respiratory function in space.

Parabolic flights have shown that the sternum is displaced in the cranial direction in microgravity and it is accompanied by an increase in diameter of the lower rib cage. This change in the position of the chest wall was predicted to cause the volume-pressure curve to lie between the standing upright and the supine position curves, with a net result of a reduction in lung volumes. In five subjects studied in a KC-135 aircraft, functional residual capacity decreased by 432 ml during microgravity. Vital capacity also reduced from a mean value of 4.72

L at 1 G to 4.35 L at 0G. Forced vital capacity and forced expiratory volume in 1s were also decreased by an average of 2.5% in the 20s of microgravity in a parabolic flight (1). During the 9 day mission of the Space Life Sciences-1, forced vital capacity and forced expiratory volume in 1s were significantly reduced on flight day 2, but were greater than pre-flight values at day 9. In comparison with standing pre-flight values, tidal volume was decreased by 15% (110 ml) in microgravity and this reduction remained during the entire space flight. Functional residual capacity and expiratory reserve volume decreased significantly in-flight by 520 ml and 370 ml, respectively, when compared with pre-flight standing values. Residual volume was less during flight by 350 ml, when compared with standing control values. This 20% reduction in the residual volume was unexpected as it is normally fairly resistant to change. Lung volumes are believed to be affected by the changes in the intra-thoracic blood volume that occurs throughout the mission, and by alterations in respiratory mechanics and the cranial displacement of the diaphragm and abdominal content that happens in the absence of gravity (2). The gravitational gradient affects the distribution of ventilation and perfusion in the upright human lung. This uneven distribution of ventilation and blood flow within the lungs leads to variations in ventilation-perfusion ratios. Cardiogenic oscillations of CO₂ decreased to approximately 60% in amplitude in microgravity (3) and there was also a significant reduction in cardiogenic oscillations of nitrogen (to 44%) and argon (to 24%) in comparison to the pre-flight standing values (4). Possible causes of the residual inhomogeneity of ventilation include regional differences in lung compliance, airway resistance and the motion of the chest wall and diaphragm. Microgravity was expected to abolish completely apicobasal differences in perfusion and its persistence is possibly related to other mechanisms not affected by gravity, such as central-peripheral differences in blood flow and interregional differences in conductance.

The diffusion capacity of the lung increased by 62% in a parabolic flight study and by 28% in sustained microgravity when values were compared with pre-flight standing values (5)(6). The standing-to-supine transition pre and post-flight caused a significant elevation in blood volume in pulmonary capillaries. Diffusing capacity of the membrane was unchanged pre-flight in the standing-to-supine transition and significantly elevated in-flight in comparison to standing (27%) and supine (21%). In microgravity, the capillary filling is uniform, which is associated with a large increase in the surface area of the blood-gas barrier. Consequently, the membrane diffusing capacity is substantially raised. This suggests an absence of sub-clinical interstitial pulmonary oedema in microgravity, as had been previously speculated (5)(6).

The overall effect of acute and sustained exposure to microgravity, although affecting the respiratory system, does not cause any deleterious effects to the gas exchange in the lungs. However, there is no current suitable method of accessing arterial blood in space. Consequently, at present, values for blood gas tensions are usually derived from measurements of respiratory gas partial pressures. To this end, the earlobe arterialized blood technique for collecting blood gas tensions has been considered for use in space (7). Access to arterial blood analysis will allow better physiological evaluations and the management of clinical emergencies in a space mission, resulting in increased safety for the crew members involved.

Paiva M, Verbanck S, Estenne M. Chest Wall Mechanics in Microgravity: Results from Parabolic Flights. 5th Eur. Symp. On Life Sciences Research in Space, 2003 August ESA SP-336.

Elliot AR, Prisk GK, Guy HJB, West JB. Lung Volumes during Sustained Microgravity on Spacelab SLS-1. J. Appl. Physiol. 1994 77: 2005-14.

Prisk GK, Guy HJB, Elliot AR, West JB. Inhomogeneity of Pulmonary Perfusion during Sustained Microgravity on SLS-1. *J. Appl. Physiol.* 1994 76(4): 1730-38.

Guy HJB, Prisk GK, Elliot AR, Deutschmann III RA, West JB. Inhomogeneity of Pulmonary Ventilation during Sustained Microgravity as Determined by Single-Breath Washouts. *J. Appl. Physiol.* 1994 76(4): 1719-29.

Vaida P, Kays C, Techoueyeres P, Lachaud JL, Riviere D. Pulmonary Diffusing Capacity and Pulmonary Capillary Blood Volume During Parabolic Flights. *Aviat. Space Environ. Med.* 1996 67: 700 (Abstract 210).

Prisk GK, Guy HJB, Elliot AR, Deutschmann III RA, West JB. Pulmonary Diffusing Capacity, Capillary Blood Volume, and Cardiac Output during Sustained Microgravity on SLS-1. *J. Appl. Physiol.* 1993 75(1): 15-26.

Russomano T, Evetts SN, Castro J, dos Santos MA, Gavillon J, Azevedo DFG, Whittle J, Coats E, Ernsting J. A Device for Sampling Arterialized Earlobe Blood in Austere Environments. *Aviation, Space, and Environmental Medicine* 2006 April 77(4): 453-55

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SA93

Musculoskeletal alterations in space – from physiology to countermeasure applications

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Space flight, as well as ground based models for it, induces reductions in muscle mass, muscle function, bone mass and thus a weakening of the skeleton's structural strength. The combination of these alterations must be expected to greatly increase the risk of falls and fractures upon re-entry into gravitational fields, with potentially deleterious consequences e.g. on Mars. Therefore, the quest to maintain musculoskeletal health in space has prompted the development of countermeasures, of which many involve physical exercise. Such developments can only be done in a rationale way if the mechanisms that underlie the alterations are understood.

With regards to muscle, the space-induced reduction in size is very prominent in the legs, somewhat less in the trunk and absent in the arms. This is mostly understood as disuse atrophy, since astronauts cannot load their leg muscles forcefully whilst in space. Accordingly, bed rest is considered as a valid ground based model of space flight (1), with obvious relevance for clinical medicine. The atrophy is most severe in those muscles that normally act against gravity, and it is also more pronounced in mono-articular muscles than in bi-articular muscles. Finally, it is also important to notice that the loss in muscle strength after space flight is in excess of the loss in anatomical cross section (2). The possible causes are currently under investigation.

With regards to bone, its structural weakening in space had initially been ascribed to endocrine disturbances, e.g. a lack of vitamin D. However, it has to be appreciated that bone losses are occurring in the lower, but not in upper extremities. We therefore argue that endocrine and nutritional effects, although important confounders, are unlikely to be the primary cause.

It is now generally accepted that bones adapt to mechanical stimuli. The mechanostat theory proposes that bone strains are regulated in the fashion of a negative feed-back loop (3),

and that bones can thereby respond to alterations in their loading. Controversy, however, still exists as to where the most important forces arise from. Whilst it is often thought that gravitational bone loading, or even weight bearing itself would constitute the primary osteogenic stimulus, biomechanical analyses suggest that the greatest forces arise from muscle contraction. In order to promote our understanding of the musculature's role in the maintenance of bone, evidence from three strings of research is accumulated.

Firstly, resistive exercise was performed as a countermeasure during bed rest, with the intention to safeguarding bone strength through the maintenance of muscle strength. In the long term bed rest (LTBR) study, resistive flywheel exercise was performed 2-3 times per week during 90 days of bed rest. This exercise regimen was highly efficient for the knee extensor, but not for the plantar flexor muscles, and it failed to achieve a significant benefit for the hip or the tibia (4). In the first Berlin bed rest (BBR) study, however, the combination of resistive exercise with whole body vibration performed 11 times per week was found to be fully efficient for plantar flexor muscle strength and for tibial bone mass (5). The second Berlin bed rest study then established the specific benefit of vibration for bone in the bed rest model.

The second line of evidence for the muscle-bone hypothesis derives from a novel model of tibial unloading that has recently been developed in our laboratory. In particular, an orthosis has been designed that replaces the function of the plantar-flexor muscle-tendon complex, but allows a normal gait pattern and therefore will maintain the tibia's gravitational loading.

The third line of evidence, finally, is a study that is currently assessing the temporal and quantitative relationship between muscle contractions and bone strains in humans. Taken together, the available evidence underlines the important role of muscle contractions for the maintenance of bone. Exercise countermeasures for the maintenance of bone in space therefore will probably have to involve forceful muscle contractions.

Pavy-Le Traon A, Heer M, Narici MV, Rittweger J, Vernikos J. From space to Earth: advances in human physiology from 20 years of bed rest studies (1986-2006). *Eur J Appl Physiol* 2007;101(2):143-194.

Narici M, Kayser B, Barattini P, Cerretelli P. Effects of 17-day space-flight on electrically evoked torque and cross-sectional area of the human triceps surae. *Eur J Appl Physiol* 2003;90(3-4):275-282.

Frost HM. Bone "mass" and the "mechanostat": a proposal. *Anat Rec* 1987;219(1):1-9.

Rittweger J, Frost HM, Schiessl H, Ohshima H, Alkner B, Tesch P, Felsenberg D. Muscle atrophy and bone loss after 90 days of bed rest and the effects of flywheel resistive exercise and pamidronate: results from the LTBR study. *Bone* 2005;36(6):1019-1029.

Rittweger J, Beller G, Armbrrecht G, Mulder E, Buehring B, Gast U, Dimeo F, Schubert H, de Haan A, Stegeman DF, Schiessl H, Felsenberg D. Prevention of bone loss during 56 days of strict bed rest by side-alternating resistive vibration exercise. *Bone* 2010;PMID: 19732856(46):137-147

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Healthcare of ESA astronauts in space and on earth

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ESA Medical Operations has a long experience in collaborating with the US American and Russian space-flight programs

since the early 1980's with more than 40 supported space missions.

As one of five International Partners, ESA is involved in the construction and operation of the International Space Station. Multilateral Memoranda of Understanding define the policies and rules of this major international endeavor. The medical support and health care of humans, living, working and researching in space, is the most important element of space flight operations. ESA is acknowledging its responsibility to preserve health and well being of its astronauts by implementing a robust medical support infrastructure, however, not neglecting the fact that this needs to be done on the basis of a multinational partnership.

