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.
The science of laughter
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Research into laughter is dwarfed by the scale of work into emotions such as fear and disgust, however it is probably one of the most frequently encountered emotional expressions. In this talk I will outline the physical bases of laughter, and aspects of its evolution. I will describe the disparities between people’s lay understanding of when we laugh, which tend to be focussed on humour with studies which reveal the social foundations of laughter. I will address the neural basis of the perception of laughter and discuss how these studies may relevant to the roles of laughter in interactions.

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

Intestinal absorption of sugars and peptides: from textbook to surprises
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Understanding the mechanisms by which nutrients are absorbed in the gastrointestinal tract has been a subject of research for more than hundred years. In particular the absorption of monosaccharides as the quantitatively most important nutrient class attracted scientists from physiology, gastroenterology and more recently also from molecular biology and genetics. Absorption of short chain peptides in intact form represents the exception to the rule according to which only the monomers of the complex food constituents are absorbed into epithelial cells. With the cloning of the membrane proteins that mediate the influx of nutrients into epithelial cells in the 1990’s a new chapter was opened and numerous wonderful studies since then have been assessing the structure and functions of the proteins. However, there are still some very fundamental aspects that are not resolved. There is for example a controversial debate on whether intestinal glucose absorption at high luminal glucose concentrations involves a second transport system – such as GLUT2 - in addition to the electrogenic Na⁺-dependent transporter SGLT1. I shall be presenting studies in mice lacking SGLT1 or lacking the facilitated glucose transporter GLUT2 to determine whether GLUT2 contributes to overall glucose and/or fructose absorption. The latter is thought to be mediated by GLUT5 in apical membranes and is also thought to be the basis of fructose malabsorption which is not yet defined on a molecular or genetic basis. Most interestingly, as shown in humans, fructose malabsorption can be overcome when simultaneously glucose is provided although the mechanisms underlying this phenomenon are not known.

Intestinal absorption of short chain peptides is mediated by the rheogenic transporter PEPT1. There is no disease associated with a malfunction of PEPT1. However, in inherited diseases of amino acid transport, such as Cystinuria or Hartnup disease, impaired intestinal amino acid absorption was shown to be compensated by uptake of the critical amino acids in peptide form via PEPT1. An interplay of SGLT1, GLUT2 and PEPT1 was proposed based on studies in rats to represent a network by which the intestine absorbs in a coordinated manner large quantities of nutrients that also represent an osmotic challenge. However, using the various transporter-deficient mouse strains we did not find any evidence that glucose and peptide transport processes are interconnected. What came as a surprise is the finding that both SGLT1 and PEPT1 as “classical” transporters have a second role and that is that of a sensor in enteroendocrine cells in the gut allowing luminal sensing for release of gastrointestinal peptide hormones, primarily of GIP and GLP1.

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Engaging students and valuing teachers
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University academic staff are under increasing pressure to deliver high-quality teaching in the face of many other demands on their time, dwindling staff-student ratios, increasing student expectations as a result of the current fees regime, and growing diversity in students’ backgrounds and abilities. There is also an increasing expectation from both professional bodies and undergraduates that medical science – including physiology – teaching in professional programmes such as medicine, dentistry, nursing and veterinary science is embedded in a context that emphasises its clinical relevance and application. This can be difficult for teaching staff who do not have the relevant clinical background and experience. In the first part of my talk I will draw on my experiences from large group lectures, laboratory practicals and project work to describe and evaluate various initiatives aimed at engaging undergraduates in the face of these challenges.

The second part of my talk will focus on teachers, rather than students, in higher education. In 2009-10, two publications 1, 2 highlighted concerns that recognition of achievements in teaching was secondary to recognition of research success in most cases of academic career progression. This imbalance was also acknowledged in the 2011 White Paper on Higher Education in England: “Students at the Heart of the System” 3, which stated that the changes being proposed would “lead to higher education institutions concentrating on high-quality teaching, and staff earning promotion for teaching ability rather than research alone”. I will describe work undertaken by The Physiological Society since 2011, some of which has been in collaboration with the Academy of Medical Sciences, the Heads of University Biosciences and the Society of Biology 4, to determine how much change there has been in this area, and consider what else can be done to ensure that teaching achievements are appropriately recognised, valued and rewarded in career progression in higher education.


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PL4

From trafficking of neuronal voltage-gated calcium channels to neuropathic pain
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Voltage-gated calcium channels (VGCCs) permit depolarisation-dependent Ca\textsuperscript{2+} entry into excitable cells to control many functions, including neurotransmitter release in neurons, and muscle contraction. There are three distinct sub-families of VGCCs, Ca\textsubscript{1.1} - 3. For the Ca\textsubscript{1.1} and Ca\textsubscript{2.2} sub-families, the channels are known to be heteromeric, consisting of an \(\alpha 1\) pore-forming subunit, associated with auxiliary subunits \(\beta\) and \(\alpha_2\delta\). These auxiliary subunits modulate the functional expression and properties of the channels. In my lecture I will describe our studies on neuronal voltage-gated calcium channel trafficking, and relate this to studies of neuropathic pain mechanisms. In particular I will concentrate on the importance of the auxiliary \(\alpha_2\delta\) subunits in Ca\textsubscript{2.2} (N-type) calcium channel trafficking and function (Cassidy et al., 2014), and the key function of the Von Willebrand factor domain in \(\alpha_2\delta\) in this process (Canti et al., 2005; Hoppa et al., 2012; Cassidy et al., 2014).

I will then describe how the \(\alpha_2\delta\) ligands gabapentin and pregabalin, which are of therapeutic use in various neuropathic pain conditions, influence the trafficking of voltage-gated calcium channels. Related to this, I will describe evidence for the role of \(\alpha_2\delta-1\) in the development of neuropathic mechanical hypersensitivity in rodent models of neuropathic pain. The mRNA for \(\alpha_2\delta-1\) is among those that are strongly up-regulated in injured dorsal root ganglion (DRG) neurons, leading to an increase in \(\alpha_2\delta-1\) protein in DRG cell bodies, their axons and their terminals; such injuries lead to a chronic neuropathic pain-state (Bauer et al., 2009). Finally I will describe experiments in which we have characterised \(\alpha_2\delta-1\) knockout mice, and demonstrated that they show a marked delay in the onset of neuropathic hypersensitivity following nerve injury, indicating that \(\alpha_2\delta-1\) is essential to the rapid development of this condition (Patel et al., 2013).


Cassidy JS, Ferron L, Kadurin I, Pratt WS, & Dolphin AC (2014). Functional exofacially tagged \(\alpha\) type calcium channel subunits elucidate the interaction with auxiliary alpha2delta\textsubscript{1} subunits. Proc Natl Acad Sci U S A 111, 8979-8984.


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PL5

Calcium microdomains in cardiac myocytes
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Excitation-contraction coupling in cardiac myocytes is the result of a transient rise in cytosolic calcium. Calcium influx through voltage-dependent calcium channels, opening during the action potential, is the trigger for release of calcium from the intracellular stores, the sarcoplasmic reticulum, which supplies the largest part of the calcium that activates the myofilaments and contraction. The signaling between the L-type calcium channels in the sarclemma, LTCC, and the calcium release channels in the sarcoplasmic reticulum, the ryanodine receptors, RyR, was proposed to be a local process (Stern, 1992). This was based on theoretical grounds and supported by the microarchitecture of LTCC and RyR in the dyadic cleft between sarclemma and sarcoplasmic reticulum, creating a structural environment for microdomains of calcium (Franzini-Armstrong et al., 1999). Modeling of fluxes through LTCC and RyR have suggested local calcium concentrations in these microdomains to be 1 to 3 orders of magnitude larger than in the global cytosolic compartment, where [Ca\textsuperscript{2+}]\textsubscript{i} can be estimated with fluorescent dyes (e.g. (Soeller & Cannell, 1997) (Shannon et al., 2004)).

Because of the restricted size of the dyadic cleft and because of the limitations of the currently available calcium indicators, direct measurements and confirmation of these predictions are not yet available. Nevertheless there is indirect evidence for these high microdomain calcium transients, as well as some more quantitative estimates, using ‘natural’ reporters, i.e. calcium-sensitive ion currents that are located within the same dyadic cleft, the LTCC and the Na/Ca exchanger, NCX (e.g. (Acsai et al., 2011;Tr Trafford et al., 1995)).

Na homeostasis, regulated by voltage-dependent Na channels and the Na/K pump, through NCX, modulates and contributes to the microdomain [Ca\textsuperscript{2+}]\textsubscript{i} (Shattuck et al., 2015) though the quantitative aspects are still under debate, awaiting more direct measurements.

RyRs are not always within the dyad and in cells where the sarclemma has fewer invaginations in the form of T-tubules, many RyR are not facing the sarclemma, such as in atrial cells or in ventricular myocytes of larger mammals. These have been called orphaned (Sham et al., 1995) or non-coupled RyR (Biesmans et al., 2011) and lead to marked loss of synchrony in the global calcium signal for excitation-contraction coupling (Heinzel et al., 2011;Heinzel et al., 2002). Loss of T-tubules
occurs in disease with consequences for alterations in microdomain signaling near RyRs (Dries et al., 2013). The calcium microdomain near RyRs is further populated with signaling molecules such as CaMKII and NADPH-oxidases. Recent advances in high-resolution microscopy are showing more details on the organization of RyR and associated signaling partners, as well as in the T-tubule structure and dyadic cleft. Ongoing studies of the structure-function relation in these calcium microdomain reveal changes with disease that impact on cardiac contractile function as well as arrhythmogenesis.


Heinzel FR, Macquaide N, Biesmans L, & Sipido K (2011). Dyssynchrony of Ca(2+)+ release from the sarcoplasmic reticulum as subcellular mechanism of cardiac contractile dysfunction. *J Mol Cell Cardiol* **50**, 390-400.


*Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.*
Functional oxygen sensitivity of astrocytes
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In terrestrial mammals the oxygen storage capacity of the central nervous system is limited and neuronal function is rapidly impaired if oxygen supply is interrupted even for a short period of time. However, oxygen tension (PO\textsubscript{2}) at the level of the peripheral (arterial) chemoreceptor is not sensitive to regional CNS differences in oxygen demands that reflect variable activity levels or local tissue hypoxia, pointing to the necessity of a functional brain oxygen sensor. Here we show that astrocytes, the most numerous brain glial cells, are highly sensitive to physiological changes in PO\textsubscript{2}. Astrocytes respond to decreases in PO\textsubscript{2} a few mmHg below normal brain oxygenation with elevations in intracellular calcium. The hypoxia sensor of astrocytes resides in the mitochondria where oxygen is consumed. Physiological decrease in PO\textsubscript{2} inhibits astroglial mitochondrial respiration, leading to mitochondrial depolarization, production of free radicals, lipid peroxidation, activation of phospholipase C, IP\textsubscript{3} receptors and recruitment of Ca\textsuperscript{2+} from the intracellular stores. Hypoxia-induced [Ca\textsuperscript{2+}] increases in astrocytes trigger fusion of vesicular compartments containing ATP. Blockade of astrocytic signaling by overexpression of ATP-degrading enzymes or targeted astrocyte-specific expression of tetanus toxin (to interfere with vesicular release mechanisms) within the respiratory rhythm-generating circuits of the brainstem reveals the fundamental physiological role of astroglial oxygen sensitivity - in low oxygen conditions (environmental hypoxia) this mechanism maintains enhanced breathing even in the absence of peripheral chemoreceptor oxygen sensing. These results demonstrate that astrocytes are functionally specialized CNS oxygen sensors tuned for rapid detection of physiological changes in brain oxygenation.

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Regulation of cerebral blood flow by capillary pericytes, in health and disease
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Active neurons control their energy supply by dilating blood vessels to increase local blood supply to active regions. As well as balancing the brain’s energy supply with demand, this “neurovascular coupling” means that changes in blood supply, detected noninvasively in humans using fMRI/BOLD imaging, can be used to indicate regions of increased neuronal activity. Understanding the mechanisms that underlie neurovascular coupling is therefore critical for understanding how brain activity is fuelled and exactly how BOLD changes relate to neuronal activity. Classically, neurovascular coupling was thought to occur when signaling molecules, released from astrocytes and neurons, dilate arteriole smooth muscle cells. More recently, however\textsuperscript{1}, contractile pericytes on capillaries have been found to constrict and dilate so could contribute to neurovascular coupling. Here (and see Ref. 2), I will show that capillaries in brain slices dilate in response to bath-applied glutamate or in response to neuronal activity. This dilation is mediated by prostaglandin E2 and is modulated by 20-HETE and nitric oxide. Capillaries also dilate in vivo, often before arterioles, suggesting that capillary pericytes are the first vascular cells to sense an increase in neuronal activity. The size of the capillary dilation, and the increased resistance of the capillary bed versus other components of the vascular tree\textsuperscript{3}, suggests that capillary dilation produces most of the increase in blood flow that is observed in response to somatosensory stimulation. Finally, pericytes constrict and die following cerebral ischaemia, indicating that pericycle dysfunction may underlie the prolonged hypoperfusion observed following cerebral ischaemia. Protecting pericytes may therefore be a useful therapeutic strategy following stroke.

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Selective astrocytic lesions spare neurons but interfere with reflex signal
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Conjugates of the toxin saporin (SAP) have been widely used to target select neurons while leaving other neurons undisturbed. Using one such conjugate we found that an antibody to dopamine-\beta-hydroxylase conjugated with SAP (antiDBH-SAP) killed catecholamine neurons in the nucleus tractus solitarii (NTS) while sparing other neurons. Bilateral injections of anti-DH-SAP into the NTS led to attenuation of baroreceptor reflexes, lability of arterial pressure, and, in some animals, sudden death. However, targeting the same neurons with 6-hydroxydopamine produced no such cardiovascular events. We hypothesized that the conjugates may target non-neuronal cells in the NTS and found that, indeed, local astrocytes were killed by the conjugates as well as by unconjugated SAP itself. SAP injections into the NTS led to loss of glial fibrillary acidic protein (GFAP). Although there were positive markers for astrocytic death, neuronal structural markers and neuronal biosynthetic enzymes were undisturbed. Our recent studies have further suggested that local neurons are physiologically intact. Nonetheless, SAP injections into the NTS significantly reduced cardiovascular responses elicited by glutamate agonists injected into the NTS. Furthermore, bilateral injections of SAP into the NTS led to attenuation of cardiovascular reflexes whose pathways pass through the NTS, lability of arterial pressure, damage to cardiac myocytes and sudden death resulting from asystole. When asystole and death followed SAP treatment the fatal arrhythmia followed progressive bradycardia. In that treated animals demonstrate altered ventilatory func-
tion, we conjecture that it is altered ventilation that leads to cardiac compromise and death.

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SA005

Metabolic sensing and support of synaptic activity by astrocytes

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Astrocytes participate in brain energy metabolism by supplying substrates to neurons from their glycogen stores and from glycolysis. Glycogen stores are unique to astrocytes in the brain and are concentrated in astrocyte processes surrounding synaptic terminals. We have investigated the molecular pathways responsible for metabolic coupling between neuronal activity and astrocytes in the hippocampus region of the brain. We discovered that a bicarbonate (HCO$_3^-$) sensor, soluble adenylyl cyclase (sAC), is highly expressed in astrocytes and becomes activated in response to HCO$_3^-$ entry via the electrogenic NaHCO$_3$ cotransporter (NBC). Activated sAC increases intracellular cAMP levels, causing glycolysis breakdown, enhanced glycolysis, and the release of lactate into the extracellular space. During periods of low glucose, lactate is subsequently taken up by neurons for use as an energy substrate. This process is recruited over a broad physiological range of [K$^+$]$_{extr}$ and also during aglycemic episodes, helping to maintain synaptic function. The mobilization of glycogen and lactate efflux from astrocytes may also lead to alterations of neurovascular coupling as lactate enhances the efficacy of the astrocyte mediated vasodilation pathway. These data reveal a molecular pathway in astrocytes that is responsible for brain metabolic coupling to neurons. Depletion of the glycogen reservoir in astrocytes may lead to metabolic stress and synaptic impairment when glucose levels or supply are compromised.

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SA006

Ion channels in the lysosome: Opening the gate to the cell’s recycling and nutrient-sensing center

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Ca$^{2+}$ efflux from the lysosome carries the signals needed for precise delivery of hydrolases and cargo, as well as timely removal of digested catabolites. Impaired homeostasis of lysosomal Ca$^{2+}$ causes lysosomal dysfunction and lysosomal storage diseases (LSDs). By directly patch-clamping lysosomal membranes, we have recently demonstrated that Mucolipin Transient Receptor Potential protein 1 (TRPML1 or ML1) is the principle Ca$^{2+}$ channel in late endosomes and lysosomes. Human mutations in TRPML1 result in type IV Mucolipidosis (ML-IV) neurodegenerative LSD, and at the cellular level, lysosomal trafficking defects and lysosome storage. Upon nutrient starvation, autophagy digests unwanted cellular components to generate catabolites that are required for housekeeping biosynthesis processes. A complete execution of autophagy demands an enhancement in lysosome function and biosynthesis to match the increase in autophagosome formation. We report that lysosomal TRPML1 channels play a central role in this quality-control process. By using Ca$^{2+}$ imaging and whole-lysosome patch clamping, lysosomal Ca$^{2+}$ release and
SA007

Emerging roles for endolysosomal TPC2 channels in LRRK2-linked Parkinson’s disease

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Mutations in LRRK2 (leucine-rich repeat kinase 2) are the most common genetic cause of Parkinson’s disease (PD). In addition, sequence variations at the LRRK2 locus increase risk for developing sporadic PD, thus highlighting the crucial role for LRRK2 in both disease entities. Pathogenic point mutations in LRRK2 segregate with familial disease in an autosomal-dominant manner, indicating a toxic gain-of-function phenotype. Indeed, most mutations increase the kinase activity of LRRK2, and this has spurred great hope for novel PD drug development approaches. However, no consistent LRRK2 kinase substrates have been identified thus far, and the cellular role(s) of LRRK2 have remained largely unknown.

Over the past years, we have investigated the cellular function(s) of LRRK2 using a variety of approaches. We have used transient expression of wildtype or mutant proteins in various tissue culture cell types, or analyzed effects of endogenous LRRK2 using patient-derived cells from either healthy controls or from patients with PD due to LRRK2 mutations. Our data indicate that pathogenic LRRK2 functions at the late endosome in a manner dependent on kinase activity. The late endosome comprises a major intracellular trafficking hub, and deficits can have profound effects on both endocytic and autophagic trafficking events.

Interestingly, we found that the observed LRRK2-mediated alterations were mimicked by NAADP, which triggers calcium release from acidic stores, and blocked by an NAADP antagonist. Subsequent studies highlighted a role for the NAADP-sensitive TPC2 channels in the LRRK2-mediated events, with mechanistic implications for PD being related to altered calcium-dependent trafficking to and from endolysosomal stores. The characteristics of the link between LRRK2 and TPC2 function, and molecular insights into its regulation will be discussed.

Together, our data indicate that TPC2 channels as well as modulators thereof may comprise additional promising drug targets for LRRK2-linked PD.


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SA008

NAADP-mediated calcium signalling: molecular mechanisms and physiological roles

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Of the established Ca\(^{2+}\) mobilizing messengers, NAADP is arguably the most tantalizing (1). It is the most potent, often working at low nanomolar concentrations. Unlike other Ca\(^{2+}\) mobilizing messengers, such as inositol trisphosphate (IP\(_3\)) and cyclic ADP-ribose, which release Ca\(^{2+}\) from the endoplasmic reticulum, NAADP mobilizes calcium from acidic stores, including lysosomes, representing a new function for this organelle.

Recent work has implicated a new class of ion channel, the two pore channels (TPCs), as key components for NAADP-evoked Ca\(^{2+}\) release (2). These channels are endolysosomal in localization where they play a role in local Ca\(^{2+}\) release.

Three distinct aspects of the NAADP-mediated Ca\(^{2+}\) signalling pathway have been identified. The first is to regulate local Ca\(^{2+}\) release that may play a role in endolysosomal functions, including fusion and trafficking. The second is to trigger global Ca\(^{2+}\) release by recruiting Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) channels at lysosomal-endoplasmic reticulum interfaces. The third is to regulate plasma membrane excitability by the targeting of Ca\(^{2+}\) release from sub-plasma membrane stores to activate plasma membrane calcium-activated channels.

In this talk, I will discuss the emerging role of endolysosomal-based NAADP-mediated Ca\(^{2+}\) release as a widespread trigger for intracellular calcium signalling in health and disease, and how studies of TPCs have enhanced our understanding of this process.


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Regulation of TRPML3 trafficking and function by palmitoylation

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TRPML3 is an organellar Ca\(^{2+}\) permeable channel that plays an important role in membrane trafficking and autophagy. Although TRPML3 shows dynamic subcellular localization during endocytosis and autophagy, the underlying mechanism by which TRPML3 traffics between intracellular compartments is not known. Here we report that TRPML3 undergoes palmitoylation at C-terminal cysteine residues (Cys549-551), and that the palmitoylation is required for the dynamic intracellular trafficking of TRPML3. Cell surface expression of TRPML3 was decreased by inhibition of palmitoylation, whereas organellar targetting and channel activity appeared not to be affected. The impaired TRPML3 trafficking by palmitoylation-site mutations altered TRPML3 function in endocytosis, leading to increased endocytosis. Upon induction of autophagy, palmitoylation mutant did not exacerbate autophagy compared with WT-TRPML3. Importantly, inhibition of TRPML3 palmitoylation markedly reduced autophagic flux in endocytosis and autophagy by regulating its trafficking between subcellular compartments.

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Two-Pore Channel 2-deficient mice are highly susceptible to Non-Alcoholic Fatty Liver Disease

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Endolysosomal organelles play a key role in trafficking, breakdown and receptor-mediated recycling of different macromolecules such as low-density lipoprotein (LDL)-cholesterol, epithelial growth factor (EGF) or transferrin. In the liver, receptor-mediated uptake of low-density lipoproteins (LDLs) and subsequent intracellular transport is essential for hepatic cholesterol homoeostasis and plasma lipoprotein metabolism. Dysfunction within this pathway results in liver disease such as non-alcoholic fatty liver disease (NAFLD), which is associated with increased cardiovascular and liver-related mortality. It has been estimated that as many as 30% of adults in these countries have NAFLD. This liver disease has thus emerged as a substantial public health concern.

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Insights into the neural control of blood pressure: Implications for the development of new antihypertensive strategies

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With an ever rising number of patients diagnosed with hypertension, the poor efficacy, compliance and tolerability of current medicines, and the apparent absence of new anti-hypertensive medications over the last 15 years there has been a major resurgence of interest in strategies focused on intervening directly with autonomic control mechanisms. Renal denervation, carotid sinus stimulation and deep brain stimulation are all examples and have all undergone trials in an attempt to curb the significant causal problem of increased sympathetic traffic to cardiovascular end organs. These strategies may all prove therapeutically useful in sub-cohorts of patients with drug-resistant high blood pressure. In this presentation, I will explore the carotid body as a potential novel target for regulating sympathetic outflow that may have considerable advantages including the ability to identify patients with pathological or hyperactive carotid bodies and to assess whether the intervention has been effective in reducing aberrant carotid body discharge as well as abating sympathetic traffic chronically. Ideally, any therapeutic intervention would be reversible, free from side effects and, importantly, would only remediate the pathology sparing physiological function. Using multiple models of hypertension and numerous animal species, and human patients, I will present our recent findings demonstrating the pathological role of purinergic receptors (specifically, P2X3) in the carotid body pertaining to conditions of autonomic imbalance and sympathetic activity excess. Using selective P2X3 receptor antagonists we may be able to provide a much needed approach for restoring carotid body physiology and autonomic homeostasis across a range of diseases including high blood pressure in which the sympathetic nervous system has become overactive.

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Nerve-to-spleen pathway is critical in the development of hypertension in the setting of chronic inflammatory disease
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Both the autonomic nervous system and the immune system have been implicated in hypertension. Because of the bidirectional communication between the two systems, there is recent interest in neuro-immune mechanisms that may also play a role in the pathogenesis of the disease. Systemic lupus erythematosus (SLE) is an autoimmune disorder associated with aberrant immune function and chronic inflammation that contributes to the prevalent hypertension. SLE is also associated with prevalent autonomic dysfunction; therefore, SLE is an ideal model to study the link between the nervous system, immune system and hypertension.

The cholinergic anti-inflammatory pathway is a vagally-mediated neuro-immune mechanism that suppresses the production and secretion of cytokines from splenic immune cells upon stimulation. The α7 subunit of the nicotinic acetylcholine receptor (α7nAChR) on these splenic immune cells is a critical component of the cholinergic anti-inflammatory pathway. Although stimulation of this pathway has been shown to be protective in chronic inflammatory diseases, it is unknown whether this novel neuro-immune pathway is important in hypertension. Our overall hypothesis is that the cholinergic anti-inflammatory pathway is impaired in hypertension and that activation of this pathway at the level of the α7nAChR would protect from the development of the disease. To test this hypothesis we measured splenic protein expression of α7nAChR and the vesicular acetylcholine transporter (VACHT) in female SLE mice with hypertension. SLE (NZBW/F1) mice had 365±30% higher splenic protein expression of α7nAChR compared to control NZW mice (0.038±0.002 vs. 0.008±0.003; n = 3; p<0.001). Similarly, splenic protein expression of VACHT was 24.4±7.6% higher in SLE mice compared to controls (0.33±0.02 vs. 0.26±0.03; n = 3; p<0.05). These data suggest the cholinergic pathway may be impaired at the level of the α7nAChR in SLE mice.

In order to investigate whether activation of the pathway is protective in SLE hypertension, female SLE and control mice were infused with nicotine hydrogen tartrate salt (2 mg/kg/day, SC) or saline for 7 days. Splenic protein expression of TNF-α and IL-6 was significantly higher in saline-treated SLE mice compared to nicotine-treated SLE mice (1.37±0.06 vs. 1.09±0.06 and 0.55±0.10 vs. 0.36±0.04; n = 3; all p<0.05), suggesting efficacy of the therapy in reducing splenic inflammation. Mean arterial blood pressure was increased in SLE mice compared to controls (140±4 vs. 114±2; n = 6-9; p<0.001). Nicotine prevented the rise in MAP in SLE mice (129±4; p=0.022), but did not alter MAP in controls (121±3). This protection from hypertension coincided with a 17±5% reduction (p=0.041) in renal TNF-α in nicotine-treated SLE mice (n=3), which is important because we have shown that renal TNF-α plays a mechanistic role in the development of hypertension during SLE. Because nicotine acts on both ganglionic and peripheral cholinergic receptors, in a subsequent study mice were administered the selective α7nAChR agonist, PNU-282987 (0.38 mg/kg/day, IP), or vehicle for 28 days. Splenic protein expression of TNF-α and IL-6 was significantly higher in saline-treated SLE mice compared to PNU-282987-treated SLE mice (0.54±0.03 vs. 0.33±0.01 and 0.86±0.05 vs. 0.40±0.08; n = 3; all p<0.05). MAP was increased in SLE mice compared to controls (138±2 vs. 122±5; n = 5). PNU-282987 prevented the rise in MAP in SLE mice (128±4), but did not alter MAP in controls (125±5).

These data suggest the anti-inflammatory effects of cholinergic agonists may protect from SLE hypertension. Taken together, the cholinergic anti-inflammatory pathway, particularly the α7nAChR in the spleen, may be an important target in patients with hypertension and other diseases of chronic inflammation.

Mathis KW et al. (2014). Preventing autoimmunity protects against the development of hypertension and renal injury. Hypertension 64(4), 792-800.


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inhibitory renorenal reflex control of ERSNA is enhanced. Conversely during low sodium dietary conditions, the renorenal reflex control of ERSNA is reduced. These are physiologically appropriate responses to maintain sodium balance during various dietary sodium conditions. Increased activation of angiotension (ANG) II type 1 receptors in the renal pelvic area and \( \alpha_2 \) adrenoceptors on the afferent renal nerve endings contribute to the suppressed responsiveness of the afferent renal nerves in low sodium dietary conditions.

In pathophysiological sodium-retaining states, characterized by increased ANG II, including hypertension and renal edema-forming diseases such as heart failure and renal failure, the inhibitory renorenal reflexes are impaired. Impairment of the inhibitory renorenal reflexes most likely contributes to the inappropriately increased ERSNA and sodium retention in these pathophysiological conditions.

When the inhibitory renorenal reflexes are suppressed, there is evidence for a prevalence of excitatory reflexes originating in the kidney in various pathological conditions involving renal injury, including hypertension, heart failure and chronic renal failure. Support for this notion is derived from studies showing that denervation of the ischemic kidney reduces ERSNA and arterial pressure in renovascular hypertension. Further, selective afferent renal denervation has been shown to reduce arterial pressure in chronic renal failure, DOCA salt hypertension and to some degree in spontaneous hypertension. Among possible mechanisms is adenosine activating renal chemosensitive nerve fibers.