The US and Russian medical support programs have a long tradition. ESA does not have its own transportation access to space and therefore heavily depends on the two nations, offering launch services. ESA's approach to define and implement a medical support concept was not to decide on one or the other or to just follow a program according to political boundaries. To the contrary, ESA Medical Operations realized that the ISS program opens the door for creative solutions, to combine established and proven concepts, to benefit from the experience of both big Partners. And even though ESA is a small Partner, we used the opportunities to propose new approaches, to critically analyze the current concepts, and to even question established processes. Our geographical location and our cultural background, combined with the experience gained in both space-flight programs enabled us to proactively facilitate such processes. Moreover, ESA by itself working in an environment of many nationalities, cultures and languages helped in positioning ourselves, to increase our reputation among the Partners and to promote a multicultural approach to space-medicine. By understanding our heritage it becomes evident that besides all attempts to harmonize and standardize medical care, preserving the cultural diversity and identity and respecting skills and experiences provided by all Partners is essential to operate the Space Station in a truly global and international fashion.

With the delivery of the ESA elements – the scientific laboratory "COLUMBUS" and the Automated Cargo Vehicle "ATV", ESA became a full member of the ISS program. This allows the Agency to have in average one ESA astronaut per year supporting a mission of about 180 days. The current mission in fact marks the 5th long-duration ISS spaceflight with ESA astronaut participation.

But a majority of their time, astronauts are living and working on this planet under normal gravity conditions. Maintaining the physical and mental health and fitness as an important element of space-medicine healthcare with the goal to "protect" this precious human asset. Therefore a continuous preventive medical program and intensive post-mission rehabilitation exercises follow careful medical selection.

As medical support is not "just waiting for the patient to see the doctor", a robust ground infrastructure and ground support team needed to be defined and implemented. Furthermore, space-medicine is not only "the doctors"; it is a carefully orchestrated team effort by many professions, enabling at the end the physician to make a clinical decision.

This presentation will provide an overview of the main elements of the ESA medical operations support program and its implementation within ESA and in coordination with the other Partners. By using live examples of the current and recent mission activities, an overview of the complexity, the problems encountered, the opportunities for the future, and possible impacts on terrestrial medical applications will be presented.

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Interstitial Cajal-like cells of the human thoracic duct

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Lymph transport is generally accepted to rely upon an intrinsic contractile activity generated locally within and along the walls of lymph vessels. The pumping behaviour of transporting lymphatic vessels resembles that of the gastrointestinal tract where interstitial cells of Cajal (ICC) are critical for coordinating contractile function. Interstitial Cajal-like cells (ICLC) are reminiscent of ICC in their morphology and ultrastructure but are found outside of the gut in other smooth muscle-containing tissues. ICLC have already been demonstrated in several spontaneously active smooth muscle-containing tissues and c-kit positive ICLC have been identified in the subendothelium of sheep mesenteric lymphatics (McCloskey *et al.* 2002). In many tissues, including animal lymphatic vessels, ICLC are speculated to coordinate electrical activity or act as pacemaker cells. While isolated segments of human thoracic duct (TD) have spontaneous and evoked pump-like contractile activity (Telinius *et al.* 2010) the anatomical architecture of this tissue is poorly understood. Given the increasing evidence of ICLC populations in many smooth muscle tissues we sought to establish the presence of ICLC in the TD while defining the histology and ultrastructure of this large lymphatic vessel.

Thoracic duct tissue was obtained from the mid-thoracic region from >40 adult patients (average age of approximately 60 years; predominantly male) undergoing surgery due to esophageal and cardia cancer at the Department of Cardiothoracic and Vascular Surgery, Aarhus University Hospital, Skejby. The TD is prophylactically ligated and resected from the mid-thoracic region during the surgery; thus the removal of TD tissue does not represent an additional procedure for the patient. The protocol was reviewed and approved by the ethical committee for Region Midtjylland Denmark and the study conducted in accordance with the principles of the World Medical Association Declaration of Helsinki. All patients received information about the study and participated with informed consent.

Using conventional histology and immunohistochemistry of thin, fixed preparations and immunofluorescence of whole-mount fixed preparations by confocal microscopy, we investigated the morphology of the TD from the mid-thorax region and the presence and localisation of ICLC protein markers (c-kit, CD34, and vimentin). Transmission electron microscopy was employed to investigate ultrastructure. ICLC were also investigated in live tissue preparations using methylene blue staining as well as with Ca^{2+} -dependent fluorophores and confocal fluorescence microscopy.

Methylene blue stained bipolar and stellate shaped cells in the media of the TD. Immunoreactivity for the ICLC protein markers c-kit, CD34 and vimentin in thin sections and whole-mount tissue localised to cells and processes associated with SMC in the media and subendothelium. Loading and imaging of the thoracic duct with Ca^{2+} -dependent fluorophores provided a similar morphological picture as observed with fixed tissues.

SMC bundles were observed in multiple orientations; and in association with these bundles, brightly fluorescent small cell bodies and processes were observed. Electron microscopy, the gold standard for identifying ICLC, unequivocally demonstrated the presence of ICLC in the human TD. ICLC with stellate or bipolar appearance were found throughout the TD wall: localised in the subendothelial region as well as in intimate association with SMC throughout the media where they were seen to envelop SMC bundles with their processes. ICLC connected to SMC in the human TD via peg-and-socket junctions and close appositions. While other types of interstitial cells were observed at the EM level, ICLC were predominant and clearly distinguishable at the ultrastructural level by the presence of caveolae, dense bands and a patchy basal lamina. During our initial functional experiments with Ca^{2+} -dependent fluorescence and confocal microscopy, we have observed global Ca^{2+} events in cells with morphology and localisation suggestive of ICLC in association with spontaneous contractions, potassium-induced depolarisation, and mechanical stretch. Our data demonstrate a novel population of ICLC throughout the human TD from the mid-thorax region. This finding is of particular relevance for future physiological investigations of the pumping activity in human lymphatics as ICLC may represent the initiator or coordinator of this activity.

McCloskey, K.D., et al. 2002. Kit-like immunopositive cells in sheep mesenteric lymphatic vessels. *Cell Tissue Res*, 310:77-84.

Telinus, N., et al. 2010. Human thoracic duct in vitro: diameter-tension properties, spontaneous and evoked contractile activity. *Am.J.Physiol Heart Circ.Physiol*, 299:811-818.

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SA96

Lymphatic muscle and endothelium: function in adulthood and elderly

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Effective lymph flow is crucial for fluid and macromolecules homeostasis, fat absorption, and immunity. Studies of the last decade significantly advanced the understanding of the basic mechanisms controlling lymph flow, and therefore all processes indicated above. The major units of lymphatic vessels, lymphangions, pump lymph in self-regulatory mode through constant adjustment of their contractions to the complicated combinations of lymph pressures and flows. Lymphatic vessels provide the energy of contractions of their muscle cells to support an effective unidirectional lymph flow. Muscle cells are able to adjust their contractile characteristics depending on variable levels of wall stretch, which determines by preload and afterload of lymphangions. Lymphatic endothelial cell are tightly involved into functional adaptation of the contracting lymphangions through wall shear-stress dependent mechanisms. At moderate levels of the wall shear stress the lymphatic endothelium generates phasic spikes of NO release, supporting the most energy-sufficient mode of the lymphatic pumping. At high levels of steady lymph flow, which reflect higher levels of lymph formation, lymphatic relaxation diminishes resistance to passive lymph flow. Regional variability of the regulatory mechanisms of lymph flow relates to the existence of the additional, not completely discovered regulatory path-

ways involved into stretch- and flow-dependent modulation of lymphatic contractility. Aging alters all of these functions, however even status of contractility and flow in aged lymph vessels is not well characterized. The role of the oxidative stress as risk factor for aging of lymph vessels are unknown.

9-mo and 24-mo old Fischer-344 rats were used. Specimens of isolated mesenteric vessels (MLV) were dissected from animal underwent anesthesia by Sodium Pentobarbital 50 mg/kg weight I.P., at the end of dissections animals were humanely euthanized by injecting Sodium Pentobarbital 100 mg/kg body weight IP. Diameters/pump indices of isolated/pressurized segments of the mesenteric lymph vessels were determined. High-speed cell microscopic video monitoring was used to calculate lymphatic diameters, contraction frequency, flow velocity, and wall shear stress in vivo. Application of the non-selective nitric oxide synthase inhibitor - L-NAME in 100 μM was implemented. Protein expression of three isoforms of superoxide dismutase (SOD) and 3-nitrotyrosine has been determined, their immunohistochemical labeling has been performed. The SOD activity and levels of lipid peroxidation were evaluated. Live vessel fluorescent imaging with dihydroethedine for whole MLV, and with Mitotracker Red – for mitochondria, allowed to compare the levels of superoxide anions in adult and aged MLV.

We found weakening of the lymphatic pump in isolated aged MLV including diminished tone, contraction amplitude, and frequency with corresponding decrease in their minute productivity. Application of L-NAME is able to enhance contractility of aged MLV. Data demonstrate diminished ability of the aged MLV to resist to oxidative stress: decreased SOD activity referred to diminished expression of its Cu/Zn isoform. High levels of superoxide anions and increased levels of lipid peroxidation confirmed in aged MLV.

Cumulatively, our findings support the idea that in aged tissues surrounding the aged MLV, the additional source of some yet unlinked lymphatic contraction-stimulatory metabolites is counterbalanced or blocked by NO release. The aging-associated increases in oxidative stress and oxidative damage is indeed present in the walls of MLV and may contribute to the aging-associated lymphatic pump dysfunction.

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Integration of pacemaker activity and motor neurotransmission in visceral smooth muscles

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Control of motor patterns in visceral organs comes from multiple layers of regulatory mechanisms that start with the properties of smooth muscle cells that permit contractile behaviour or precondition cells for contraction or relaxation when other stimuli are overlaid. Like other motor systems in the body, visceral organs including the gastrointestinal (GI) tract are controlled by a variety of neural and non-neural factors. For example, spontaneous electrical activity, intrinsic to many

smooth muscle tissues, is generated by a specialized population of cells called interstitial cells of Cajal (ICC) that are electrically coupled to the smooth muscle cells. In addition to this level of control, inputs from the enteric nervous system (ENS), hormonal influences, and paracrine factors regulate motor activity during normal physiological responses. Inflammatory mediators contribute additionally during the course of pathophysiological conditions. Smooth muscle cells integrate the inputs from all levels of control and respond in normal individuals with appropriate contractile responses.