Removal of both renal sympathetic and afferent renal nerves most likely contributes to the arterial pressure reduction following renal denervation in treatment resistant hypertensive patients, at least initially. Sympathetic nerves eventually reinnervate renal tissue. Therefore, it was thought that the long-term pressure reduction following renal denervation in patients was due to lack of afferent renal innervation. However, recent findings in rats and sheep show that the afferent nerves reinnervate renal tissue in a similar time-dependent fashion as the sympathetic nerves following renal denervation. These findings suggest that additional mechanisms, possibly related to the initial removal of the renal sympathetic/afferent nerves, are likely to contribute to the long-term arterial pressure reduction observed in drug-resistant hypertensive patients following renal denervation.

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

**Peripheral cardiac sympathetic dysfunction in the pre-hypertensive spontaneously hypertensive rat**

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Hypertension is associated with an increase in cardiac sympathetic transmission, although the exact mechanism underlying this is unknown. The adult spontaneously hypertensive rat (SHR) has increased cardiac sympathetic activity compared to Wistar Kyoto (WKY) controls. Recent studies in 4 week old pre-hypertensive SHR have shown increased calcium transients in isolated stellate ganglion neurons compared to age matched WKYS. In an isolated organ bath atrial preparation with intact right stellate ganglion (37±0.5°C) there was a greater heart rate response to stellate stimulation (5 and 7Hz, 30sec, 20V, 1msec) in SHRs compared to WKYS, and a significantly greater release of \(^{3}H\)-NE to field stimulation (5Hz, 20V, 1msec) of right atria. Whereas, ventricular weight/body weight ratios and mean arterial pressure measured via the left carotid artery (under 2% isoflurane) demonstrated that SHRs were without left ventricular hypertrophy and were normotensive at this age compared to WKYS.

This neural phenotype observed might result from defective NET-1 transport in the SHR preventing rapid termination of the noradrenaline signal. A novel flute assay of the noradrenaline reuptake transporter (NET) was used on isolated sympathetic postganglionic neurons. NET rate was significantly impaired in cardiac stellate sympathetic neurons from the pre-hypertensive and hypertensive SHR compared to age matched normotensive WKY. However, no reduction in transporter rate was observed at either age in the other major non-cardiac sympathetic ganglia. To test the effect of neuronal excitability on NET activity preparations were depolarised by electrical field stimulation (EFS). Electrical field stimulation potentiated the difference in NET rate observed within stellate neurons of the hypertensive SHR and age matched WKY (SHR: 2.59±0.30 au/min, n=20, WKY: 4.25±0.47 au/min, n=19, P<0.01). NET is known to be highly regulated by a number of kinase and phosphatases, modulating both its intrinsic activity and surface expression therefore allowing for a dynamic modulation of the main system responsible for the termination of the action of noradrenaline. Previous studies have indicated a role for protein kinase C inhibition in facilitating the translocation of NET to the presynaptic membrane. The PKC inhibitor calphostin C caused a significant dose dependent increase in NET activity in WKY neurons (100nM n=13, P<0.05). Similarly NET activity in SHR neurons was also increased in the presence of calphostin C compared its control (100nM n=15, P<0.05). Moreover, there was no difference in NET rate between SHR and WKY neurons after calphostin C. Therefore reduced NET activity in the SHR may be due to increased PKC expression and increased phosphorylation of NET transporters in the cytosol that prevents insertion of the transporter into the presynaptic membrane. Strategies that up regulate NET activity may help rescue the sympathetic hyperactivity observed in this model system.

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**PVN Gα12-proteins and the neural control of blood pressure: A new paradigm for the treatment of salt-sensitive hypertension?**

R.D. Wainford

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Salt-sensitive hypertension is a critical component of essential hypertension (the leading global non-communicable cause of mortality), but the exact role of sodium in the pathogenesis of hypertension remains unclear. In normotensive, salt-resistant subjects, endogenous neural and renal water- and sodium-retaining mechanisms are suppressed to counter the influence of salt intake on blood pressure regulation. At present, there exists no clear understanding of the integrated sodium-sensitive signal transduction mechanisms that operate between the brain and the kidney to facilitate sodium homeostasis and normotension. The brain pathways that mediate salt-resistant responses to high salt-intake involve activation of G-protein

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**SA015**

**PVN Gα12-proteins and the neural control of blood pressure: A new paradigm for the treatment of salt-sensitive hypertension?**

R.D. Wainford

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Salt-sensitive hypertension is a critical component of essential hypertension (the leading global non-communicable cause of mortality), but the exact role of sodium in the pathogenesis of hypertension remains unclear. In normotensive, salt-resistant subjects, endogenous neural and renal water- and sodium-retaining mechanisms are suppressed to counter the influence of salt intake on blood pressure regulation. At present, there exists no clear understanding of the integrated sodium-sensitive signal transduction mechanisms that operate between the brain and the kidney to facilitate sodium homeostasis and normotension. The brain pathways that mediate salt-resistant responses to high salt-intake involve activation of G-protein
coupled receptors (GPCR) and signal transduction via Gzα-subunit proteins. Our laboratory has demonstrated that endogenous brain Gz12-subunit protein-gated signal transduction pathways mediate the acute renal sympathoinhibitory and natriuretic responses to GPCR activation evoked by both pharmacological and physiological stimuli (volume expansion, acute sodium loading). Significantly, we have observed endogenous up-regulation of PVN Gz12 proteins in salt-resistant animal models in response to increased salt-intake, a response that is required to potentiate endogenous renal nerve dependent sodium excreting mechanisms to counter the development of salt-sensitive hypertension. Further, we have identified that failure to up-regulate PVN Gz12 proteins in response to salt-intake contributes to the pathophysiology of salt-sensitive hypertension via renal nerve dependent mechanisms to impact the activity and expression of the sodium chloride co-transporter (NCC). Our recent advances in this area have identified a requirement of PVN Gz12 protein signal transduction in mediating neuronal activation, in response to both acute and chronic sodium challenge, within the parvo-cellular neurons of the PVN to facilitate sympathoinhibition, sodium homeostasis and normotension. Collectively, our studies provide the first direct evidence of a central molecular signal transduction mechanism, PVN Gz12 protein-gated pathways, that influences central sympathetic outflow to the kidney to impact sodium homeostasis and long-term blood pressure regulation.

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SA016

Methods for assessing microvascular flowmotion in humans

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Flowmotion is the rhythmic alteration of vascular diameter that occurs, in many if not all, arterial vessels. In vivo, it results in the oscillation of microvascular perfusion (flowmotion) in many tissues. Although it has been recognised for a long time that flowmotion, theoretically, enables greater delivery of nutrients it has only recently become apparent that alterations in flowmotion have other important physiological roles. Alterations in the patterns of flowmotion may be a major underlying mechanism linking endothelial dysfunction to cardiovascular disease states such as insulin resistance and hypertension.

Flowmotion can be measured in intact tissues and isolated vessels with microscopy, but microvascular flowmotion in vivo is harder to observe and quantify. In humans, intravital videomicroscopy can be used to observe the nailfold microcirculation but has limited use in other tissues. Laser Doppler Flowmetry (LDF) using skin surface probes has been the most commonly used technique to observe microvascular flowmotion in humans. The LDF flux signal, which is a quantity proportional to the product of the average speed of blood cells and their number can be analysed to determine the microvascular flow rhythms. Application of Fourier or wavelet analysis to LDF signals from human skin has revealed 5 distinct peaks in the frequency domain between 0.009-1.6Hz. These peaks have been associated with the following activities: endothelial: 0.009-0.2Hz (~1 cycle/100s); neurogenic: 0.02-0.06Hz (~1 cycle/25s); myogenic: 0.06-0.15Hz (~1 cycle/10s); respiratory: 0.15-0.4Hz (~1 cycle/5s); cardiac: 0.4-3Hz (~1 cycle/s). LDF can also be used to measure flowmotion in other tissues but this involves invasive needle probes to be inserted into the tissue of interest with the concomitant risk of disrupting normal blood flow patterns and thus has limited use to mostly skin. A method based on contrast-enhanced ultrasound (CEU) using gas-filled microbubbles is potentially a new minimally-invasive technique to assess microvascular flowmotion in a number of tissues. Developments in ultrasound technology have allowed real-time CEU measurement of microvascular volume that like LDF can be analysed to determine microvascular flowmotion rhythms.

Application of these two techniques (LDF and CEU) will allow microvascular flowmotion to be investigated in a number of pathological states and provide a new understanding of the origin and consequences of cardiovascular diseases.

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SA017

Determination of skeletal muscle microvascular flowmotion with contrast enhanced ultrasound

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Across muscle tissue the rhythmic variation in blood flow (flowmotion) is such that at any one time only a proportion of capillaries that supply nutrients to the muscle are perfused. Different factors control blood flow variation, with each contributing factor occurring at a specific frequency; endothelial (0.006-0.02Hz), neurogenic (0.02-0.06Hz), and myogenic (0.06-0.3Hz). A change in the contribution of any of these factors to blood flow regulation can lead to an overall increase or decrease in nutrient delivery to the myocytes, thereby potentially altering the metabolic activity of the muscle tissue. Determining flowmotion within in vivo skeletal muscle is difficult and previously assessment has been performed by invasive studies with implanted Laser Doppler Flowmetry (LDF) probes. LDF only allows flowmotion assessment in a small proportion of skeletal muscle tissue, however this study uses novel real-time contrast enhanced ultrasound (RT-CEU) imaging in the assessment of microvascular blood flow across whole skeletal muscles in vivo.

Male Sprague Dawley rats were anaesthetised (i.p sodium pentobarbital, 50mg/100g body weight) and cannulas placed into jugular veins and carotid artery. Anaesthesia was maintained via intravenous infusion (sodium pentobarbital 0.6mg/ min/kg body weight) throughout experiment. Phospholipid microbubbles (average 4um diameter) where infused into the jugular vein and RT-CEU flowmotion assessment in the calf muscle was made with a Philips IU22 ultrasound machine using a L9-3 transducer. QLab (Philips) software was used to analyse regions of interest and wavelet transformation (MATLAB) was used to determine the contribution of endothelial,
neurogenic and myogenic induced vasomotion. LDF, oxygen saturation and total haemoglobin measures where simultaneously determined via a probe (CP3-HP, Moor Instruments) placed directly onto the tibialis muscle and compared to RT-CEU measures.

A strong neurogenic contribution to flowmotion was seen in both LDF and RT-CEU measures. Treatment with the adrenergic antagonist phentolamine produced a marked reduction in neurogenic flowmotion contribution while concurrently increasing total blood flow and microvascular perfusion to the skeletal muscle to a similar extent of that seen during muscle contraction.

This study shows that RT-CEU is a new minimally invasive technique which may be used to determine in vivo skeletal muscle flowmotion. Application of RT-CEU in animal models and human participants may lead to determination of any changes in flowmotion which occur during alterations in metabolism, such as during insulin infusion. Additionally RT-CEU may lead to greater understanding of the causes of vascular regulation defects which occur during pathological conditions affecting the microvascular such as hypertension, insulin resistance and Type 2 Diabetes.

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SA018

Linking microvascular vasomotion and capillary recruitment

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Insulin has been shown to regulate its own delivery to skeletal muscle interstitium via actions on the microvasculature (1,2,3). In fact, these vascular effects are thought to be rate-limiting for insulin-stimulated glucose uptake by skeletal muscle (1). Insulin’s influence on microvascular vasomotion - a rhythmic change in vascular diameter (4) - is one of the suggested levels of modulation. Insulin-induced changes in pre-capillary arteriolar vasomotion would result in a rhythmical variation in blood flow downstream (flowmotion). An increase in such preferential blood flow towards a particular capillary bed i.e. capillary recruitment will increase local capillary endothelial surface area for solute exchange.

Although inferred upon several times (2,5), a direct link between changes in vasomotion and changes in capillary recruitment had yet to be demonstrated. We therefore addressed this very relationship within a group of healthy volunteers displaying a wide, continuous range in BMI and, as it turned out, insulin-sensitivity.

Changes in vasomotion and capillary density were determined by laser Doppler flowmetry (LDF with spectral analysis) and capillary videomicroscopy in skin, respectively, before and during a hyperinsulinenemic euglycemic clamp in 19 healthy volunteers. An insulin-induced increase in the neurogenic vaso-motion domain was positively related to insulin-augmented capillary recruitment (r=0.51, P=0.04), and both parameters were related to insulin-mediated glucose uptake (r=0.47, P=0.06 and r=0.73, P=0.001, respectively). Furthermore, the change in insulin-augmented capillary recruitment could, at least statistically, largely explain the association between the neurogenic domain and insulin-me-
diated glucose uptake in a regression analysis, supporting the suggested physiological framework.


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SA019

Flow motion as a target for therapeutic intervention

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Flow motion is the periodic oscillation of blood flow in the microvascular network imparted by local and central control mechanisms. Spectral analysis of the component frequencies of the blood flow signal reveals the influence of endothelial (0.0095 – 0.02Hz), sympathetic (0.02 – 0.06Hz) and myogenic (0.06 – 0.15Hz) activity in the vessel wall and of respiration (0.15 – 0.4Hz) and heat beat (0.4 – 1.6Hz) (1). The role of flow motion to maintain microvascular perfusion and tissue homeostasis is much debated and the pathophysiological relevance of disturbed flow motion poorly understood.

The simultaneous non-invasive measurement of skin blood flow (BF) and parameters of tissue oxygenation (oxyHb, deoxyHb, totalHb and SO2) using a combined laser Doppler and white light spectroscopy probe (Moor Instruments, UK) and spectral analysis over the range (0.0095 – 1.6Hz) has provided new insight into the intrinsic variability of blood flux and tissue oxygenation signals and the relationship between them (2). In healthy skin we have demonstrated a strong correlation between simultaneously recorded skin BF, tissue SO2 and total spectral power across a wide range of blood flows and multiple time varying oscillations. There is also a significant positive correlation between the contributions in each of the three low frequency bands to the BF and SO2 signals (2). However, differences in the relative contribution of the component frequencies to flow motion activity, as evidenced by a relatively small contribution of respiratory and cardiac activities to the SO2 signal, suggests a significant dissociation between the higher frequency oscillations. Exploration of the dynamic characteristics of flow motion using frequency coherence
demonstrates considerable concordance within the endothelial and neurogenic (low frequency components) of the BF and SO2 signals suggesting that they are modulated in a similar manner; although causality has yet to be proven. These relationships are diminished and the intrinsic variability in flow motion signals lost in individuals with cardiovascular and metabolic disease contributing to a reduced ability to respond to changes in the local or systemic environment (3). However, our understanding of whether flow motion can be improved with therapeutic intervention is far from complete. While the concept of skin microcirculation as a peripheral index or surrogate of vascular health remains contentious, monitoring local tissue perfusion in combination with tissue oxygen parameters, could provide an early indicator of compromised tissue function and an effective tool to augment the diagnosis, treatment and management of conditions across a number of clinical specialties in which microvascular dysfunction plays a role.