In the GI tract ICC, usually located along the intermuscular plane between the circular and longitudinal muscle layers, generates pacemaker potentials that are conducted passively into the adjacent muscle layers via gap junctions where they produce rhythmic membrane potential changes that regulate phasic contractions (1). The mechanical activity of GI smooth muscle cells, can be altered by autonomic, or enteric, nerves innervating them. Previously it was thought that neuroeffector transmission occurred simply because neurally released transmitters acted directly on smooth muscle cells. However, in several, regions of the GI tract, it appears that nerve terminals, rather than communicating directly with smooth muscle cells, preferentially form synapses with ICC and these relay information to neighbouring smooth muscle cells (2). Thus a set of ICC, which are distributed amongst the smooth muscle cells of the gut, are the targets of neurotransmitters released by intrinsic enteric excitatory and inhibitory nerve terminals. In some regions of the GI tract, the same set of ICC also augment the waves of depolarisation generated by pacemaker ICC. Similarly in the urethra, ICC, distributed amongst the smooth muscle cells, generate rhythmic activity and also appear to be the targets of autonomic nerve terminals (3).

A second interstitial cell, recently identified based on the expression of platelet-derived growth factor receptor- α (PDGFR α), which previously referred to as "fibroblast-like" were observed in close association with enteric nerve fibers (4) PDGFR α + cells express SK3 potassium channels which are sensitive to the bee venom apamin. Activation of inhibitory motor nerves produces a post-junctional response that is mediated through purines acting on P2Y1R receptors and is inhibited by apamin. Further, isolated PDGFR α + cells, unlike smooth muscle cells, produce robust outward currents in response to purines which were blocked by apamin and selective P2Y1R antagonists (5). It is therefore likely that PDGFR α + cells generate inhibitory post-junctional responses and like ICC contribute to neuroeffector motor neurotransmission in GI muscles. There is also a significant body of functional immunohistochemical evidence that reveals ICC and now a second population of interstitial cells that are PDGFR α +, are transducers and integrators of motor neurotransmitter signals. Thus, neuromuscular regulation of GI muscles is likely to include multiple modes of neurotransmission (volume and synaptic) and several cell types may receive and transduce inputs from motoneurons.

In summary, motor activity and inputs from excitatory/inhibitory motor nerves in visceral smooth muscles is fundamental to normal organ function. An understanding how this motor activity is properly coordinated by inputs from pacemaker ICC and by intramuscular interstitial cells (ICC and PDGFR α + cells) that act as intermediaries in motor transmission is critical for a complete understanding of how visceral organs perform their functions in health, before we attempt to unravel the changes that occur in the pathophysiological states of disease.

Sanders KM (1996). *Gastroenterology* 111:492-515.

Beckett EA, Takeda Y, Yanase H, Sanders KM, Ward SM (2005). *J Comp Neurol* 493:193-206.

Thornbury KD, Hollywood MA, McHale NG, Sergeant GP (2011). *Acta Gastroenterol Belg* 74: 536-542.

Iino S, Horiguchi K, Horiguchi S, Nojyo Y (2009). *Histochem Cell Biol* 131:691-702.

Kurahashi M, Zheng H, Dwyer L, Ward SM, Koh SD, Sanders KM (2011). *J Physiol* 589: 697-710.

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Role of Kv7 channels in β -adrenoceptor mediated relaxation

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Voltage-gated potassium channels encoded by KCNQ 1-5 (termed Kv7.1 – 7.5) have been identified as crucial regulators of neuronal membrane potential and cardiac action potential. However, Kv7 channels are not the preserve of these two cell types as extensive recent studies have revealed Kv7 channels especially Kv7.4 and Kv7.5 promote vascular smooth muscle dilatation and relax non-vascular smooth muscles such as uterus, colon or bladder. These activities are consistent with activation of low threshold potassium channels leading to stabilization of a negative membrane potential. This lecture will provide an overview of this emergent area of research. In addition it will highlight more recent discoveries that Kv7 channel function is impaired considerably in cardiovascular disease and that activation of Kv7.4 underlies vasorelaxant responses to agonists of β -adrenoceptors and other cAMP linked receptors (eg CGRP). The combination of these two findings provides an explanation for the generalized reduction in vasodilatory effects in hypertension. These findings consolidate the view that Kv7 channels may be master regulators of vascular smooth muscle contractility. They are also likely to be as important in receptor-mediated regulation of visceral smooth muscle although the existence of multiple cell types in visceral tissues makes the situation more complicated.

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The bladder wall: multiple cellular components and their complex interactions

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The wall of the urinary bladder comprises a complex cellular structure whose complement of specific cell types and physiological cellular interactions have been shown in recent years to be more extensive than previously understood. Classically,

the urothelium was considered as a passive barrier providing an important separation of urine and bladder smooth muscle and nerves. Yet, the urothelial cells are now known to participate in bladder function by sensing and responding to changes in the environment such as pH, temperature and stretch by releasing signalling molecules including acetylcholine and ATP (1). Much attention has been given to the role of afferent nerves and how they interact with urothelial cells, interstitial cells and smooth muscle (2). In the last decade, much work on the novel interstitial cells which occupy the sub-urothelial lamina propria layer (IC-LP) and the detrusor layer (intramuscular IC-IM and interbundle IC-IB) has provided new avenues of research into normal and dysfunctional bladder physiology (3). The dual functions of bladder filling and emptying require coordination between the activities of smooth muscle, nerves, interstitial cells and the urothelium. During filling, relaxation of detrusor smooth muscle is important to enable urine storage at low intravesical pressures. Yet, low-level spontaneous, myogenic activity occurs during filling in detrusor smooth muscle which is considered to maintain the bladder wall in the optimal shape for efficient emptying. This activity appears to occur in discrete areas of the bladder wall which remain uncoordinated during filling. When a conscious decision has been made to void, the smooth muscle must then contract in a highly coordinated fashion and this is under parasympathetic neuronal control. Recent insights on the role of the mucosal layer on spontaneous activity will be presented with particular reference to interstitial cells. It has been shown that the urothelium provides inhibitory signalling to detrusor smooth muscle through urothelium derived inhibitory factor (4). Conversely others have shown that the urothelium and potentially other sub-urothelial components enhance detrusor contractility (5). Work from our laboratory on animal models of neurogenic dysfunctional bladder including spinal cord injured (SCI, 6) and denervated bladders indicates different physiological roles for IC-LP and detrusor IC. In chronic SCI, loss of IC-LP and detrusor IC was associated with a hypercompliant phenotype and long-duration spontaneous contractions. In denervated bladders, maintenance of IC-LP but loss of detrusor IC was associated with high frequency, increased amplitude spontaneous contractions, indicative of an overactive phenotype. These observations suggest that (a) IC-LP may have a pacemaker-type function, modulating or driving smooth muscle spontaneous activity and (b) detrusor IC may have an inhibitory function, limiting spontaneous contractions of the detrusor during filling. Physiological evidence in support of these hypotheses include the findings that IC-LP exhibit spontaneous depolarizing calcium activated chloride currents (7), not found in detrusor IC which display hyperpolarizing spontaneous transient outward BK currents (RMJ Cunningham and KD McCloskey, unpublished observations).

The current state of knowledge in bladder basic research will be discussed with significant gaps in our knowledge highlighted and key areas of future research suggested.

Birder LA. (2011) *Handb Exp Pharmacol.* 202:207-31.

Kanai A, Andersson KE. (2010) *J Urol.* 183(4):1288-95.

McCloskey KD. (2011) *Handb Exp Pharmacol.* 202:233-54.

Hawthorn MH et al, (2000) *Br J Pharmacol.* Feb;129:416-9.

Sui GP et al, (2008) *Am J Physiol Renal Physiol.* 295:F688-97.

Johnston L et al, (2012) *J Cell Mol Med.* doi: 10.1111/j.1582-4934.2011.01410.x.

Wu C, Sui GP, Fry CH. (2004) *J Physiol.* 559:231-43.

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SA100

Gasotransmitters: Insights from comparative physiology

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The ability of non-respiratory gases, such as nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H₂S) to function as signaling moieties (gasotransmitters) has only recently been appreciated, due in part to their ephemeral existence in biological tissues. These gases dominated the prebiotic Earth and were major contributors to the origin of life and eukaryotes, but they disappeared as ambient oxygen levels rose and their importance in metabolism declined. Yet cells retained many of the metabolic pathways and now use them for signaling. This lecture provides an overview of these gases from the perspective of Comparative Physiology.

NO is synthesized from L-arginine by nitric oxide synthase (NOS) which is present in all metazoans. Three NOS isoforms have been identified in mammals, neuronal (nNOS), inducible (iNOS) and endothelial (eNOS). Both nNOS and iNOS isoforms have been identified in fish and amphibians, whereas there is no evidence for eNOS in either vertebrate. Furthermore, fish and amphibian endothelial cells lack both nNOS and iNOS and are incapable of NO synthesis. Perivascular nitrergic nerves expressing nNOS are found in many arteries and veins of both teleost (bony) fish and amphibians suggesting that they may contribute to tissue perfusion. NO may also be generated by hemoglobin (Hb)- or myoglobin (Mb)-catalyzed nitrite (NO₂⁻) reduction, although the physiological importance of this in aquatic animals, where ambient nitrate and nitrite concentrations may be elevated and highly variable, remains to be determined. NO is vasodilatory in most vertebrates, although vasoconstrictory responses have been reported in a few primitive fish. NO is a positive inotrope in many fish hearts. NO activation of guanylyl cyclase has been shown in all vertebrates, whereas the effects of NO on ion channels remains to be examined in fish. NOS isoforms and mechanisms of action in reptiles and birds appear to be generally similar to those reported in mammals. CO is synthesized from heme by membrane-bound heme oxygenases (HO). Three isoforms have been identified in mammals, HO-1, HO-2 and HO-3. HO-1 is inducible and stimulated by various chemical and physiological stressors. HO-2 is constitutive and activated by stimuli such as glucocorticoids. HO-3, may be a splice variant of HO-2; its function is unclear. Stress-induced HO-1 transcription has been shown in fish and birds suggesting it is well conserved in vertebrates. Hypoxia stimulates HO-1 expression in the hypoxia-tolerant goldfish. CO (presumably from HO-2) dilates fish vessels, and it has been implicated in a variety of neuronal signaling mechanisms in amphibians, reptiles and birds.