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SA020

Noncoding RNAs in vascular cells and implications for cardiac disease

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Noncoding RNAs including microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) regulate a substantial fraction of the genome and most cellular functions in health and disease. It has been shown that noncoding RNAs are both diagnostically and therapeutically of highest relevance. In this talk, their role as potential players in different vascular cells (e.g. endothelial and smooth muscle cells) will be discussed. This includes the role of anti-angiogenic and pro-apoptotic miR-24 for outcome after ischemic cardiac disease in small and large animal models. Additionally, the importance of hypoxia-related lncRNAs will be highlighted to introduce new aspects of regulatory RNA biology.

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14q32 non-coding RNAs in vascular remodelling

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Cardiovascular disease is the leading cause of morbidity and mortality in the Western world. A disturbed balance between processes of vascular remodeling, including atherogenesis and neovascularization often forms the basis of cardiovascular pathology. We aim to study the role of non-coding RNAs in vascular remodeling. Using www.targetscan.org, we performed a reverse target prediction on two sets of genes involved in vascular remodeling: 197 genes involved in neovascularization on the one hand and 165 genes involved in atherosclerosis on the other. We found enrichment of binding sites for 27 microRNAs (miRNAs) from a single noncoding RNA gene cluster, located on human chromosome 14q32 in the neovascularization gene set. Strikingly, we also found enrichment of eleven 14q32 miRNAs in the atherosclerosis gene set. The 14q32 cluster forms the largest known non-coding RNA gene cluster, containing 54 microRNAs, 41 small nucleolar RNAs (snoRNAs) and 3 long non-coding RNAs (lncRNAs). This cluster is highly conserved, but in mammals only. SnoRNAs from the 14q32 cluster are of the C/D-box subtype and although they clearly have the same structure and sequence motif conservation of other snoRNAs, they have no known RNA targets. The function of these snoRNAs is likely non-canonical and remains to be elucidated. The 14q32 lncRNAs MEG3 and MEG8 (Rian in mice) likely regulate imprinting and transcription of the cluster and are upregulated in activated endothelial cells under hypoxia1.

We have previously shown that 14q32 miRNAs are regulated in three different expression patterns during post-ischemic vascular remodeling2. One third of the 14q32 miRNAs was upregulated in murine muscle tissue within 24 hours after induction of ischemia (early responders), one third was upregulated within 72 hours after induction of ischemia (late responders) and one third of the 14q32 miRNAs was not regulated at all after ischemia (non-responders). Similar to the microRNAs, we now show that the 14q32 snoRNAs follow the same three expression patterns after ischemia, namely early responders, late responders and a non-responder. LncRNAs MEG3 and Rian on the other hand were rapidly downregulated after induction of ischemia. Inhibition of 14q32 miRNAs miR-329, miR-487b, miR-494 and miR-495 using Gene Silencing Oligonucleotides (GSOs) increased both atherogenesis and angiogenesis, leading to a 40% increase in blood flow recovery in a model for hindlimb ischemia in mice. In addition, we could confirm upregulation of multiple target genes after inhibition of 14q32 microRNAs. We then investigated the effects of 14q32 microRNA inhibition on atherosclerosis. When looking at expression patterns of 14q32 “angiomiRs”, we found that specifically miR-494 was abundantly expressed in murine tissues involved in atherosclerosis, including the liver, spleen and carotid arteries. When looking at human carotid artery lesions, we found that miR-494 expression was doubled in vulnerable plaques compared to plaques of a stable phenotype. Again, we used GSOs to
inhibit miR-494 in hypercholesterolemic ApoE-/- mice, after placing semi-constrictive collars around both carotid arteries for 28 days to induce atherosclerotic lesion formation. Atherosclerotic lesion formation was significantly reduced in mice treated with GSO-494, while plaque stability was increased, determined by both a decrease in necrotic core size and an increase in plaque collagen content. Furthermore, inhibition of miR-494 resulted in increased cholesterol efflux in vitro. Indeed, in vivo, plasma total cholesterol levels and LDL fractions were decreased after miR-494 inhibition. These data demonstrate an important role for the 14q32 non-coding RNA cluster in vascular remodeling and cardiovascular pathology. Furthermore, we showed that inhibition of 14q32 miRs has great therapeutic potential in the prevention and treatment of atherosclerotic disease.


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SA022

Non-coding RNA in cardiovascular aging
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Aging is the major risk factor for developing cardiovascular complications like endothelial dysfunction, aneurysm formation, acute myocardial infarction and heart failure. The mechanisms involved in cardiovascular aging are poorly understood, but non-coding RNAs, including microRNAs, have emerged as key biological regulators. We have identified that age-induced miR-29 has a causal role in aneurysm formation by regulating the expression of extracellular matrix proteins in the aorta. Furthermore, we recently described the crucial role for miR-34a in cardiac aging, which regulates cardiomyocyte apoptosis and telomere length. Current studies in the laboratory focus on determining the role of long non-coding RNAs (IncRNAs) in aging of the endothelium and how these affect organ homeostasis and cellular metabolism. We firstly showed that many IncRNAs (>200 nt) are expressed in endothelium and that the IncRNA MALAT1 is required for endothelial proliferation in vitro and in vivo. Several IncRNAs are also regulated during endothelial ageing, most notably Meg3 and H19, which are up- and downregulated during ageing, respectively. Inhibition of Meg3 and H19 in vitro affects endothelial function and senescence and ongoing mechanistic studies will elucidate the mechanism by which these IncRNAs affect endothelial ageing.

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SA023

Posttranscriptional regulation of microRNA expression
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MicroRNAs (miRNAs) are conserved non-coding RNAs that regulate gene expression by targeting partially complementary sequences in the mRNAs. Each miRNA potentially regulates hundreds of miRNA targets, thus controlling a variety of biological processes, including mammalian cellular differentiation and development. In spite of widespread efforts to understand the roles of individual miRNAs, little progress has been made towards unravelling the regulation of their biogenesis. My group has focused on elucidating the cis and trans-acting factors of tissue-specific miRNA biogenesis in mammalian cells. We have identified factors that regulate the production of brain-enriched and brain-specific miRNAs, as well as factors that are responsible for the selective uridylation and degradation of miRNA precursors in embryonic cells. We have demonstrated that the expression profile of brain-enriched miRNA-7, which is processed from a ubiquitous pre-mRNA transcript coding for hnRNP K protein, is achieved by inhibition of its biogenesis in non-brain cells. By identifying MSI2 and HuR proteins as inhibitors of miRNA-7 maturation in non-brain cells, we provided the first insight into the regulation of brain-enriched miRNA processing by defined tissue-specific factors. Furthermore, we showed that brain-specific miRNA-9 is regulated transcriptionally and post-transcriptionally during neuronal differentiation. We revealed that Lin28a, an RNA-binding protein progressively switched off during differentiation, inhibits the processing of brain-specific miRNA-9 by inducing the degradation of its precursor transcript during early stages of neuronal differentiation.

Our findings have far reaching consequences for our understanding of how RNA-binding proteins commit to a specific molecular function and how, through targeting miRNA bio genesis pathway, they contribute to control of gene expression in mammalian cells.

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SA024

Regulation of muscle mitochondrial biogenesis by acetylation
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In recent years, reversible acetylation, which is a post-translational modification (PTM) in which an acetyl group is added to a lysine residue, has been proposed to link metabolic flux to cellular signaling and the adaptive response. This is due to the fact that two fundamental metabolites central to cellular/energy metabolism, NAD+ and acetyl CoA, are key substrates for the sirtuin (SIRT) deacetylases (DACs) and acetyltransferases (ACTs), respectively. DACs remove acetyl groups from lysine residues, whilst ACTs add them. Given that exercise results in fluctuations in acetyl CoA and NAD+, reversible acetylation presents as an interesting molecular signalling mechanism for regulating protein activity and transcriptional
SA025

Role of HIFs in skeletal muscle adaptation to training

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Skeletal muscle is essential to life as it provides the mechanical power and the structure for locomotion, posture and breathing. Beyond these vital functions, it also plays an essential role in the regulation of whole body metabolism. Loss of muscle mass and disturbed metabolic function leads to and/or exacerbates a number of chronic diseases, including coronary heart disease, obesity, and type 2 diabetes. To date, no medicine has proven to be more efficient than exercise to improve those pathological states. Understanding the molecular mechanisms behind exercise-induced health benefits will be crucial for the development of effective drugs or to an effective lifestyle program aiming at decreasing the number of persons suffering from muscle wasting and/or chronic diseases. During exercise, oxygen levels in skeletal muscle are fluctuating. Activation of the oxygen sensing hypoxia inducible factor (HIF) pathway is critical to cell adaptation whenever oxygen becomes limited, as it initiates a wide range of responses aimed to restore oxygen homeostasis. Despite the overwhelming amount of papers describing a crucial role for HIFs during development and disease, very little is known on how HIFs control muscle metabolism and exercise adaptations. Indeed, only few studies have reported that HIF-1α is stabilized during acute exercise in human skeletal muscle and no data exist so far showing HIF-2α stabilization. Moreover, few studies have tested the hypothesis whether HIF-1α/R-related processes could be relevant to exercise-induced skeletal muscle metabolism and adaptation, and the precise interaction with other molecular regulators of muscle metabolism such as the mechanistic target of rapamycin (mTOR), the mitogen-activated protein kinase (MAPK) pathways, AMP-activated kinase (AMPK), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1α), the sirtuins, the histone deacetylases (HDAC) and more recently autophagy might be unraveled. During the presentation, the data accumulated for the last years on HIFs stabilization and their role in exercise-induced health benefits will be presented. A specific attention will be paid to the role of HIFs in mitochondrial biogenesis.

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SA026

Defining the role of BRCA1 in skeletal muscle metabolic regulation

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BRCA1 is classically known as a tumor suppressor gene that acts to maintain genomic stability in mammary cells. However, BRCA1 mRNA expression is found in a variety of cell types, including skeletal muscle, without a defined physiological role in those cells. The purpose of this study was to use a translational approach to determine if BRCA1 plays a critical role in the regulation of physiological and metabolic function of skeletal muscle. We found that BRCA1 mRNA and protein is expressed in skeletal muscle biopsies from humans and adult mice. To determine if BRCA1 is critical for skeletal muscle function, we generated inducible skeletal muscle specific BRCA1 knock out mice (BRCA1KOsm). Further, using shRNA approaches we significantly reduced BRCA1 expression in cultured human myotubes (BRCA1sh). Using in vivo and in vitro approaches, our data suggest that induced deletion of BRCA1 expression leads to significant dysfunction of adult skeletal muscle. When compared to age-matched wild-type (WT) mice, BRCA1KOsm develop visible kyphosis and detectable muscle weakness as measured by in situ muscle contractions. In addition, we found reductions in mitochondrial oxygen consumption and alterations in glucose dynamics of BRCA1KOsm and BRCA1sh when compared to the WT mice or control myotubes, respectively. Finally, using immuno-precipitation approaches we have identified that BRCA1 can complex with the phosphorylated form of acetyl CoA-carboxylase. Our data show that loss of BRCA1 specific to skeletal muscle leads to the development of muscle weakness and reductions in mitochondrial function. When considering the high degree of susceptibility of BRCA1 to genetic mutation our data suggest that further human genetic association studies are potentially warranted. Collectively, our results suggest the BRCA1 pathway is a novel target that is necessary for optimal function of skeletal muscle.

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SA027

14-3-3 proteins as potential signalling integrators for exercise induced adaptations

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Exercise can be broadly split into two modes; endurance and resistance exercise. Each mode of exercise leads to mode specific adaptations such as increased muscle mass with resistance exercise and improved fatigue resistance with endurance exercise. Data from some animal and ex vivo studies suggested that there were a series of kinase cascades responsive to specific modes responsible for the respective adaptations. For instance, the AMP-activated protein kinase (AMPK) was thought to be specifically responsive to endurance exercise whilst the mammalian target of rapamycin complex 1
Adipose tissue: The brown, the white and the brite


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Two types of adipose tissues, brown and white, coexist in mammals. WAT stores energy in the form of triglycerides while BAT has the ability to dissipate energy through adaptive thermogenesis and has a common embryological origin with skeletal muscle. A particular type of adipocytes sometimes occurs within classical WAT depots. These adipocytes manifest several classical brown adipocyte characteristics, most notably the presence of UCP1, but do not express the novel molecular markers characteristic of classical brown adipocytes and have completely independent developmental origin. This particular adipose cell-type cannot be classified either as brown or white; it represents a distinct - "brite" (brown like-in-white) - adipose cell-type (sometimes referred as beige, inducible or convertible adipocytes). Subcutaneous white-fat depots (i.e. inguinal depot) are particularly prone to browning under prolonged cold exposure. In mitochondria isolated from the inguinal "white" adipose depot of cold-acclimated mice, UCP1 protein levels almost reach those in brown-fat mitochondria. The UCP1 is thermogenically functional - these mitochondria exhibit UCP1-dependent thermogenesis with lipid or carbohydrate substrates with canonical guanosine diphosphate (GDP) sensitivity and loss of thermogenesis in UCP1 knockout (KO) mice. The thermogenic density (UCP1-dependent oxygen consumption per g tissue) of inguinal white adipose tissue is maximally one-fifth of that in brown adipose tissue, and the total quantitative contribution of all inguinal mitochondria is maximally one-third of all interscapular brown-fat mitochondria. Thus, the classical brown adipose tissue depots would still predominate in thermogenesis.

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

Adipose tissue metabolism and its contribution to metabolic health

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The pre-diabetes and type 2 diabetes spectrum is associated with disordered hormonal regulation of adipose tissue fatty acid metabolism. Resistance to insulin-stimulated dietary fatty acid storage has been described over the past fifteen years and is now clearly documented using a wide array of isotopic tracer methods, including our recent non-invasive positron emission tomography (PET) method able to quantify whole-body organ-specific fatty acid partitioning. Another less well recognized feature of adipose tissues' metabolic inflexibility is their resistance to catecholamine-stimulated intracellular lipolysis and mobilisation of non-esterified fatty acids (NEFA) to lean organs. Recently, brown adipose tissue (BAT) has been rediscovered in adult humans using 18-fluoro-deoxyglucose PET. Reduced BAT glucose uptake is clearly associated with obesity and type 2 diabetes. However, fatty acids from intracellular triglyceride lipolysis are the main energy source of BAT. Although BAT clearly contributes to cold-induced thermogenesis, its potential for energy dissipation and role in the development of obesity and type 2 diabetes are not elucidated at this time. We found that white adipose tissue NEFA mobilization is linked to total body BAT metabolic capacity. Whether BAT displays metabolic inflexibility with regards to dietary fat utilization and intracellular fatty acid mobilization or whether adipose tissues in type 2 diabetes is an intriguing hypothesis under investigation in our laboratory.

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

The origins and development of brown adipose tissue: The role of BMP-signalling and other mechanisms of lineage determination

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Obesity represents a severe challenge to medical care systems worldwide. Adequate treatment strategies are urgently needed to counter not only pathological overweight but also its associated complications such as diabetes and cardiovascular disease. Brown adipose tissue (BAT) dissipates energy in the form of heat and has therefore been proposed as a target for anti-obesity therapies. Recently, it has become clear that metabolically active brown fat cells can be observed in adult humans, thus supporting the notion of a viable treatment approach for obesity by targeting BAT (Nedergaard et al., 2007).

Two types of brown adipose tissue can be found in mice and in all likelihood also in human subjects (Cypess et al., 2013): The
classical brown adipose tissue that develops during embryogenesis and that persists throughout life and, secondly, the brite (brown-in-white) adipocytes that are recruited either through transdifferentiation of mature white adipocytes or through differentiation of progenitor cells present in white adipose tissue depots. Previous studies have shown that these two tissue types arise from distinct developmental origins, altogether suggesting that classical brown adipocytes are more closely related to the myogenic satellite cells of the muscle lineage than to white adipogenic progenitors (Seale et al., 2008).

We and others recently demonstrated that bone morphogenetic protein (BMP)-7 and other BMPs play an important role in brown adipogenesis (Tseng et al., 2008; Schulz et al., 2013b). Specifically, BMP-signalling has been implicated in the process of brown adipogenic lineage determination and differentiation as well as on the systemic level where BMPs affect thermogenesis and the sympathetic activation of brown adipocytes. To determine the role of BMPR1A in brown fat development, we generated a mouse model in which BMPR1A is deleted in classical brown adipocyte progenitor cells using the Cre/loxP system (Schulz et al., 2013a). Interscapular brown fat mass was significantly reduced in knockout (KO) mice whereas skeletal muscle that arises from the same cellular lineage appeared essentially normal. As a result of loss of classical brown adipose tissue, a compensatory browning of white adipose tissue depots was observed that was mostly due to increase sympathetic input to white fat. Of note, compensatory browning not only led to a full recovery of thermogenesis in the knockout mice but also led to improved insulin sensitivity suggesting that brite adipocytes might possess additional metabolic benefits. In summary, these findings establish an essential role of BMP signalling network in the development of brown fat depots and suggest that targeting BMP signalling may present a strategy to counteract obesity by increasing brown fat mass and function.


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responses to stress and adrenocorticotropic (ACTH) identifies individuals that have increased propensity to gain adipose tissue and obesity. In this model, increased predilection to obesity is associated with a number of metabolic, behavioural and neuroendocrine differences that ultimately lead to increased propensity to obesity [3-5]. A key metabolic feature of high cortisol responders is an inherent reduction in post-prandial thermogenesis in skeletal muscle [4]. In contrast to this, genetic selection for differences in adiposity are associated with differences in post-prandial thermogenesis in adipose tissue and not skeletal muscle. Thus, we demonstrate that skeletal muscle and adipose thermogenesis can be differentially regulated. Metabolic adaption to under-nutrition and/or altered propensity to obesity do not lead to global differences in thermogenesis, but in fact can lead to site and tissue specific changes.


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SA032

Developmental regulation of adipose tissue and its role on energy balance through the life cycle

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There are now three types of adipose tissue, brown, beige (or brown in white) and white, which each have different tissue-specific molecular markers. Brown adipose tissue (BAT) is the least abundant fat in the body but is characterised as possessing uncoupling protein (UCP)1 which has the capacity to generate up to 300 times more heat than any other tissue (Symonds 2013). Adipose tissue is one of the last tissues to develop in the fetus and, in particular, has the essential feature of enabling the newborn to effectively adapt to cool exposure of the extra-uterine environment (Symonds, et al. 2012c).

Significant depots of BAT are present both around central organs such as the kidney and heart but BAT is also present in the supraclavicular region. The extent to which these BAT depots are replaced by white adipose tissue, or are transformed to a mix of beige and white adipocytes, remains a current focus of academic debate (Pope, et al. 2014). These processes can be manipulated by environmental challenges to the fetus and/or neonate, offering the potential to promote BAT function in the newborn as well as into later life (Symonds, et al. 2010).

Advances in our ability to understand tissue, or depot, specific roles of fat together with its impact on whole body energy regulation (Sacks and Symonds 2013) will be critical in developing effective early life strategies designed to prevent obesity. To this end, we have now developed the technique of thermal imaging in order to quantify potential changes in BAT activity through the life cycle in free-living subjects (Symonds, et al. 2012a). This has shown that BAT activity is much greater in children than adults and is negatively associated with body mass index (Robinson, et al. 2013; Robinson, et al. 2014). We are now beginning to further explore the impact of other factors on BAT function including diet and genetics (Symonds, et al. 2012b). These advances bring new opportunities to quantify, and manipulate, BAT development in early life, not only promote survival of the newborn but also to prevent excess adiposity through into later life (Symonds and Budge 2012).


Symonds ME & Budge H 2012 How promising is thermal imaging in the quest to combat obesity? Imaging in Medicine 4 589-591.


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SA033

Development and function of human cerebral cortex neuronal networks from pluripotent stem cells

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The formation of functional neuronal networks is one of the key aspects of neuronal network development. During development, neurons typically undergo a phase of over-connectivity followed by synaptic pruning and a reduction in neuronal connectivity. Similarly, network activity undergoes different phases of synchronous oscillatory firing before complex firing patterns emerge. The functional role of this stereotypic oscillatory network activity is not fully understood however it is believed that synchronous activity is important to shape neuronal connectivity and the development of mature neuronal networks.

Human pluripotent (PSC) stem cells can be terminally differentiated into cerebral cortex neurons in vitro. These neurons express marker from all cortical layer, form functional synapses and acquire mature electrophysiological properties over time. We used calcium imaging to monitor neuronal network activity in these neurons over weeks. We found that human
PSC-derived cortical neurons form large-scale networks in vitro that reflect those found in the developing cerebral cortex. Synchronised oscillatory networks developed in a highly stereotyped pattern over several weeks in culture. An initial phase of increasing frequency was followed by a phase of decreasing frequency, before giving rise to non-synchronous, highly recurrent, mature firing pattern. Blocking AMPA or NMDA receptors indicated that synchronous firing is a result of excitatory network activity. We investigated single neuron connectivity using a trans-synaptic rabies tracing technique. Most neurons received inputs from only a few other neurons. A small subset of hub-like neurons however received a large number of synaptic inputs indicating that the connectivity in these networks follows a power law distribution. These data shows that the formation of PSC-derived cortical networks in vitro mimics cortical network development and function and can be used to study network dynamics in health and disease.

Down syndrome (DS) is the most common form of intellectual disability and in the majority of cases is caused by Trisomy 21 (TS21). To date, little is known about the developmental and functional basis of this neurological disorder. We used TS21 stem cell derived cortical neurons to model cortical network development in DS and compared it to euploid control cells. We found that TS21 neurons displayed deficiencies in cortical network synchronisations as well as in complex network dynamics suggesting that aberrant cortical network activity, is a contributing factors to the neurological phenotypes found in DS. Taken together these results demonstrate that human stem cell derived cortical networks can serve as an ideal model system to study human cortical network development and function.

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SA034

Electrophysiological assessment of the maturation of human pluripotent stem cell-derived neurones

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The ability to generate regionally defined neuronal populations from human pluripotent cell stem cells (hPSCs) in vitro provides an increasingly utilised experimental resource for the investigation of human neuronal physiology and neurological disorders. Despite this, the physiological properties of such neurones have remained understudied. In this regard, we have developed a protocol that efficiently generates populations of excitatory cortical neurones derived from hPSCs (hECNs) and examined the functional properties of these neurones with respect to previously determined regionally-specific features and, importantly, well-defined foetal and adult properties in their native mammalian counterparts.

To generate hECNs, in vitro embryonic or induced hPSCs are neuralsed using an approach based upon the default model of neurogenesis that minimizes extrinsic and intrinsic signals that lead to alternative cell fates (Bilican et al. 2014). Neural stem cells (NSCs) generated with this protocol express anterior brain markers FOXG1 and OTX2. When NSCs are dissociated and plated as monolayer they terminally differentiate to an enriched population of VGLUT1+-hECNs that express cortical layer markers CTIP2, SAT2B or Reelin. Glia and GABAergic interneurones account for <10% of the cell population. Electrophysiological assessment of the intrinsic properties of hECNs indicates that they become progressively more excitable with time; after 5 weeks in culture >95% of cells fire action potentials. During this period of maturation we also observe that hECNs show a reduction in their input resistance and an increase in whole-cell capacitance in association with changes in excitability..

To ascertain whether hECNs expressed ligand-gated ion channels (LGICs) with immature- or mature-like properties, we carried out both biophysical and pharmacological characterisation of major neurotransmitter LGICs. Our analysis indicates that hECNs express GABAARs with a likely subunit combination of α2/3β3γ2, (James et al. 2014) while the majority of NMDARs are diheteromeric assemblies of GluN1 and GluN2B subunits. In the rodent cortex these combinations are predominately associated with early postnatal development. Interestingly our data indicate that for AMPARs, hECNs express subunit combinations that are more typical of a mature adult cortex (Livesey et al. 2014). In the mammalian CNS, the developmentally-regulated GluA2 subunit is subjected to post-transcriptional editing where a M2 pore-lining glutamine (Q) codon is edited to arginine (R), which imparts a reduced single-channel conductance (γ), insensitivity to polyamine block and reduced Ca2+-permeability to the AMPAR complex. Measurements of AMPARγ1 in hECNs at week 2 (~11 pS) and week 5 (~4 pS) are consistent with a developmental shift from GluA2(R)-lacking to GluA2(R)-containing AMPARs. Consistent with changes in unitary conductance, AMPAR-mediated currents recorded from Week 5 hECNs are insensitive to polyamine block. Interestingly, an equivalent upregulation of GluA2(R)-containing AMPARs over an equivalent time period is observed when human cortical neurones are differentiated in vitro from native human foetal cortical NPCs suggesting that the human AMPAR development time-course might be distinct from that of seen in rodents.

Using a similar experimental approach, data will be presented showing that motor neurones derived from hPSCs exhibit a rapid up regulation of the GluA2-edited AMPAR in vitro. The editing of AMPARs is a major focus for motor neurone disease (MND) research where human motor neurones show abnormalities in AMPAR editing to yield an increase in Ca2+-permeable GluA2(Q)-containing AMPARs that are thought to give rise to glutamate-mediated excitotoxicity in affected adult neurones. Data investigating functional AMPAR regulation in cortical and motor neurones derived from MND patients will be presented.

In vitro hPSC-derived neurones offer tremendous opportunities to gain mechanistic insight into human neurological disorders however it has become apparent from our own and others’ work that the neurophysiological properties of such neurones need to be characterised carefully in order to exploit them to their full potential in studies disease-modelling. Bilican B, Livesey MR, Haghi G, Qiu J, Burr K, Siller R, Hardingham GE, Wyllie DJ, (2014) Ionotropic GABA and glycine receptor subunit composition in human pluripotent stem cell-derived excitatory cortical neurones. J Physiol 592:4535-63.


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SA035

Modelling Huntington’s Disease with neurons differentiated from patient-derived induced pluripotent stem cells

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Huntington’s Disease (HD) is a neurodegenerative disorder characterised by selective loss of medium spiny neurons (MSNs) in the striatum, resulting in a decline in motor control and behavioral disinhibition. Currently, besides palliative care, there are no treatments which alter either the onset or the course of this devastating, terminal illness. HD is remarkable in that it is a genetic disorder affecting a single exon in an identified gene to produce a predictable glutamine expansion (polyQ) in a known protein (huntingtin, Htt), yet we still do not know with any certainty the causal link between the polyQ expansion in mutant Htt and the selective death of MSNs in the striatum (Zuccato et al., 2010).

Although transgenic models have provided most of the information which is currently known about the pathophysiology of HD, hallmarks of the human disease are often manifest either poorly or late in transgenic rodents. Thus, to address fundamental questions about the roles of mHtt in the human disease, we have used induced pluripotent stem cells (iPSC) from HD patients with polyQ expansions of 33, 60, and 180 (The HD-iPSC Consortium., 2012; Ebert et al., 2013), and differentiated them using a novel protocol which accelerates differentiation and synaptogenesis, such that cells demonstrate functional and synaptic marker profiles characteristic of mature central neurons within 21 days of plating (Rushton et al., 2013).

Remarkably, these patient-derived neurons show many of the polyQ-dependent disease phenotypes, including differential and BDNF-dependent glutamate-induced neuronal excitotoxicity, calcium dyshomeostasis, caspase activation, and mitochondrial dysfunction, which characterise HD. Recently, this rapid maturation system has been applied to hydrogel-supported, 3-D cultures of iPSC-derived neurons and the allelic sequence has been enhanced to include more disease-relevant polyQs, from 38 to 50. The challenge now is to translate this highly tractable disease model to high-throughput platforms in order to identify small molecules which can rescue these disease-appropriate phenotypes, paving the way for rational design of novel therapeutic interventions.


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SA036

Functional investigation of nociceptor-like neurons derived from human pluripotent stem cells

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The development of new stem cell-based technologies has occurred at a remarkable pace in recent years, and adoption of these technologies is yielding significant impacts throughout the biosciences. In neuroscience much effort has been applied to developing protocols which drive the production of both neurons and glial cells from stem cell sources. With regard to the former, extensive effort has been directed to specifying cells that resemble the multitude of neuronal subclasses found within the central nervous system. Our work, however, has been focussed elsewhere, specifically on the production, and subsequent functional characterization, of peripheral neurons from stem cell sources. We have been especially interested in producing human cells akin to primary sensory neurons, in particular human induced pluripotent stem cell (iPS)-derived nociceptors (hiPS-dNC).