Cysteine is generally thought to be the source of most tissues H₂S production via cytosolic enzymes cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) or the sequential activity of mitochondrial cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfur transferase (3-MST). CBS and CSE produce H₂S outright, whereas release of H₂S from 3-mercaptopyruvate requires endogenous reducing disulfides such as thioredoxin (Trx) or dihydrolipoic acid (DHLA). H₂S may also be "recovered" from thiosulfate (S₂O₃²⁻) during hypoxia. Although H₂S biosynthesis appears ubiquitous in vertebrate tissues, there is considerable controversy surrounding H₂S concentrations in tissues and blood. Most recent measurements suggest that H₂S does not exist in physiologically

significant concentrations in blood or tissues under normoxic conditions and that it most likely functions as an autocrine or paracrine signal. Vascular responses to exogenous H_2S are variable as it may constrict, dilate or produce multi-phasic responses. The vasoactive effects of H_2S and the inverse relationship between H_2S and O_2 concentration in tissues has led to the hypothesis that H_2S is the enigmatic O_2 "sensor" that directly couples tissue PO_2 to a variety of appropriate physiological responses. In the cardiovascular system this enables pulmonary and systemic blood vessels to directly match perfusion to either ventilation or metabolism, respectively, and it appears to be a mechanism for chemoreceptor transduction of blood and environmental PO_2 . This O_2 -sensing mechanism consists of a simple balance between H_2S production in the cytoplasm and its PO_2 -dependent oxidation by the mitochondria. Additional evidence for H_2S -mediated O_2 sensing in non-vascular smooth muscle is found in the gastrointestinal and urinary tracts.

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SA101

Interplay of CO and H_2S in O_2 sensing by the carotid body

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The chemosensing glomus cells of the rat carotid body express two O_2 -sensitive K^+ channels, BK_{Ca} channels and a TASK-like leak K^+ channel. Inhibition of these channels by hypoxia causes cell depolarization, voltage-gated Ca^{2+} influx and neurotransmitter release. Although the identity of the upstream O_2 sensor is still controversial, that the activity of BK_{Ca} is regulated not only by O_2 but also by the gaseous compounds CO and H_2S , suggests that integration of these signals may be crucial to the physiological response of the tissue. Thus, the ability of BK_{Ca} to respond to lowered O_2 is enhanced by its co-localization with hemeoxygenase-2, an enzyme which generates CO in the presence of O_2 . Since CO is a potent BK_{Ca} channel opener, its reduced concentration during O_2 deprivation results in channel closure, thus conferring a degree of O_2 -sensitivity to the BK_{Ca} channel. Conversely, H_2S is a potent BK_{Ca} channel inhibitor. H_2S is produced endogenously by cystathionine- β -synthase and cystathionine- γ -lyase (both being expressed in rat carotid body), and its intracellular concentration is dependent upon the balance between its enzymatic generation and its mitochondrial breakdown. During hypoxia, mitochondrial oxidation of H_2S in many tissues is reduced, leading to hypoxia-evoked rises in its concentration. This may be sufficient to inhibit BK_{Ca} channels and lead to carotid body excitation. Consequently, it appears possible that carotid body function may be heavily dependent upon regulated production/breakdown of two gases which have been classically thought of by their toxic properties, suggesting that the integration of signals from these newly emerging gasotransmitters may refine, or even define, responses of this tissue to hypoxia.

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SA102

Carbon monoxide and hydrogen sulphide induce vasodilation by distinct ion channel-mediated signaling mechanisms

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Carbon monoxide (CO) and hydrogen sulphide are a gaseous vasodilators produced in the vascular wall by the enzymes heme oxygenase (HO) and cystathionine γ -lyase, respectively. Elucidating mechanisms by which these gases induce vasodilation improves understanding of physiological control of vascular contractility and could lead to the development of novel therapies for cardiovascular diseases, including hypertension and stroke. I will summarize our recent studies and compare and contrast mechanisms by which CO and H_2S modulate arterial smooth muscle cell ion channel activity to dilate cerebral arterioles.

Our data indicate that HO-2-derived CO activates large-conductance calcium (Ca^{2+}) activated potassium (BKCa) channels by binding to reduced inhibitory heme attached to a conserved heme-binding domain in the channel C-terminus. CO binding to heme elevates BKCa channel apparent Ca^{2+} -sensitivity, leading to enhanced coupling to Ca^{2+} sparks in piglet cerebral arteriole smooth muscle cells. The elevation in coupling increases transient BKCa current frequency and amplitude, leading to membrane hyperpolarization, a reduction in voltage-dependent Ca^{2+} channel activity, a decrease in global intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and vasodilation. Glutamate stimulates CO production by wild-type astrocytes (HO-2 $^{+/+}$), but not by astrocytes of HO-2 knock out (HO-2 $^{-/-}$) mice. Glutamate does not affect BKCa channel activity in smooth muscle cells alone, but activates single BKCa channels and transient BKCa currents in smooth muscle cells that are in contact with astrocytes. HO-2 inhibition or genetic ablation of HO-2 prevents glutamate-induced BKCa channel activation. In brain slices, glutamate activates Ca^{2+} sparks and reduces global $[Ca^{2+}]_i$ in arteriole myocytes, leading to vasodilation, and these effects are blocked by HO-2 inhibition. Brain slice arteriole dilation to glutamate is blocked by astrocyte toxin and BKCa inhibition. Collectively, data indicate that endogenous CO binds to heme on BKCa channels, leading to enhanced coupling to Ca^{2+} sparks in arteriole smooth muscle cells. In the neurovascular unit, glutamate-induced astrocyte-derived CO produces vasodilation to couple blood flow to increased neuronal activity.

In contrast to the relatively selective mechanism of vasodilation activated by CO, our data indicate that H_2S dilates cerebral arterioles by activating both ATP-sensitive potassium (KATP) and BKCa channels in piglet cerebral arteriole smooth muscle cells. Pinacidil, a KATP channel activator, and H_2S activate K^+ currents at physiological steady-state voltages in cerebral arteriole smooth muscle cells. Glibenclamide, a selective KATP channel inhibitor, fully reverses pinacidil-induced K^+ currents, but only partially reverses H_2S -induced K^+ currents. Pinacidil dilates pressurized (40 mmHg) piglet arterioles and glibenclamide fully reverses this effect. Na 2 S also dilates cerebral arterioles, but this is only partially reversed by glibenclamide. Western blotting indicates that cerebral arterioles express Kir6.1 and sulfonylurea receptor 2B (SUR2B) KATP channel subunits. Pinacidil and H_2S -induced vasodilation is smaller in cerebral arterioles of SUR2 null mice than in wild-type controls. Based on these data, we tested the hypothesis that H_2S dilates cerebral arterioles by modulating local and global intra-

cellular Ca^{2+} signals in smooth muscle cells. High-speed confocal imaging revealed that H₂S increases Ca^{2+} spark frequency and decreases global intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) in cerebral arteriole smooth muscle cells. H₂S does not alter Ca^{2+} wave frequency. H₂S increases the frequency of transient BKCa currents, but does not alter the amplitude of these events. In contrast, H₂S does not alter the activity of single KCa channels recorded in the absence of Ca^{2+} sparks. H₂S elevates SR Ca^{2+} load ($[\text{Ca}^{2+}]_{\text{SR}}$), which leads to Ca^{2+} spark activation in arterial smooth muscle cells. H₂S also hyperpolarizes the membrane potential of pressurized arterioles and this is partially reversed by iberiotoxin, a selective BKCa channel blocker. Iberiotoxin and ryanodine, a ryanodine receptor inhibitor, also partially reverse H₂S-induced vasodilation. In summary, these data indicate that H₂S can dilate cerebral arterioles by activating both KATP channels containing SUR2B subunits and by stimulating Ca^{2+} sparks, which activate BKCa channels, in arterial smooth muscle cells. Collectively, these studies provide evidence that astrocyte-derived CO and H₂S, an endothelial-derived gas transmitter, dilate cerebral arterioles through distinct signaling mechanisms that activate ion channels in smooth muscle cells.

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SA103

CO regulation of Na^+ and Ca^{2+} channels

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Our awareness of the range of signalling pathways which can be influenced by CO continues to grow, and in recent years ion channels have become recognised as a major family of effectors for many of the biological actions of this gasotransmitter (Peers, 2011; Wilkinson and Kemp, 2011).

We have reported that CO inhibits L-type Ca^{2+} channels in cardiac myocytes in a splice variant-dependent manner via redox modulation of key cysteine residues in the C-terminal region of the channel protein. Redox modulation involved CO increasing the production of reactive oxygen species (ROS) from mitochondria (Scragg et al., 2008). We suggested at the time that this action may account for the known protective effects of heme oxygenase-1 (HO-1) expression, following myocardial ischemia (Peers and Steele, 2012). However, subsequent studies have revealed that CO can exert pro-arrhythmic effects, and this appears to occur via modulation of cardiac Na^+ channels. Thus, following exposure to CO, the normally rapidly inactivating Na^+ current is reduced in amplitude, but inactivation is dramatically slowed, giving rise to a sustained 'late' Na^+ current. This effect appears to arise due to stimulation of NO formation, and subsequent S-nitrosylation of the channel protein (Nav1.5). As a consequence of this, we often observed early afterdepolarization-like arrhythmic events which can be prevented by ranolazine, an inhibitor of the late Na^+ current. Clearly, the overall effect of CO in the myocardium depends on the net effect of its action at different ion channels, as well as other potential targets.

Most recently we have identified T-type Ca^{2+} channels as a new site of action of CO. Inhibition of T-type channels by CO is only reversed by exposure to a reducing agent such as dithiothreitol, suggesting a ROS-mediated action. However, we have

not been able to inhibit this effect of CO using a range of inhibitors of various intracellular sources of ROS. CO inhibition of T-type channels has implications in a number of physiological and pathological scenarios. Since T-type channels can strongly influence cell proliferation (Lory et al., 2006), we are investigating the effects of CO in proliferation of vascular smooth muscle and in cancer cells. Our preliminary studies suggest that proliferation can be slowed either by known blockers of T-type channels (e.g. mibefradil), induction of HO-1, or by application of CO donors such as CORM-3. Importantly, HO-1 induction or CO donors have no additional effect in the presence of mibefradil. These findings suggest that CO regulation of T-type channels may have clinically useful potential in the treatment of proliferative disorders and, given the numerous roles of T-type channels in the nervous system, there is further potential for exploiting this pathway.

Lory P, et al (2006) *Cell Calcium*, 40, 135-146

Peers C (2011) *Exp Physiol*, 96, 836-839

Peers C and Steele DS (2012) *J Mol Cell Cardiol*, 52, 359-365

Scragg JL et al (2008). *J Biol Chem*, 283, 24412-24419

Wilkinson WJ and Kemp PJ (2011) *J Physiol*, 589, 3055-3062

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SA104

NO and CO regulation of alveolar fluid clearance

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The gaseous mediators nitric oxide (NO) and carbon monoxide (CO) are widely implicated in the lung disease, and have been identified as candidate therapeutic targets and biomarkers of lung disease. Both NO and CO are endogenously produced in mammalian cells, including the epithelial cells lining the lung alveoli. As such, these mediators are well positioned to modulate lung epithelial cell function, including the regulation of alveolar fluid volume, which is undertaken by the concerted action of a series of ion channels, notably, the epithelial sodium channel (ENaC) as well as ion pumps, notably, the sodium/potassium ATPase (Na/K-ATPase). Our recent studies have addressed (i) what are the effects of the gaseous mediators NO and CO in alveolar ion and fluid transport, and (ii) by what mechanism do NO and CO impact alveolar ion transport. Endogenous NO is a key signalling molecule in the lung through the action of nitric oxide synthases on the amino acid arginine. The use of NO donors papaNONOate and deaNONOate demonstrated that NO can rapidly inhibit amiloride-sensitive short-circuit currents across polarised H441 cell monolayers as assessed by Ussing chamber studies. This phenomenon could be neutralised by NO scavengers, was independent of soluble guanylate cyclase signalling such as [1,2,4]oxadiazolo[4,3-]quinoxalin-1-one. Both the sodium channel and sodium pump components of the alveolar epithelial transport systems were targeted by NO. Permeabilisation of the basolateral membrane of H441 cell monolayers, it was evident that NO targeted highly-selective, amiloride-sensitive Na^+ channels in the apical membrane, although was without effect on human $\alpha\beta\text{ENaC}$

expressed in *Xenopus* oocytes, leading to speculation that NO may impact the cellular regulatory mechanisms of ENaC activity in H441 cells. In contrast, apical permeabilisation of H441 cell monolayers revealed that NO also targets the Na/K-ATPase on the basolateral membrane, where NO appeared to drive S-nitrosylation of the α subunit of the Na/K-ATPase in H441 cells, and hence, impair Na/K-ATPase function.

Exogenous CO has been proposed as an inhalative treatment for lung disease, and has been explored – to date – primarily as an anti-inflammatory strategy in experimental animals models. Furthermore, endogenous CO, generated by the action of haemoxygenases, has been proposed as an endogenous modulator of alveolar ion transport. Interestingly, CO, when applied exogenously to isolated, ventilated, and perfused rabbit lungs has been demonstrated to impair alveolar sodium transport, and hence, impede the clearance of alveolar oedema fluid. These studies are supported by data from cell-culture studies, where exposure of polarised monolayers of bronchial epithelial cells or alveolar type II cells either to CO-donor molecules, or to CO gas directly, impairs the sodium transport capacity of the cell monolayer. This has been attributed to perturbed amiloride-sensitive ENaC activity, since CO was able to rapidly decrease the amiloride affinity of ENaC, without impacting (i) soluble guanylate cyclase/cyclic guanosine monophosphate signalling, (ii) ENaC trafficking, or (iii) Na/K-ATPase activity. The precise mechanism(s) by which CO inhibits ENaC activity remains unclear.

These data support a role for NO and CO in regulating alveolar ion transport, and hence, lung fluid balance, and underscore the importance of gaseous mediators in regulation lung epithelial cell function. The ease of local administration of gaseous mediators to the lung underscores their potential usefulness as interventional agents in the management of lung disease.

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SA105

NO regulation of glutamate receptors via S-nitrosylation

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Glutamate is the major excitatory neurotransmitter of the central nervous system. NMDA-type glutamate receptors have been implicated in multiple physiological processes, including neuronal development, plasticity, and long-term potentiation. However, as seen in both acute and chronic neurodegenerative disorders, overstimulation of NMDA receptors can cause excessive Ca^{2+} influx, free radical generation, and abnormal enzymatic activity, and can thus contribute to synaptic injury and neuronal death. Here, we present a mechanism whereby excessive NMDA receptor activity can be preferentially abated during hypoxia and other CNS insults, but less so under physiological conditions. Under ambient air conditions, NO inhibits NMDA receptor activity via S-nitrosylation predominantly by reacting with a critical thiol group on its NR2A subunit at Cys 399 along with two additional cysteine pairs if their disulfide bonds are reduced to free thiol (these additional cysteines are on the NR1 subunit at positions 744 and 798, and on NR2 subunits at cysteine residues 87 and 320). We demonstrate that relative hypoxia enhances this S-nitrosylation reaction of NMDA receptors by a unique mechanism involving an “NO-reactive oxygen sensor motif” whose determinants include

C744 and C798 of the NR1 subunit. Redox reactions involving these two thiol groups sensitize other NMDA receptor sites to S-nitrosylation and consequent receptor inhibition, while their own nitrosylation has little effect on NMDA receptor activity. The crystal structure of the ligand-binding domain of NR1 reveals a flexible disulfide bond (C744-C798), which may account for its susceptibility to reduction and subsequent reaction with NO that is observed with biochemical techniques. These thiols are S-nitrosylated preferentially during increasing hypoxia or other CNS insults, thus preventing excessive activity associated with cytotoxicity while avoiding blockade of physiologically active NMDA receptors.

Takahashi H, Shin Y, Cho S-J, Zago WM, Nakamura T, Gu Z, Ma Y, Furukawa H, Liddington RL, Zhang D, Tong G, Chen H-SV, Lipton SA. Hypoxia enhances S-nitrosylation-mediated NMDA receptor inhibition via a thiol oxygen sensor motif. *Neuron* 2007;53:53-64.

Lei SZ, Pan Z-H, Aggarwal SK, Chen H-SV, Hartman J, Sucher NJ, Lipton SA. Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex. *Neuron* 1992;8:1087-1099.

Lipton SA, Choi Y-B, Pan Z-H, Lei SZ, Chen H-SV, Sucher NJ, Singel DJ, Loscalzo J, Stamler JS. A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature* 1993;364:626-632.

Sullivan JM, Traynelis SF, Chen HSV, Escobar W, Heinemann SF, Lipton SA. Identification of two cysteine residues that are required for redox modulation of the NMDA subtype of glutamate receptor. *Neuron* 1994;13:929-936.

Stamler JS, Toone EJ, Lipton SA, Sucher NJ. (S)NO signals: translocation, regulation and a consensus motif. *Neuron* 1997;18:691-696.

Kim W-K, Choi Y-B, Rayudu PV, Das P, Asaad W, Arnelle DR, Stamler JS, Lipton SA. Attenuation of NMDA receptor activity and neurotoxicity by nitroxyl (NO⁻). *Neuron* 1999;24:461-469.

Choi Y-B, Tennesi L, Le DA, Ortiz J, Bai G, Chen H-SV, Lipton SA. Molecular basis of NMDA receptor-coupled ion channel modulation by S-nitrosylation. *Nature Neurosci* 2000;3:15-21.

Lipton SA, Choi Y-B, Takahashi T, Zhang D, Li W, Godzik A, Bankston LA. Cysteine regulation of protein function – as exemplified by NMDA-receptor modulation. *Trends Neurosci* 2002;25:474-480.

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SA106

Physiological genomics and complex disease traits

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The salt-sensitive Dahl S (SS) rat model and genetically engineered variations of this model have been used to understand the regulation of cardiovascular phenotypes associated with the complex disease of hypertension. Building upon our initial development of the first genomic scale map of determinants of cardiovascular and renal function, molecular genetic technologies including microarray, DNA and RNA sequencing and proteomics have been applied to understand mechanisms that determine blood pressure, salt-sensitivity and renal injury. Studies are based upon the overall concept that a causal gene may impact on one or more intermediate molecular pathways which both diverge and converge to form larger tree-like branches or intermediate physiological pathways ultimately determine blood pressure. We began identifying such pathways related to kidney function by studying whole organ func-

tion and whole tissue gene expression searching for differences in SS and salt-resistant normotensive control strains. Studies then moved from whole tissue analyses (containing many cell types) to a focus on a single kidney cell type, the medullary thick ascending limb of Henle (mTAL). Our physiological studies have confirmed the mTAL as a major site of the excess O₂- and H₂O₂ production which together with reduced bioavailability of NO contributes importantly to the reduced medullary blood flow, sodium retention, hypertension, and renal injury found in the outer medulla of the SS rat. Transcriptome profiles, RNAseq and microRNA sequencing, together with Bayesian model analysis of mTAL epithelial cells, have begun to reveal novel genes, microRNAs and regulatory pathways responsible for altered function and renal injury of SS rats. LC/MS proteomic techniques have identified differentially expressed proteins in mTAL mitochondria which point to deficiencies of mTAL energy production and oxygen utilization in SS rats contributing to salt-induced hypertension and renal medullary oxidative stress. We have recently developed a rat model with a null mutation of p67^{phox} on the SS background using Zinc Finger Nuclease (ZFN) technology and found the p67^{phox} cytosolic subunit of NAD(P)H oxidase in the mTAL contributes importantly to the excess free radical production in this nephron segment. ZFN technology has now enabled us to reveal functional relevance of candidate genes found to be associated with hypertension in human GWAS studies.