Our cell generation protocols are based upon those described originally by Chambers and colleagues (2012). The protocol involves dual SMAD inhibition to induce neuroectoderm formation, followed by inhibition of GSK-3, γ-secretase, and vascular endothelial growth factor receptor/fibroblast growth factor receptor, which drives fate specification toward a sensory phenotype. Neuronal maturation is achieved with a cocktail of growth factors (brain-derived neurotrophic factor, glial derived neurotrophic factor, neuronal growth factor, and NT3). The resultant cells express a range of key sensory neuron markers such as TAC1 (substance P), neurokinin A precursor, SLC17A6 (VGLUT2), SCN9A (Na1.7), and SCN10A (Na1.8). hiPS-dNC can be prepared in this way from iPSC cells collected from both “normal” individuals and those who have known sensory disorder-related genetic mutations.

Functionally we have performed extensive neurophysiological analysis of hiPS-dNC derived from multiple donor sources using patch clamp methods at physiological temperatures. With approximately physiological recording solutions somatic resting membrane potentials were between -55 and -60 mV - in alignment with values recorded in rodent nociceptors in vivo. Most cells did not fire action potentials (APs) at rest. Input resistance values averaged <100 MW. Injection of depolarizing current stimuli resulted in TTX-sensitive AP generation in ~90% of cells. Mean rheobase varied from ~250 to ~550 pA dependent on donor. hiPS-dNC could support high rates of AP firing (>50 Hz) for extended periods. Mean AP threshold was between ~30 and ~40 mV and AP zeniths were ~30 mV. AP upstrokes were very fast, averaging around 400 V/s, and in some cases exceeding 600 V/s. Such values are more in line with non-cultured mammalian neurons, such as those recorded in tissue slices. Commensurate with this Na+ current densities were very high (as in mammalian slice)- and at physiological temperature faithful voltage clamp could only be achieved in nucleated macropatches, which had peak Na+ current densities of >500 pA/PF and K+ current densities at
purified cultures of astrocytes showed a significant decrease in glucose uptake after treatment with 2 and 0.2 µmol/L Aβ at all time points investigated (p < 0.01). In addition, a significant increase in the glycogen content of cells was also measured. Mixed neuron and astrocyte co-cultures as well as pure astrocyte cultures showed an initial decrease in glycogen levels at 6 hours compared with control at 0.2 µmol/L and 2 µmol/L P < 0.01. These changes were accompanied by changes in NAD+/NADH (P<0.05), ATP (P<0.05), and glutathione levels (P<0.05), suggesting a disruption in the energy-redox axis within these cultures. The high energy demands associated with neuronal functions such as memory formation and protection from oxidative stress put these cells at particular risk from Aβ-induced hypometabolism.

As numerous cell types interact in the brain it is important that any in vitro model developed reflects this arrangement. Our findings indicate that stem cell derived neuron and astrocyte networks can communicate, and so have the potential to interact in a tripartite manner as is seen in vivo. This study therefore lays the foundation for further development of stem cell derived neurons and astrocytes into therapeutic cell replacement and human toxicology/disease models. More recently our data provides evidence for a detrimental effect of Aβ on carbohydrate metabolism in both neurons and astrocytes. As a purely in vitro system, human stem cell models can be readily manipulated and maintained in culture for a period of months without the use of animals. In our laboratory cultures can be maintained in culture for up to 12 months months thus providing the opportunity to study the consequences of these changes over extended periods of time relevant to aspects of the disease progression time frame in vivo. In addition, their human origin provides a more realistic in vitro model as well as informing other human in vitro models such as patient-derived iPSC.


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revisited. An interesting aspect of epigenetic modification in response to environmental conditioning is the question of the stability of these resulting effects. Here, the capacity for trans-generational transfer of these modifications will be discussed, in the context of nutrition, stress and diabetes.

With this talk, our particular focus will be on obesity and the related inflammatory state, as well as effects of exercise. The rising prevalence of obesity globally is linked to concurrent increases in inflammatory diseases as well as pathologies with inflammation as an aetiological factor. Epigenetic mechanisms have been proposed to be the cause of this inflammatory state observed in obesity. In mouse adipocytes for example, over-expression of DNMT3A resulted in increased expression of pro-inflammatory genes in obese mice. Similarly, epigenetic modifications have also been illustrated in models of dietary obesity. In this context, rats fed a high energy diet had significantly higher methylation in the leptin gene promoter region, which was associated with lower circulating leptin levels. At least some of the epigenetic modifications associated with dietary obesity-related inflammation is transgenerationally transferred – an upregulation of NLRC4, which is critical in the formation of the inflammasome, was reported in both first and second generation offspring in high-fat fed mice. From the studies reviewed, excessive inflammatory states seem to result from DNA hypomethylation, which leads to an upregulation of inflammatory gene expression.

This poses an interesting question: are high-performance athletes at risk of inflammation-induced epigenetic modification? Although results from human studies in the context of habitual exercise are emerging to inform on the effect of exercise on the epigenome, the models used and focus parameters are quite varied, so that we have more questions than answers at this stage. The burning issues in the field will be highlighted. Finally, some interesting recent findings on IL-6 signalling from separate human exercise studies performed in our own and collaborator laboratories will be discussed in terms of modifications at both protein expression and epigenetic level, in an attempt to answer this question.

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SA039

Global DNA methylation and gene expression changes with long-term endurance training

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Regular endurance exercise training induces beneficial functional and health effects in human skeletal muscle. The putative contribution to the training response of the epigenome as a mediator between genes and environment has not yet been clarified. We have investigated the contribution of DNA methylation and associated transcriptomic changes in a well-controlled human intervention study. The response to a three-month supervised one-legged knee-extension endurance training program (n=23) was evaluated using performance tests and metabolic enzyme activity assays. DNA methylation and transcriptomic profiling were performed on vastus lateralis muscle biopsies, using Illumina 450k beadchip and RNA-seq. Transcriptome data was investigated for a subset of the subjects (n=12) that participated in a follow-up training intervention one year later training both legs, one at a time, at exactly the same work load as the first period.

A signature of DNA methylation and gene expression separated the samples based on training and gender. The training effects were mirrored by alterations in DNA methylation and gene expression. We detected 4919 differentially methylated positions (DMPs) and 4076 differentially expressed genes (FDR<0.05). DMPs were predominantly found in enhancers, gene bodies and intergenic regions and less in CpG islands or promoters. Ontology analysis demonstrated that muscle related processes e.g. myogenesis, muscle energetics and tissue remodelling were enriched. The analysis identified transcriptional regulator binding motifs of Myogenic Regulatory Factors, Myocyte Enhancer Factor 2 and the ETS group in the proximity of DMPs. An integrative analysis identified positive or negative correlations between methylation and expression, suggesting coordinated training-induced modifications. A transcriptional network analysis revealed modules with distinct ontologies, and the overall direction of methylation changes within each module was inversely correlated to expression changes, lending further support to a coordination between the epigenome and the transcriptome. From second training period, we found neither evidence of any remaining transcriptome effects from the first training period, nor any difference in response between the previously trained leg compared to the previously untrained leg.

In conclusion, this data provides a valuable and novel perspective on the fields of human physiology and environmental epigenomics, showing that a physiological stimulus can induce highly consistent and associated modifications in methylation and expression concordant with the observed health-enhancing phenotypic adaptations. This could represent a mechanism for variability in individual response to lifestyle interventions or disease susceptibility.

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SA040

The impact of exercise on the epigenome of human skeletal muscle

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Skeletal muscle is a highly adaptable and plastic organ that responds to a single acute exercise bout by inducing the expression of genes involved in numerous functions, such as oxidative metabolism, protection against oxidative stress or improvement of the contractile apparatus. Several epigenetic mechanisms are mediators of contraction-induced gene expression, notably histone modification and changes in DNA methylation on exercise-responsive genes. These transient changes suggest that epigenetic mechanisms are not restricted to early stages of human development, but are
broad dynamic controllers of genomic plasticity in response to environmental factors.

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SA041

MicroRNA expression in the development of insulin resistance

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(Mal)adaptive responses to nutrition and energy balance are driven by the regulation of gene expression. Whereas these (mal)adaptive responses are reasonably well characterised at the genome level, regulation by the epigenome is less well understood. Epigenetic control mechanisms include microRNAs (miRNAs), and since the discovery of miRNAs two decades ago (Lee et al., 1993), an increasing number of research groups have started to investigate miRNA regulation of gene expression in various contexts.

miRNAs exist in both tissue and the circulation, although their mechanism of action is confined to tissue. Given that skeletal muscle accounts for ~75% of non-oxidative glucose disposal in the postprandial state (Björnholm & Zierath, 2005), measurements of gene expression and post-translational modification in skeletal muscle are often made in studies investigating insulin sensitivity / resistance. However, post-transcriptional regulation, and in particular miRNA regulation, of gene expression has been less well studied within the context of insulin sensitivity. Only in the past 5 years or so has the role of miRNAs within type 2 diabetes (T2D) pathology been highlighted. Some researchers have now even suggested that T2D may be considered a miRNA-related disease (Guay et al., 2011). Thus, the impact of skeletal muscle miRNA regulation in insulin resistance pathology is apparent.

Adipose tissue, liver and pancreatic β cells, like skeletal muscle, are involved in the regulation of energy excess and T2D pathology. Considering (mal)adaptive responses to energy excess span several tissue types, plasma miRNAs may be involved in this whole-body response - essentially communicating between insulin-responsive tissues (Ortega et al., 2014). miRNAs have been implicated in cell-cell communication (Turchinovich et al., 2013) and are actively secreted into the circulation, rather than released in a non-specific manner, from tissue (Mittelbrunn et al., 2011; Rayner & Hennessy, 2013). For these reasons, plasma miRNAs may be useful biomarkers of a variety of adaptations.

Data are beginning to accumulate to suggest that different miRNAs may be involved in the regulation of gene expression along the continuum between initial development of insulin resistance and manifest T2D. However, the impact of short-term high-fat energy excess (HFEE) as a model for insulin resistance development and / or the impact of glucose consumption on plasma and skeletal muscle miRNA levels in humans is currently unknown. We investigated changes (basal and following oral glucose consumption (OGTT)) in miRNA levels following 6 d HFEE (150 % habitual energy intake; 60 % of energy from fat) in non-active, healthy males (n = 20). Ten of twenty participants consumed 10 % of total fats from fish oil (FO) sources. FO consumption was included in this subset of participants to ascertain whether changes in dietary fat consumption modified insulin sensitivity change and / or altered miRNA profiles. We hypothesised that plasma and skeletal muscle miRNA levels would be altered by HFEE (both basal and the OGTT response) and that some associations would exist between miRNA levels in different sample types and insulin sensitivity change.

We demonstrate that levels of a number of miRNAs were significantly altered by HFEE in skeletal muscle (miR-106b-5p, miR-214-3p, miR-215) but not plasma. Certain miRNAs were responsive to the OGTT including miR-145-5p (plasma) and miR-193a-3p, miR-206 (skeletal muscle). No miRNAs in either tissue type were solely group-responsive; however, several group interactions existed with HFEE and / or the OGTT (miR-7-5p, miR-27a-3p (plasma), miR-18a-5p, miR-145-5p, miR-214-3p (skeletal muscle)). Basal levels of miR-145-5p (plasma) and miR-204-5p (skeletal muscle) significantly predicted 16-23 % of the change in HFEE-mediated insulin sensitivity. Certain miRNAs may be useful markers of the high-fat overfed state (with or without FO), the response to oral glucose consumption and / or the combined influence of these. However, clear associations between plasma and skeletal muscle levels of these miRNAs are lacking, suggesting that investigation of these plasma miRNAs within the context of HFEE may not inform functional outcomes within skeletal muscle, but may better relate to other tissue types.


Ortega FJ et al. (2014). Diabetes Care 37, 1375–1383.


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SA038

Non-coding RNA expression in diet, exercise and disease

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Epigenetics is the study of heritable changes in phenotype that are the result of things other than changes in the DNA sequence itself. The field includes histone modifications, DNA methylation patterns and non-coding RNA expression. All of which are involved in the regulation of gene expression. Whilst the central dogma of genetics may suggest that all RNA is translated into protein, non-coding RNAs have been known of for many years. The most obvious examples would be ribosomal RNA (rRNA) or transfer RNA (tRNA). However, recent projects such as ENCODE (1) report that whilst only ~3 % of the genome is translated into protein, in the region of 62-85 % of the genome is transcribed into RNA. This means that the vast majority of RNA is non-coding. This non-coding RNA has been divided into multiple classes including: rRNA; tRNA; PIWI-interacting RNA (piRNA); small nucleolar RNA (snoRNA); long non-coding RNA (lncRNA); microRNA (miRNA); transcribed ultra-conserved regions (T-UCR); and, anti-sense transcripts. There are also many failed / stalled transcripts
leading to debate about the exact amount of overall transcription that is functional. However, it is clear that the vast majority of RNA produced in the cell is non-coding and that much of this is functional.

The recent focus on non-coding RNA has largely been on miRNA. miRNAs are short (~22 nucleotide) RNA molecules that primarily bind to the 3' UTR of messenger RNA (mRNA) molecules either preventing translation of mRNA or targeting the mRNA for degradation. They were first discovered in *C. elegans* in 1993 but are now known to be widespread throughout animals, plants and viruses. In humans there are only 1881 known miRNAs (http://www.mirBase.org accessed 2015 Mar 27). However, each miRNA binds to multiple mRNAs and each mRNA is bound by multiple miRNAs. Thus, miRNAs comprise a highly complex network for the fine tuning of gene expression. MiRNAs have been shown to be altered by diet and exercise and are likely to be involved in the normal physiological response to a number of stimuli (2). They have also been shown to be consistently altered in many disease states suggesting that they are part of the physiological response to disease, or involved in the development of disease itself (3).