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SA107

Polygenic and diabetic cardiomyopathy in the Drosophila model

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We have created numerous models of congenital heart disease and cardiomyopathies using the Drosophila heart as a genetic model system. These include models for long-QT cardiac arrhythmias, for dilated and restrictive cardiac disorders, for haplo-insufficiencies and polygenic interactions of transcription factors, exhibiting pronounced aggravation with age, and involving genetic pathways that are conserved from fly to human. For example, insulin/TOR and SREBP signaling, known to modulate growth and (lipid) metabolism in many organisms, can act autonomously within the myocardium to fine-tune cardiac performance that deteriorates with age or due to a high fat diet. Among the new genetic pathways, we discovered to be critical for establishing adult heart function, involve cardiogenic determinants, such as tinman/Nkx2-5, and microRNAs (miR) to control not only cardiac development but also functional properties of the mature heart. In a genetic modifier screen for mutations that cause cardiac dysfunction in a weak tinman mutant, we identified the Rho-GTPase encoded by Cdc42. We found evidence that tinman/Nkx2-5 regulates Cdc42 function via miR-1 in the adult heart of flies as well as mice. This shows that the Drosophila heart serves as an efficient discovery tool for conserved (polygenic) modulators of heart disease. Cdc42 is also critical for cardiac morphogenesis in the embryo, where it functions to control localized non-muscle myosin accumulation at the leading edge of cardiomyocytes during heart tube assembly and lumen formation.

We recently also developed a Drosophila model for High Fat Diet-induced (HFD) obesity and heart dysfunction associated with excessive cardiac fat accumulation, involving nutrient-sensitive TOR signaling. In a screen for other modifiers of obesity-associated heart dysfunction, we found a role for the fly homolog of PPAR- γ Coactivator-1 genes, PGC-1/Spargel in protecting the heart from HFD-induced cardiomyopathy: Flies with reduced PGC-1/Spargal function show elevated obesity and exasperated heart dysfunction in response to a HFD, whereas overexpression, even restricted to the heart protects from cardiomyopathy phenotypes. Thus, PGC-1 plays an important role in HFD-induced obesity and heart dysfunction.

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SA108

Cardiac remodelling from a fish perspective

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A model system that has the potential to enhance our understanding of cardiac hypertrophy is the response of the fish heart to thermal acclimation. Salmonid fishes (like rainbow trout, *Oncorhynchus mykiss*) are ectothermic and remain active in waters that vary seasonally between 4°C (winter) and 20°C (summer). In response to prolonged cooling there is an increase in relative ventricular mass that is thought to help compensate for the influence of low temperature on contraction and the increased work required to pump cold viscous blood about the body. We exploited this natural physiological hypertrophic system and assessed functional, morphological, and gene/protein expression changes associated with thermal acclimation in trout ventricle. Trout were thermally acclimated to 4, 11 or 18°C for a minimum of 1 month prior to experimentation. Fish were humanely killed in accordance with UK legislation and hearts excised and either used for functional studies or to investigate protein and gene expression. We show a significant upregulation ($P < 0.05$, 2-way ANOVA) of a number of pro-hypertrophic markers after cold acclimation including ventricular myosin heavy chain (VMHC), muscle LIM protein (MLP) and smooth muscle light chain (SMC2) and regulator of calcineurin (RCAN), a marker of pro-hypertrophic NFAT signalling. These gene expression changes are associated with significant ($P < 0.05$, 2-way ANOVA) cold-induced increases in collagen and decreases in elastin, that correlate well with functional changes in ventricular compliance. We also show cold induced remodelling of conduction pathways with associated changes in gap junction mRNA level changes. These results suggest the trout heart is a good model for mammalian hypertrophy. Because the hypertrophic response in fish is transient (seasonal) we believe this system can provide insight into pathways for hypertrophic recovery in mammals.

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SA109

Phenotyping of genetically engineered rodent: An underexploited research tool?

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Genetically engineered rodent models have become an essential tool for understanding of gene functions in the whole organism, for unraveling disease etiology and pathogenesis, and for preclinical testing of diagnostic, preventive and therapeutic approaches. Clinically-relevant successful animal models are expected to have properties common with human disease, such as pathological features, molecular mechanisms, course of disease progression, competent immune system, and involvement of identical cell lineages and tissues. Accurate identification and validation of such properties requires comprehensive phenotyping, which, to a large extent, is based on extensive knowledge of pathology, a discipline that studies causes and mechanisms of a disease. Pathologists are medical professionals who are trained in the integration of clinical presentation of disease with associated structural and functional changes in the whole organism, as well as in its component organs, tissues, cells and molecules. Unfortunately, the predominant majority of animal modelers are insufficiently prepared for interactions with pathologists. In fact, few principal investigators and members of their laboratories have adequate understanding of principles and methods of pathology, and some even do not recognize the key role of a pathologist in comprehensive animal phenotyping. Another major limiting factor in efficient validation of murine models for human disease has been inadequate number of human and veterinary pathologists sufficiently trained in rodent pathology. Further complicating situation, some diagnostic pathologists do not have appropriate knowledge of contemporary biomedical research, thereby endangering the efficacy of their communications with investigators. During recent decade these shortcomings have begun to attract increasing attention, and some solutions, such as workshops and courses, have been developed. However, the situation remains far from optimal and requires additional measures, such as inclusion of basic histology and pathology subjects into graduate school curricula, focused facilitation of human and veterinary pathologists' training in murine pathology, and active promotion of authentic collaborations between animal modelers and diagnostic pathologists. Success of such activities should ensure that comprehensive phenotyping of genetically engineered rodents will become truly efficient research tool entirely satisfying the needs of rapidly developing biomedical research.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

SA110

New insights from animal models into the central molecular mechanisms that determine salt-resistance vs. salt-sensitivity

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Fluid and electrolyte homeostasis is integral to blood pressure regulation. However, the central molecular mechanisms regulating the neural control of sodium excretion remain unclear. We have previously demonstrated brain $G\alpha i2$ -subunit protein gated-pathways mediate the natriuretic response evoked by α_2 -adrenoceptor activation in-vivo. Consequently, we examined the role of brain $G\alpha i2$ -proteins in the neural mechanisms facilitating fluid and electrolyte homeostasis in response to an acute i.v. 5% bodyweight volume expansion (VE) in conscious Sprague-Dawley (SD) rats (N=6/group). Compared to a control scrambled (SCR) oligodeoxynucleotide (ODN), $G\alpha i2$ ODN mediated down-regulation of brain $G\alpha i2$ proteins abolished the renal sympathoinhibitory response (peak Δ RSNA [% control] SCR 47 ± 5 vs. $G\alpha i2$ 94 ± 8 , $P < 0.05$) and attenuated the natriuresis (peak Δ UNaV [μ eq/ μ l] SCR 45 ± 6 vs. $G\alpha i2$ 25 ± 4 , $P < 0.05$) to an i.v. VE. Confirming the role of brain $G\alpha i2$ proteins in the regulation of RSNA $G\alpha i2$ ODN pre-treatment failed to alter the natriuretic response to an i.v. VE in bilaterally renal denervated (RDNX) rats. Extending these studies beyond an acute experimental paradigm we have examined the role(s) of central $G\alpha i2$ proteins in fluid & electrolyte homeostasis and blood pressure regulation following elevated dietary salt-intake - which is known to evoke salt-sensitive hypertension in the Dahl salt-sensitive (DSS) rat. In response to a 21-day high salt (HS) challenge (8% NaCl diet) to fluid & electrolyte homeostasis we observed a site specific increase in hypothalamic paraventricular nucleus (PVN) $G\alpha i2$ protein levels in "salt-resistant" SD and Dahl Salt-Resistant rats (5.8 fold, and 7.6 fold respectively, $P < 0.05$) that was not observed in DSS rats (N=6/group). In SD rats ODN-mediated prevention of $G\alpha i2$ up-regulation in the brain caused renal nerve-dependent sodium & water retention (24h Na⁺ balance [meq] SCR + HS 0.3 ± 0.1 , $G\alpha i2$ + HS $2.9 \pm 0.3^*$, $G\alpha i2$ RDNX + HS 0.8 ± 0.4), global sympathoexcitation (plasma NE [nmol/L] SCR + HS 49 ± 5 , $G\alpha i2$ + HS $98 \pm 8^*$, $G\alpha i2$ RDNX + HS 73 ± 5) and salt-sensitive hypertension (MAP [mmHg] SCR + HS 128 ± 3 , $G\alpha i2$ + HS $147 \pm 3^*$, $G\alpha i2$ RDNX + HS 132 ± 2) (N=6/group). In these animals we observed no difference in the parasympathetic or sympathetic control of heart rate following i.v. atropine (1mg/kg) or propranolol (1mg/kg) administration (N=6/group). Ganglionic blockade with hexamethonium (30mg/kg i.v.) resulted in a significantly greater reduction in blood pressure in $G\alpha i2$ -ODN infused rats faced with a high salt challenge indicating elevated sympathetic nervous system activity contributes to the hypertension observed in this group (N=6/group). In DSS rats chronic central $G\alpha i2$ protein down-regulation significantly exacerbated the magnitude of hypertension (MAP [mmHg] SCR + HS 164 ± 3 vs. $G\alpha i2$ + HS $187 \pm 3^*$), sodium retention and global sympathoexcitation (plasma NE [nmol/L] SCR + HS 88 ± 8 vs. $G\alpha i2$ + HS $115 \pm 9^*$) in response to 21-days of HS intake (N=6/group). ODN-mediated PVN specific $G\alpha i2$ down-regulation, but not i.c.v. miss-injection or PVN SCR ODN pre-treatment, caused sodium retention, global sympathoexcitation (plasma norepinephrine [nmol/L] i.c.v. $G\alpha i2$ + HS 20 ± 4 , PVN $G\alpha i2$ + HS $75 \pm 9\tau$) and hypertension (MAP [mmHg] i.c.v. $G\alpha i2$ + HS 127 ± 3 , PVN $G\alpha i2$ + HS $143 \pm 4\tau$) in

Sprague-Dawley rats maintained on a high salt intake for 7-days (N=6/group). Owing to the location of the *Gai2* gene on chromosome 8, which is implicated in DSS rat hypertension, we believe the *Gai2* gene is a new target for rat genomic models investigating the pathophysiology of hypertension. Therefore, from multiple animal models we conclude that PVN *Gai2* protein-gated pathways represent a central molecular pathway acting to regulate renal nerve-dependent sodium excretion to facilitate sodium homeostasis and maintenance of a salt-resistant phenotype. Owing to a report linking SNP's in the *GNAI2* gene to hypertension in human subjects we speculate that our findings may have wider significance in relation to the long-term regulation of blood pressure and fluid and electrolyte homeostasis. R01HL107330. **p*<0.05 vs. SCR + HS, *tp*<0.05 vs. i.c.v. *Gai2* + HS.

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SA111

Use of different experimental hypertension models to decipher the intratubular renin-angiotensin system

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Studies using various models of experimental hypertension have shown that subtle increases in circulating angiotensin II (Ang II) cause an augmentation of the intrarenal/intratubular renin-angiotensin system (RAS) which contributes to enhanced sodium reabsorption in proximal and distal nephron segments and the progressive development of hypertension and tissue injury. These models exhibit increases in proximal tubule angiotensinogen mRNA and protein, angiotensin converting enzyme (ACE), renin/prorenin in principal cells, and prorenin receptor (PRR) in intercalated cells of collecting ducts (CD). The intrarenal RAS activation leads to increased excretion of angiotensinogen, as well as renin and Ang II, in the urine. The chronic Ang II infused models in rats and mice have been used to show dissociation between plasma and juxtaglomerular (JG) cell renin which are suppressed, and the CD renin which is increased and has been associated with enhanced distal tubular Ang II levels and sodium reabsorption. In this particular model, principal cells of the CD secrete renin into the urine, which contribute to augment intratubular generation of Ang II in the distal nephron segments. Because plasma and JG renin are markedly suppressed, their contribution to the increased urinary renin is unlikely. Likewise, chronic Ang II infusions stimulate angiotensinogen mRNA and protein which is then secreted into the tubular lumen by the proximal tubule cells and spilled into the distal nephron segments providing substrate for Ang I formation by renin. Augmentation of CD renin secretion along with the upregulation of PRR in CD intercalated cells enhances renin/prorenin enzymatic activity. Additionally, the soluble form of PRR (sPRR) is secreted into the lumen contributing further to angiotensinogen cleavage thus making more substrate available for Ang II formation. The 2-kidney 1-clip hypertensive model has been used to discriminate between the effects of the increases in renal perfusion pressure and high intrarenal Ang II content on the stimulation of renin and ACE in the CD. In this model, both the clipped kidney and the unclipped kidney, which is subjected to the increased arterial pressure, show an increase in renin expression in CD segments indicating that the prevailing blood pres-

sure is not directly regulating CD renin/prorenin. This model has also been important to demonstrate that enhancement of CD renin in both the clipped kidney and the unclipped kidney is associated with reciprocal changes in Ang II and Ang 1-7 content and ACE and ACE2 activities. The Ang II infused rats and the 2-kidney 1-clip hypertensive model have demonstrated that the effects of Ang II on CD renin upregulation are mainly due to direct activation of the AT1 receptors present in the distal nephron as these are blocked by AT1 receptor antagonists. The effects on CD renin upregulations are independent of sodium reabsorption or mineralocorticoid receptor activity. The Cyp11a1-Ren2 transgenic rat model of inducible ANG II-dependent hypertension has shown that stimulation of increased renin formation of non-renal origin which stimulates circulating Ang II also leads to increased CD renin levels and increased urinary excretion of angiotensinogen, renin and Ang II. These findings are consistent with the ability of elevated circulating ANG II levels, secondary to increased extrarenal renin formation, to stimulate intratubular formation of ANG II. Transgenic mouse models with lack of ACE expression and activity either systemically or within the kidney have been used to demonstrate the importance of intrarenal ACE and intrarenally formed Ang II in the development of Ang II dependent hypertension. The ability of intrarenally formed Ang II to stimulate endogenous intrarenal angiotensinogen expression and the importance of tubular AT1 receptors in the development of hypertension has been shown using mice harboring human angiotensinogen and renin transgenes that are expressed systemically or in the kidney only. Collectively, the data obtained from different experimental models of hypertension have led to the conceptual development of a robust intrarenal/intratubular RAS which, if inappropriately stimulated, contributes to an augmented tubular sodium reabsorption and the progressive development of hypertension and tissue injury.

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SA112

Free fatty acids and their receptors in diabetes

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It has long been appreciated that free fatty acids can serve as metabolic substrates in the pancreatic β -cell and that they are able to regulate the secretory activity of these cells by coordinating the oxidation of relevant fuel molecules within the mitochondria [1]. It has also been proposed that fatty acids can propagate intracellular signalling by, for example, altering the formation of acyl-CoA species within β -cells or by modulating the availability of eicosanoids and/or other metabolites [1,2]. In addition, excessively high concentrations of free fatty acids are known to exert detrimental effects on β -cells in that they cause both a loss of glucose-induced insulin secretion and a subsequent decline in cell viability [3]. As such, it has widely been argued that elevated levels of circulating free fatty acids may contribute to the decline in glucose homeostasis seen in patients with type 2 diabetes.

Until recently, it was considered likely that fatty acids exert the majority of their actions upon entry into the β -cell. However, it is now clear that they can also influence cell function by acting more directly from the extracellular environment.

This is because β -cells express various members of a family of G-protein coupled receptors that can bind fatty acids in the extracellular space and which transmit signals to the intracellular environment [4]. These molecules were originally identified as orphan receptors but it is now established that their endogenous ligands are either free fatty acids per se or, in some cases, fatty acid derivatives. Such receptors are implicated in mediating at least part of the stimulatory actions of free fatty acids on insulin secretion and they have also been proposed as mediators of altered β -cell viability in type 2 diabetes. Among this family of receptors, those members which have received most attention in relation to their ability to control β -cell function are FFA1 (formerly GPR40) and GPR119, although more recent work has also begun to focus on GPR120. Both FFA1 and GPR119 play a role in the early potentiation of glucose-induced insulin secretion by exogenous free fatty acids and each is expressed in the β -cell. By contrast, the role of GPR120 is more controversial and uncertainties remain about the role and expression of this molecule in β -cells.

From a therapeutic perspective, the finding that FFA1 and GPR119 can each enhance glucose-induced insulin secretion, has prompted a range of investigators to develop selective agonists that might be useful as modulators of β -cell secretory activity in vivo. However, it is also possible that such molecules might exert a second beneficial effect by preserving β -cell viability in an analogous manner to that proposed for agonists of a different GPCR, the GLP-1 receptor. In this case, it is well established that addition of GLP-1 leads to a potentiation of glucose-induced insulin secretion but there is also evidence that activation of the GLP-1 receptor may lead to a cytoprotective response in β -cells [5]. Therefore, it is of interest to establish whether a similar process may ensue following activation of relevant free fatty acid receptors in β -cells. To address this issue, we have studied, in more detail, the possibility that GPR119 might mediate a cytoprotective action in β -cells using a putative endogenous ligand for this receptor, oleoylethanolamide (OEA) [6].

Treatment of cultured pancreatic β -cells with exogenous palmitate (C16:0) led to a dose-dependent loss of viability during periods of >24h and this response was attenuated very effectively by co-incubation with various mono-unsaturated fatty acids, including oleate (C18:1). Oleate alone was well-tolerated by the cells. Like oleate, OEA, also failed to exert any obvious cytotoxic actions in rodent β -cells but it dramatically attenuated the loss of viability caused by palmitate and was apparently more potent than oleate. The response to OEA was not reproduced by any of several synthetic agonists of GPR119 and, more surprisingly, it was abrogated by inclusion of inhibitors of fatty acid amide hydrolase (FAAH). Taken together, the data imply that OEA is not, itself, the active cytoprotective species but that it is rapidly converted to free oleate (by the action of FAAH) which then mediates cytoprotection. This, in turn, implies that the cytoprotective response is unlikely to be mediated by GPR119. Indeed, it seems probable that oleate mediates this effect from an intracellular site rather than via a cell surface receptor.

Prentki M & Madiraju SR (2012) *Mol Cell Endocrinol* **353**, 88-100

Luo P & Wang MH (2011) *Prostaglandins Other Lipid Mediat.* **95**, 1-10.

Morgan NG & Dhayal S (2010) *Prostagland. Leukotri. Essent. Fatty Acids* **82**, 231-236.

Kebede MA, Alquier T, Latour MG & Poitout V (2009) *Diabetes Obes. Metab.* **11** Suppl 4:10-20.

Drucker DJ (2003) *Mol Endocrinol.* **17**, 161-171.

Stone VM, Dhayal S, Smith DM, Lenaghan C, Brocklehurst KJ & Morgan NG (2012) *Br J Pharmacol.* **165**, 2758-2770.

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SA113

Regulation and mechanism of action of FFAR1/GPR40

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The G-protein-coupled receptor GPR40 is specifically expressed in pancreatic beta-cells and has been identified as a long-chain fatty acid receptor (1;2). Since fatty acids do not initiate insulin secretion in the absence of glucose but strongly potentiate glucose-stimulated insulin secretion, GPR40 has received considerable interest as a potential therapeutic target for type 2 diabetes. Several pharmaceutical companies have active programs towards that goal, including one recently completed phase II trial (3).

Previous work performed in our laboratory has demonstrated that GPR40 mediates approximately 50% of the effect of fatty acids in potentiating glucose-induced insulin secretion (4). Deletion of GPR40 renders mice more susceptible to high-fat-diet induced diabetes (5), indicating that the receptor is important for beta-cell compensation for insulin resistance and supporting the concept that GPR40 agonists have therapeutic potential in type 2 diabetes.

In a recent study we observed that glucose stimulates GPR40 gene transcription in pancreatic beta-cells via increased binding of pancreas-duodenum homeobox-1 (Pdx-1) to the A-box in the HR2 region of the GPR40 promoter (6). Mutation of the Pdx-1 binding site within the HR2 nearly abolishes glucose activation of GPR40 promoter activity. The stimulation of GPR40 expression and Pdx-1 binding to the HR2 in response to glucose are mimicked by N-acetyl glucosamine, an intermediate of the hexosamine biosynthesis pathway, and involve phosphatidylinositol 3-kinase-dependent O-GlcNAcylation of Pdx-1 in the nucleus. We demonstrated that O-GlcNAc transferase (OGT), the enzyme that modifies proteins by O-GlcNAcylation, interacts with the product of the PI3K reaction, phosphatidylinositol 3,4,5-trisphosphate (PIP3), in the nucleus. This interaction enables OGT to catalyze O-GlcNAcylation of nuclear proteins, including Pdx-1. We concluded from this study that glucose stimulates GPR40 gene expression at the transcriptional level through Pdx-1 binding to the HR2 region and via a signaling cascade that involves an interaction between OGT and PIP3 at the nuclear membrane (6).