More recently, miRNAs have also been observed in plasma (4). Initially, these were thought to be debris from damaged or dying cells; however, they are also released by healthy cells and are consistently altered in specific disease states. Furthermore, they appear to be actively secreted, with miRNAs released by cells in differing proportions to the miRNAs within those cells. These circulating miRNAs (c-miRNAs) have been shown to be taken up by recipient cells and alter gene expression within the recipient cells, suggesting that they are involved in cell-cell communication. Thus, they are not only useful biomarkers of disease but may also have some function in the circulation. One of the most intriguing miRNA findings in recent years was the identification of plant miRNAs in the plasma of individuals who consume large quantities of rice. However, we and others have failed to replicate this and debate remains about the functionality of such trans-kingdom miRNAs. Like tissue miRNAs, c-miRNAs have also been shown to be altered by exercise (5) and disease (4).

We have shown that c-miRNAs differ between endurance athletes and strength athletes (6). We have also shown c-miRNAs to respond to 6 weeks of olive oil supplementation in humans (unpublished). In the space of a few years we have moved from having little understanding of miRNAs with most research focusing on their involvement in cancer, to having vast amounts of data on miRNA involvement in numerous situations. As we recognise more sub-classes of non-coding RNA and begin to investigate their response to common stimuli, it seems likely that more non-coding RNA molecules will be implicated in the physiological response to diet, exercise and disease. T-UCRs are a class of long non-coding RNA that have been shown to be dysregulated in cancer (7). There are 481 known human T-UCRs. However, little data exists on their normal cellular function, or on their response to common stimuli.

We investigated the expression of T-UCRs from skeletal muscle biopsies of human volunteers (n=20) at 0 hrs and 2 hrs of an oral glucose tolerance test, before and after a period of high-fat feeding. We showed that 53% of T-UCRs are expressed at appreciable levels in skeletal muscle and using a pooled sample approach, identified T-UCRs which either responded to glucose feeding during the OGTT, or to hyper-calooric high fat feeding.

Understanding the response of the myriad of non-coding RNA molecules to common stimuli will be crucial to developing a full understanding of human physiology.


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Regulation and function of polycystin-2

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Polycystin-2 (PC2) is a calcium permeable Transient Receptor Potential (TRP) channel activated and regulated by changes in cytoplasmic calcium. PC2 mutations are responsible for ~20% of Autosomal Dominant Polycystic Kidney Disease (ADPKD). This presentation will address two questions: first, how does calcium open the channel formed by PC2 and second, how do mutations in PC2 lead to cyst formation in ADPKD. For the first question, we noted that the C-terminal cytoplasmic tail of PC2 has been shown to contain calcium-binding EF-hand domains and the C-terminal tail of human (hPC2) contains two EF hand motifs, but only the second binds calcium. We proposed that these EF hand motifs serve as a calcium sensor responsible for the calcium-dependence of PC2 function. Using NMR and bioinformatics, we found that the overall EF hand fold is highly-conserved, but in evolutionarily earlier species, both EF hands bind calcium. To test if the EF hand motif is truly a calcium sensor controlling PC2 channel function, we altered the number of calcium binding sites in hPC2. Mutant PC2 channels unable to bind calcium via the EF-hand are inactive in single-channel planar bilayers. hPC2 that was modified to bind an additional calcium ion, as confirmed by NMR studies, demonstrated a shift in the calcium dependence in single channel recordings and enhanced calcium transients in imaging studies using fluorescent calcium-sensitive dyes (compared with wild-type hPC2). However, the channel was only functionally active if the second (native) calcium binding EF hand was intact. These results suggest that the number and location of calcium binding sites in the EF hand are important for normal PC2 channel activity and cellular function. For the second question we examined the effect of altered calcium signaling on cyst formation using 3D cultures of kidney epithelial cells. Stable cell lines were created with calcium signaling altered by decreased expression of PC2 or inositol 1,4,5 trisphosphate receptor (InsP3R) isoforms and monitored for the number and size of the resulting cysts. When these modified cells were maintained for 8 weeks in 3D cultures, all knockdown cell lines had more cysts and the cysts were larger than the control cells. Knockdown of InsP3R type 1 lead to the largest cysts. The primary cilia has been proposed to be essential for cyst growth, however the cysts with InsP3R type 1 knockdown continued to enlarge even though they had lost their cilia after 4 weeks. These studies suggest that calcium binding to PC2 is necessary for function and that altered calcium transients, due to changes in PC2 or other intracellular calcium channels, will lead to cyst formation and growth. We propose that the alterations in intracellular calcium signaling that occur when mutated PC2 is expressed are a critical component of the pathophysiological changes that lead to ADPKD.


*Wardle SL et al. PLoS One. 2015; 23P.*


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

SA043
Mechanotransduction is the process by which mechanical stimuli are converted into biological activity. Piezos are mechanically activated (MA) cation channels conserved through evolution and act as mechanotransducers in various biological processes. The single Piezo gene in flies is involved in nociception; zebrafish and mouse Piezo2, in touch sensation; zebrafish Piezo1, in red blood cell volume regulation; and mouse Piezo1, in vascular development. In humans, mutations that alter channel gating of Piezo1 and 2 are linked to various disorders with dominant inheritance. Mutations that alter channel gating of Piezo1 and 2 are linked to various disorders with dominant inheritance. Basic questions regarding Piezo topology and the location of the ion permeation pathway remain unanswered, and yet these questions are crucial for a mechanistic understanding of how the channel is gated by mechanical forces, and how human disease-related point mutations affect channel function. We used a combination of bioinformatics and detection of inserted tags and phosphorylated sites to study mouse Piezo1 transmembrane topology. Using a chimeric approach followed by site directed mutagenesis, we identified a residue that when mutated impacts ion permeation properties. We propose that this amino acid is either in the pore or closely associates with the pore.

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

SA044

Structural features of piezo channels
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SA045

Role of TRPA1 channels in thermal nociception and inflammatory pain
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TRPA1 are calcium permeable, non-selective cation channels expressed in somatic and visceral nociceptors and many non-neuronal cells, including fibroblasts, odontoblasts, endothelial and glial cells. These channels are rapidly emerging as critical participants in the biological response to physical stimuli (temperature and pressure) and natural and synthetic environmental irritants, including many reactive electrophiles, oxidants and inflammatory mediators. Abnormal activation of TRPA1 has been linked to the pathogenesis of neuropathic pain, itch, atopic dermatitis, headache and asthma. Moreover, these channels are activated by clinically relevant drugs (e.g. dihydropyridines, chloroquine, clotrimazole, phenytoin) and this activation may result in unwanted side effects, but could also serve as a useful chemical template for the design of novel activators.

The activation of TRPA1 by temperature has been controversial but evidence for its role in noxious thermosensation has gained significant weight since its description as a noxious cold sensor in 2003 by Patapoutian and colleagues. Recently, we uncovered a novel role of TRPA1 channels as sensors of lipopolysaccharide (LPS) (2), a toxic Gram-negative bacterial product, leading to pain and neurogenic inflammation. The ancient nature of LPS and the observation of a conserved role of TRPA1 in chemical avoidance underscore the evolutionary significance of this alert mechanism in animals.

There is evidence that two pore channels (TPCs), a family of ion-channel localised to acidic stores, may be responsible for potently releasing Ca⁡²⁺ ions. Nicotinic acid adenine dinucleotide phosphate (NAADP) is a recently identified activator of these channels. NAADP gains significant weight since its description as a noxious cold sensor in 2003 by Patapoutian and colleagues. Recently, we uncovered a novel role of TRPA1 channels as sensors of lipopolysaccharide (LPS) (2), a toxic Gram-negative bacterial product, leading to pain and neurogenic inflammation. The ancient nature of LPS and the observation of a conserved role of TRPA1 in chemical avoidance underscore the evolutionary significance of this alert mechanism in animals.

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SA046

Recombinant human TPC2 and TPC1 are both NAADP-regulated ion channels but display important functional differences
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Nicotinic acid adenine dinucleotide phosphate (NAADP) potently releases Ca²⁺ from acidic intracellular Ca²⁺ stores. There is evidence that two pore channels (TPCs), a family of ion-channel localised to acidic stores, may be responsible for...
TRPM2 channels in ischemia

B.A. Miller and J.Y. Cheung

TRPM2 is the second member of the melastatin subfamily of TRP channels to be cloned. Previously called LTRPC2, the human channel consists of 32 exons encoding a protein of 1503 amino acids with a predicted molecular mass of ~170 kDa. The amino acid sequence of human and mouse TRPM2 are 82% identical. TRPM2 channels are widely expressed and are activated by extracellular signals including oxidative stress, TNFα and amyloid β-peptide. Stimulation of cells with these extracellular signals results in production of ADP-ribose (ADPR), which activates the channel by binding to the TRPM2 C-terminal NUDT9-H domain. TRPM2 is also positively regulated by intracellular Ca^{2+} and calmodulin and is inhibited by acidification. TRPM2 channels function as tetramers and the association of splice variants can modulate its function, especially the short isoform TRPM2-S, which is missing four C-terminal transmembrane domains and the putative Ca^{2+} pore. TRPM2 plays an essential role in susceptibility to oxidative stress in a number of tissues including heart. Early reports suggested that during hypoxia, ROS are produced that enhance ADPR production, which activates TRPM2 channels, leading to elevations in [Ca^{2+}i], cytokine production, inflammation and cell death. However, recent work suggests that this is not necessarily the case. In TRPM2 knock-out (KO) mice injected intraperitoneally with endotoxin, survival was 5 times worse than wild-type (WT), due to enhanced NADPH oxidase-mediated ROS production by KO phagocytes. In the heart, TRPM2 is expressed in the sarcolemma and transverse tubules. Compared to WT mice, baseline cardiac function (+dP/dt, ejection fraction) was not different in either global KO (gKO) or cardiac-specific KO (cKO) mice. After ischemia reperfusion (I/R), both gKO and cKO hearts had significantly lower +dP/dt compared to WT while ROS levels were significantly higher. Superoxide dismutases (SODs) and their upstream regulators (forkhead box transcription factors and hypoxia-inducible factors) were lower. Proteomes of WT-I/R and gKO-I/R hearts showed the largest differences were in mitochondrial dysfunction. Western blots confirmed reduced expression of Complex I subunits and other mitochondrial associated proteins in the KO. KO myocytes and hearts had lower mitochondrial membrane potential (γm), Ca^{2+} uptake, ATP production, and O2 consumption rates (OCR), but higher mitochondrial superoxide levels. Reduced mitochondrial Ca^{2+} uptake was due to both lower γm and mitochondrial Ca^{2+} uniporter (MCU) activity. Genetic rescue of gKO myocytes with WT TRPM2 followed by H/R reduced superoxide production. This required Ca^{2+} influx through TRPM2 since the loss-of-function TRPM2 mutant (E960D) was ineffective. The mitochondrial superoxide scavenger MitoTempo but not the cytosolic scavenger (Tempol) reduced superoxide levels and restored γm in KO-H/R myocytes. These studies demonstrate that TRPM2 protects heart from I/R and H/R injury by decreasing generation of and enhancing scavenging of ROS in heart. In addition, TRPM2 channels are important in maintaining mitochondrial function and provide the necessary Ca^{2+} for normal mitochondrial bioenergetics. TRPM2 channel expression in cells expressing TRPM2-S was found to contribute to decreased expression of genes involved in glycolysis (lactate dehydrogenase A and enolase 2), oxidant stress (FOXO3a), angiogenesis (VEGF), and mitochondrial function (BNIP3 and NDUFA4L2). The decrease in BNIP3 plays a role in reduced mitophagy, resulting in accumulation of dysfunctional mitochondria and increased ROS, reducing cell viability and tumor growth. Reduced expression of other mitochondrial proteins and reduced Ca^{2+} entry further compromise mitochondrial function, resulting in lower ATP production and contributing to increased ROS. The finding that TRPM2 is important in mitochondrial function in both protection of cardiac cells from ischemia and in tumor growth.
and response to chemotherapy suggest it has a basic role in mitochondrial bioenergetics which may apply to a number of physiological systems.


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SA048

Hypothalamic tanycytes in metabolic regulation

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The survival of an organism relies on its ability to promptly, effectively and reproducibly communicate with brain networks that control food intake and energy homeostasis. To achieve this, circulating factors of hunger and satiety reflecting nutrient availability must cross the blood-brain barrier (BBB) to reach effector neurons. A defect in this process invariably leads to uncontrolled body weight. Here we will discuss the key role played in this process by a peculiar type of glial cells named tanycytes, which have their cell bodies lining the floor of the third ventricle and their endfeet contacting the pial surface of the brain. Recent studies indeed suggest that tanycytes, besides regulating hypothalamic BBB plasticity according to nutrient status, capture metabolic signals such as leptin from the bloodstream and transport them towards their cell body for release into the cerebrospinal fluid. Blockade of this conduit for peripheral metabolic factors into the brain of obese individuals is thought to contribute to the pathophysiology of central hormonal resistance.

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SA049

Tanyocytes excite neurons of the hypothalamic arcuate nucleus in mice

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The hypothalamus controls the feeding/appetite and energy expenditure in mammals. One region of the hypothalamus, the median eminence, which is a circumventricular organ, has unusual structural features. Fenestrated capillaries in the region have a vital role in allowing entry of peripheral signals into the brain. Peripheral hormones and nutrients from the blood stream can then be easily sensed and consequently regulated by the central nervous system. The blood brain barrier in the region is regulated by a particular glial cell, called tanycytes. Their cell bodies line the third ventricle, contact the cerebrospinal fluid, and project a single process towards hypothalamic nuclei, such as the arcuate, ventromedial and dorsomedial nuclei.

Our laboratory recently demonstrate that tanycytes can directly sense glucose, artificial sweeteners and L-amoeno acids via activate specific receptors to induce Ca2+ waves in an ATP receptor-dependent manner. Changes in the tanyctic blood brain barrier were also observed according to peripheral signals. Tanycytes therefore can act as cellular integrators of macronutrient signals. An important question is whether these signals in tanycytes are also communicated to hypothalamic neurons?

To demonstrate tanycyte-neuron communication we expressed a calcium permeable version of channelrhodopsin2 (CaCh) specifically in tanycytes and recorded from neurons of the arcuate nucleus, with patch clamp methods. Our data suggest, that optogenetic activation of tanycytes can excite a range of neurons in the arcuate nucleus. This suggests that tanycytes can convey information about the macronutrient status of an individual to the hypothalamic neural networks that control energy homeostasis. They must therefore be considered as an integral and important component of these neural networks.

We thank the Medical Research Council for support.