Activation of GPR40 by fatty acids does not appear to modulate intracellular fuel metabolism in islets (7), but triggers phospholipase C-mediated hydrolysis of membrane phospholipids downstream of $G_{\alpha q}$. We have recently completed a study aimed to determine the mechanisms of GPR40-dependent potentiation of GSIS by fatty acids (Ferdaoussi M et al., manuscript in preparation). We observed that the fatty acid oleate potentiates the second-phase of glucose-stimulated insulin secretion in perfused islets, and that this effect is largely dependent upon GPR40. Accordingly, oleate induces F-actin remodeling in wild-type but not in GPR40^{-/-} islets. Oleate induces phosphorylation of protein kinase D at residues Ser-744/748 and Ser-916 in wild-type but not GPR40^{-/-} islets. Importantly, oleate potentiation of glucose-induced insulin secretion

tion is lost upon pharmacological inhibition or deletion of *pkd1*. We concluded from this study that the signaling cascade downstream of GPR40 activation by fatty acids involves activation of protein kinase D, F-actin depolymerization, and potentiation of second-phase insulin secretion.

Collectively, these findings provide important mechanistic information on the biology of GPR40, a novel drug target for type 2 diabetes.

Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, Ogi K, Hosoya M, Tanaka Y, Uejima H, Tanaka H, Maruyama M, Satoh R, Okubo S, Kizawa H, Komatsu H, Matsumura F, Noguchi Y, Shinohara T, Hinuma S, Fujisawa Y, Fujino M: Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature* 422:173-176, 2003

Briscoe CP, Tadayyon M, Andrews JL, Benson WG, Chambers JK, Eilert MM, Ellis C, Elshourbagy NA, Goetz AS, Minnick DT, Murdock PR, Sauls HR, Jr., Shabon U, Spinage LD, Strum JC, Szekeres PG, Tan KB, Way JM, Ignar DM, Wilson S, Muir AI: The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *J Biol Chem* 278:11303-11311, 2003

Burant CF, Viswanathan P, Marcinak J, Cao C, Vakilynejad M, Xie B, Leifke E: TAK-875 versus placebo or glimepiride in type 2 diabetes mellitus: a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet*, 2012

Latour MG, Alquier T, Oseid E, Tremblay C, Jetton TL, Luo J, Lin DC, Poitout V: GPR40 is necessary but not sufficient for fatty acid stimulation of insulin secretion in vivo. *Diabetes* 56:1087-1094, 2007

Kebede M, Alquier T, Latour MG, Semache M, Tremblay C, Poitout V: The fatty acid receptor GPR40 plays a role in insulin secretion in vivo after high-fat feeding. *Diabetes* 57:2432-2437, 2008

Kebede M, Ferdaoussi M, Mancini A, Alquier T, Kulkarni RN, Walker MD, Poitout V: Glucose activates free fatty acid receptor 1 gene transcription via phosphatidylinositol-3-kinase-dependent O-GlcNAcylation of pancreas-duodenum homeobox-1. *Proceedings of the National Academy of Sciences of the United States of America* 109:2376-2381, 2012

Alquier T, Peyot ML, Latour MG, Kebede M, Sorensen CM, Gesta S, Ronald Kahn C, Smith RD, Jetton TL, Metz TO, Prentki M, Poitout V: Deletion of GPR40 impairs glucose-induced insulin secretion in vivo in mice without affecting intracellular fuel metabolism in islets. *Diabetes* 58:2607-2615, 2009

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SA114

Complexities of free fatty acid receptor pharmacology

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Three closely related, FFA1-3, and two further, GPR84 and GPR120, G protein-coupled receptors recognise and respond to free fatty acids of varying chain length [1]. FFA2 and FFA3 respond to an overlapping group of short chain fatty acids produced largely by microbial fermentation of non-digestible carbohydrates in the gut. The C2 fatty acid acetate displays a degree of selectivity for human FFA2 over FFA3 and has been used, therefore, to predict specific roles for FFA2. However, this selectivity is not preserved for the rodent orthologues of these receptors, eliminating the usefulness of this ligand as a selective tool in rodent cells and tissues. A series of selective allosteric agonists of FFA2 have been described [2] and although of use, they may activate the receptor in a manner distinct from the endogenous orthosteric agonists. Bovine

FFA2, but not FFA3, displays markedly different pharmacology than human and rodent FFA2, responding to fatty acids of substantially longer chain length. We used combinations of homology modelling and mutagenesis to define the basis for this difference and to engineer a form of human FFA2 with 'bovine-like' pharmacology that also fails to respond to C1-C4 fatty acids. A series of more potent and selective orthosteric FFA2 ligands have also recently become available and these are likely to provide novel insight into the specific roles of this receptor.

FFA1 and GPR120, although only distantly related to each other, respond to a similar group of medium and longer chain free fatty acids [3]. Screening and medicinal chemistry programmes have resulted in the identification of moderately potent and selective FFA1 agonists and antagonists and such an agonist is currently undergoing late stage clinical trials to control hyperglycaemia [4]. Recently, a novel series of markedly selective agonists for GPR120 has also been described. Rapid progress in understanding the basis of fatty acid binding by these various G protein-coupled receptors [5] is resulting in the production and availability of a number of new selective pharmacological tools and these are likely to provide novel insights into the function of the individual receptors.

Stoddart LA, Smith NJ, Milligan G. International Union of Pharmacology. LXXI. Free fatty acid receptors FFA1, -2, and -3: pharmacology and pathophysiological functions. *Pharmacol. Rev.* 60, 405-417(2008).

Smith, N.J., Ward, R.J., Stoddart, L.A., Hudson, B.D., Kostenis, E., Ulven, T., Morris, J.C., Trankle, C., Tikhonova I.G., Adams, D.R. and Milligan, G. (2011) Extracellular loop 2 of the free fatty acid receptor 2 mediates allosterism of a phenylacetamide ago-allosteric modulator. *Mol. Pharmacol.* 80, 163-173.

Hudson, B.D., Smith, N.J. and Milligan, G. (2011) Experimental approaches to targeting poorly characterized GPCRs: Uncovering the therapeutic potential for Free Fatty Acid receptors. *Advances Pharmacol.* 62, 175-217.

Burant CF, Viswanathan P, Marcinak J, Cao C, Vakilynejad M, Xie B, Leifke E: TAK-875 versus placebo or glimepiride in type 2 diabetes mellitus: a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet*. 2012 Feb 24. [Epub ahead of print]

PMID:22374408

Milligan, G., Stoddart, L.A. and Smith, N.J. (2009) Agonism and allosterism: the pharmacology of the free fatty acid receptors FFA2 and FFA3. *Br. J. Pharmacol.* 158, 146-153.

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SA115

Fat detection in the mouth is mediated by GPR40 (FFAR1) and GPR120

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The taste system is made of taste cells clustered in taste buds which are embedded in taste papillae at the surface of the tongue. Taste sensations are elicited when sweeteners, salts, acids, bitter or umami molecules activate specific receptors expressed in taste cells and initiate a signal transduction cascade. The perception of dietary fat is based mainly on texture, viscosity and olfaction, but there is increasing evidence that

the taste system also plays a role. Triglycerides, the main components of oils and other dietary fats are hydrolyzed by the lingual lipase into free fatty acids, which are detected by the gustatory system. GPR40 and GPR120 are G-protein coupled receptors (GPCRs) that respond *in vitro* to medium and long chain fatty acids. We showed by immunohistochemistry and PCR that both GPCRs are expressed in mouse taste cells. GPR120 is expressed in circumvallate, foliate and fungiform papillae, mainly in type II cells. GPR40 is expressed in circumvallate and foliate but very rarely in fungiform papillae, mainly in type I cells. To determine if those GPCRs play a role in fat taste signal transduction, we carried out behavioral and nerve recording studies on GPR40 and GPR120 knockout mice. Compared with control mice, single knockout mice have diminished preference and intake for intralipid, oleic acid and linoleic acid (n=10-15 per group). Whole taste nerve recordings showed a diminished response to lingual application of several fatty acids in the glossopharyngeal and chorda tympani nerves for GPR120 KO mice and only in the glossopharyngeal nerve for GPR40 KO mice compared to wild type mice (n=6-9 per group). Preference tests comparing wild type, single and double KO mice showed that GPR40 is the main GPCR mediating fat preference in mice (n=10 per group).

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ment and signalling mechanisms. Through the actions of PUFAs on membranes, on signalling, and on intracellular sensors they can alter patterns of gene expression and protein production. Thus, the nature of the PUFAs to which cells are exposed influences their function and their ability to respond to signals.

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SA116

Non-GPCR mediated effects of fatty acids

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Omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFAs) are biologically active. The main biologically active n-6 PUFA is arachidonic acid. Most cell membranes contain fairly high amounts of arachidonic acid within different phospholipids. The arachidonic acid is mobilised by phospholipase enzymes upon cellular stimulation and the free arachidonic acid is itself a signalling molecule whilst also acting as the primary substrate for synthesis of the eicosanoid family of lipid mediators. These are produced through various pathways notably those involving cyclooxygenase, lipoxygenase and cytochrome P450 enzymes. The mediators produced have diverse roles in regulating inflammation, immunity, smooth muscle contraction, platelet function, blood clotting etc. The major biologically active n-3 PUFAs are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Both these fatty acids are readily incorporated into cell membranes where they partially replace arachidonic acid. They also inhibit arachidonic acid metabolism so reducing the production of the principal families of eicosanoids. However EPA and DHA are themselves precursors for synthesis of lipid mediators. Eicosanoids produced from EPA are typically biologically weak, while resolvins produced from EPA and DHA and protectins produced from DHA seem to be very potent inflammation resolving agents. PUFAs and PUFA-derived lipid mediators appear to be ligands for peroxisome proliferator activated receptors and through this mechanism may alter lipid metabolism, insulin sensitivity, adipocyte development and inflammation. Incorporating EPA and DHA into cell membranes can disrupt formation of lipid rafts so modulating the early stages of signal transduction. Theoretically a higher PUFA content of cell membranes alters membrane order which may impact on protein move-

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