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SA050

FGF-responsive tanycytes: Adult hypothalamic stem/progenitor cells

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Recent evidence has shown that adult neurogenesis is sustained in the hypothalamus, a region of the brain that is the central regulator of homeostasis. While studies support a role for adult neurogenesis in energy balance, the identity of the neural stem/progenitor cell remains contested. Tanycytes present possible candidate(s) due to their radial-glial like morphology and position adjacent to the 3rd ventricle. We have investigated the properties of alpha-tanycytes. Our studies show that the embryonic neural stem/progenitor characteristics of radial glia, including expression profile, a basal process and an apical primary cilium, are maintained in alpha-tanycytes during adulthood. In addition, alpha-tanycytes are multipotent in vivo and contribute to the other tanyocyte populations. A neurosphere assay adds further validity to the idea that alpha-tanycytes are a self-renewing stem cell, and suggest that there is heterogeneity in progenitor status within tanycyte subpopulations. We show, additionally, that alpha-tanycytes are responsive to FGF-signalling, a crucial regulator of proliferation and differentiation during embryogenesis. In current studies we are analysing the response of alpha-tanycytes to glucocorticoids, and asking whether glucocorticoids provoke long-term changes in the hypothalamus.

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Neuroendocrine pulsatility: Role of the hypothalamic periventricular area
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Pituitary hormones are released in the blood stream in pulses. This pulsatility prevents both secretory machinery fatigue and target tissue desensitization, and reflects the intermittent electrical activity of hypothalamic neuroendocrine cells. It has proved difficult to decipher the mechanisms shaping the activity of neuroendocrine cells because they are often anatomically dispersed or integrated into complex networks. In terms of structure-function relationship, neuroendocrine cells are distributed along the third ventricle and can be viewed as secretomotor output units linked to their target organs via a neurovascular synapse. It is thought that their afferent command either partially or fully shape the pulsatile rhythm, and a hypothesis (Brain Res Rev 41:153) posits that a determinant set of periventricular nuclei harbour neuroendocrine central pattern generator (CPG) networks driving the neuroendocrine cells. Over the passed years ([Neuroph 76:2772, JN 18:6641, EJN 11:1960 and 17:2619, JN 28:385, NatCommun 5:3285), we have developed an in vitro model that appeared suited to address neuroendocrine pattern generation. This model is an organotypic culture of thick (350um) frontal slices through the anterior hypothalamic area including the supraoptic nucleus (SON) from new-born rats. In these cultures, surprisingly, the neuroendocrine magnocellular oxytocin (OT) neurons of the SON spontaneously have the same rhythmic electrical activity that they naturally display in adult lactating female rat in response to pup suckling, i.e., during the milk-ejection reflex. This rhythmic activity is typically made of high-frequency (20-50 Hz) bursts (3-8 sec) of action potentials occurring every 3-10 min in a coordinated fashion in all the OT neurons. This electrical activity allows in vivo the release of a bolus of OT in the circulation to act at the mammary gland. We thus have used these organotypic cultures to test the hypothesis that a female-specific network is entirely driving the rhythmic activity of OT neurons, analogous to the female-specific expression of rhythmic (ovulatory cycles) activity in the hypothalamo-pituitary-gonadal axis. Our results indeed suggest that a female-specific CPG network drives the pulsatility of OT neurons of the SON. This CPG is active in both sexes at birth but is subjected to postnatal apoptotic-like silencing in males during the period of brain sexual differentiation when estradiol produced from aromatized circulating testosterone sculpt brain reproductive networks. Furthermore, in accord with the above hypothesis, removing the periventricular area from the tissue slices harvested for organotypic culture completely abolished the bursting activity of OT neurons. In conclusion, the periventricular area comprises a neural network important for driving the expression of a female-specific pulsatile behaviour in neuroendocrine in OT neurons.

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The role of synaptic plasticity in relation to energy intake (glucose sensing) in the hypothalamus
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Synaptic re-organization of hypothalamic feeding circuits in response to metabolic shifts involves neurons as well as astrocytes, cells that can directly respond to the metabolic hormone, leptin, in vitro. The role of glia cells in hypothalamic synaptic adaptations had been unclear. We have recently shown that leptin receptors are expressed in hypothalamic astrocytes and that conditional, adult deletion of leptin receptors in astrocytes leads to altered glial morphology, decreased glial coverage and elevated synaptic inputs onto hypothalamic arcuate neurons such as pro-opiomelanocortin (POMC)- and Agouti-related protein (AgRP)-producing neurons. Leptin-induced suppression of feeding was reduced, while rebound feeding after fasting or ghrelin administration was elevated in mice with astrocyte-specific leptin receptor deficiency. These data unmask an active role of glial cells in the initiation of hypothalamic synaptic plasticity and neuroendocrine control of feeding by leptin.

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Mice are not little rats: differences in breathing, chemoreflexes and thermoregulatory control mechanisms in mouse and rat models of serotonin dysfunction
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Introduction: Feedback control systems that regulate breathing require a flexible, dynamic system capable of responding to acute ventilatory challenges including hypercapnia and acidosis. This reflex arc must also be adaptable to additional environmental changes, including shifts in ambient temperature. Brainstem serotonergic (5-HT) neurons play integral roles in acute arterial CO2/pH regulation, but also participate in cold defense mechanisms and facilitate thermogenesis. How is the CO2/pH chemoreflex altered in mammals with varying environmental or brain temperature? And, are there cellular correlates to these unique interactions of temperature and CO2 chemoreception? Methods: We study breathing using whole body plethysmography, and thermoregulation with a variety of thermal imaging and temperature detection techniques. These were applied to genetically modified mouse models of 5-HT neuron lesions and/or dysfunction. Results: Moderate or near-complete genetic deletion of central 5-HT-producing neurons in conscious mice leads to selective but severe deficits in ventilatory responses to hypercapnia, and failure to generate sufficient heat to defend core body temperatures during a cold challenge. Mild environmental cooling in these models led to small but significant drops in core temperature which negatively affected both the hypercapnic and hypoxic ventilatory responses, nearly exclusively in male but not female mice. Infusing additional 5-HT into the
CNS "rescued" the CO2 chemoreflex but failed to alter cold defense failure. Direct cellular recordings identified a binary phenotype – 5-HT neurons either respond to increased temperature or CO2/pH directly in acute slices from young mice. Finally, knockout of CNS 5-HT in rats, while retaining the neurons that would otherwise make 5-HT, has no effects on either the CO2 chemoreflex or thermoregulation in conscious rats. Conclusions: We conclude that brainstem neurons that synthesize both at the cellular and organismal level contribute to defenses against hypercapnia and acidosis and cold environments, but this may relate to additional neurotransmitters produced by these neurons and not 5-HT itself.

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SA054

Orexinergic modulation in amphibians and mammal

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Orexins (or hypocretins) play an important role in the modulation of respiratory control in mammals, but there are no data describing the role of the orexinergic system in the peripheral and central chemoreception of non-mammalian vertebrates. Thus, the aim of this study was to examine the localization of orexin-immunoreactive neurons in the brain of toads (*Rhinella schneideri*) and to investigate the contribution of orexin receptor-1 (OX1R) and hypercapnic (5%CO2) ventilatory responses during light and dark phases by intracerebroventricular (i.c.v.) injections of SB-334867 (OX, R selective antagonist; dose: 5 and 10mM).

In addition, we injected SB-334867 (OX1R antagonist, 5 mM) into the locus coeruleus (LC) of male *Wistar* rats and measured ventilation (VE) using a whole-body plethysmograph, together with EEG and EMG during normocapnia and hypercapnia. In toads, our results demonstrated that the SB-334867 attenuated the ventilatory response to hypercarbia during the dark phase in 54.8% by acting on tidal volume only (vehicle: 230.9 ± 11.2% of baseline; SB-334867: 114.2 ± 5.3% of baseline) and breathing frequency (vehicle: 218.3 ± 24.1% of baseline; SB-334867: 134.8 ± 7.8% of baseline), while during the light phase, attenuated the ventilatory response to hypoxia in 51.6% by acting on tidal volume only (vehicle: 230.9 ± 33.4% of baseline; SB-334857: 127.5 ± 4.9% of baseline). As to mammals, SB-334867 injection caused a significant attenuation of the hypercapnic ventilatory response during wakefulness (△VE vehicle= 1010 mL·kg⁻¹·min⁻¹ vs △VE vehicle= 793 mL·kg⁻¹·min⁻¹) but not during sleep (△VE vehicle= 747 mL·kg⁻¹·min⁻¹ vs △VE vehicle= SB-334867 = 703 mL·kg⁻¹·min⁻¹). We conclude that in *R. schneideri*, orexinergic neurons are located in the suprachiasmatic nucleus and OX1R contributes to hypercarbic and hypoxic chemoreflexes. In rats, OX action on OX1R in the LC exerts an excitatory modulation in the hypercapnic chemoreflex during wakefulness. Financial support: FAPESP,INCT-FisComp, UNESP and CNPq.

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SA055

Cold-acclimation reduces CO2/pH chemosensitivity of locus coeruleus neurons in the American bullfrog, *Lithobates catesbeianus*

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American bullfrogs, *Lithobates catesbeianus*, are air-breathing ecothermic vertebrates that use both pulmonary and cutaneous gas exchange to meet their metabolic needs. Elevated metabolic rates at warm temperatures require pulmonary ventilation for O2 acquisition and CO2 elimination in frogs. At temperatures below ~5°C metabolism is reduced such that cutaneous gas exchange should be sufficient to sustain aerobic metabolism. As a result, lung ventilation diminishes in the cold. American bullfrogs overwinter for 3-5 months (depending on latitude and location) significantly decreasing activity of the respiratory control system that drives lung ventilation for an extended period. In fact, overwintering in aquatic habitats covered with ice (e.g., frozen ponds) completely prevents aerial respiration. In the spring, water temperatures can rise rapidly (Tattersall & Ultsch, 2008) and cutaneous gas exchange will no longer be sufficient to sustain metabolically expensive spring behaviors such as calling. The system that controls lung ventilation that has been quiescent must function properly to match both metabolic demands for O2 and for CO2 elimination requirements. How does the respiratory control system that drives lung ventilation resume operation following months of disuse and work at near maximal levels? Given the importance of central chemosensing for ventilatory control of acid-base balance, we addressed this question by investigating how cold-acclimation alters cellular CO2/pH sensitivity. The locus coeruleus is a chemoreceptive brain region that contributes to control of pulmonary ventilation of anuran amphibians in vivo (Noronha de Souza *et al.*, 2006). We assessed chemosensitivity of locus coeruleus neurons using whole-cell patch clamp electrophysiology (methods described in Santin *et al.*, 2013) from bullfrogs acclimated to either ~22°C or ~2°C for two months. We determined that chemosensitive locus coeruleus neurons from cold-acclimated bullfrogs had reduced sensitivity to hypercapnia [increase from 1.3% CO2 (normocapnia) to 5% CO2 (hypercapnia); cold-acclimated: 92±29% (n=13) vs. control: 221±41% (n=7); p=0.025; two-tailed unpaired t test]. We showed that 86% (30/35) of neurons had chemosensitive responses (Santin *et al.*, 2013); however, only 54% (7/13) of locus coeruleus neurons from cold-acclimated frogs exhibited CO2/pH-sensitive firing rates (p=0.0002; Fisher exact test). Baseline firing rates did not differ between control and cold-acclimated animals (p=0.70; two-tailed unpaired t test), suggesting that normocapnic excitability is unaltered by cold-acclimation. These results provide evidence that critical components of the respiratory control system that regulate lung breathing (e.g. neuronal chemosensitivity) are maintained, although at a reduced capacity, despite several months of reliance on cutaneous gas exchange. Maintenance of central or peripheral components of the respiratory control system in the near or complete absence of lung breathing may be essential for temperate frogs to resume breathing sufficiently to meet metabolic demands upon warming in the spring after overwintering. Understanding how overwintering frogs switch from extended use of exclusively cutaneous gas exchange to pulmonary ventilation will provide new insights into robustness of the respiratory system that controls lung breathing.
The effect of changing temperature on central chemosensing and metabolism in the vertebrate brain

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Regulation of intracellular and extracellular pH is a fundamental homeostatic requirement for vertebrates, and metabolic perturbations to an animal’s acid-base status are primarily compensated by adjusting ventilation. Ventilation is controlled via negative feedback with O₂, CO₂, and pH as primary signals; peripheral (arterial) and central (brainstem) chemoreceptors sense these signals through activation of CO₂/pH-sensitive neurons; respiratory rhythm is set in the integrator and modulated by negative feedback control via the breathing muscles (effectors). These feedback systems have been most fully elucidated for mammals where constant pH is normal, chemosensory structures are well defined, and the cellular mechanisms underlying chemosensitivity are under active investigation. In contrast, a significant gap in our knowledge of ventilatory control is that we know relatively little of the mechanisms underlying chemosensitivity in poikilotherms. These animals thus provide an ideal model for studying the relation between cellular chemosensitive responses and respiratory pattern generation.

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

SA056

Virtual dissection and human patient simulator use in Life Sciences

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Simulation in teaching is an ever-expanding field, providing opportunities for safe and experiential learning across all fields of education (1).

The University of Bradford is home to the Faculty of Life Sciences Integrated Learning Centre (ILC), phase I consisting of the Simulation Lecture Theatre (completed October 2013). This purpose-built facility is dedicated to the use of technology and simulation in Life Sciences teaching, and integrates the Anatomage Table® and the high fidelity human patient simulator (HPS, iStan®) with high-end audio-visual equipment. The facility is designed to enhance learning and teaching across areas of anatomy, physiology, pathology and pharmacology within all Faculty of Life Sciences programmes, and provides inter-Faculty links with programmes such as Radiography, Physiotherapy, and Nursing.
Undergraduate curriculum frameworks and employer needs dictate that students should have a wide knowledge base, and also the ability to apply that knowledge in real-life situations. However, “safe” environments for students to practice and apply their knowledge are very limited, and with recent changes limiting animal use in undergraduate teaching, and ethical issues associated with working with patients, alternative methods must be used. In the ILC, new and existing technologies provide interactive and experiential learning opportunities in a comfortable environment.

The HPS is a sophisticated manikin, controlled using the anatomy without access to dissection room facilities. The use of models and specimens, allows us to teach “real” 3d anatomical dissection, with specific clinical cases and the ability to provide bespoke teaching materials. This, along with the use of models and specimens, allows us to teach “real” anatomy without access to dissection room facilities.

The HPS is a sophisticated manikin, controlled using the Muse Software(2) which can be programmed to simulate different patients, different physiological/disease states, and the effects of a wide range of different drugs. Use of the HPS allows students to “learn by doing” - exploring the ways in which drugs affect the body, the often fine line between therapeutic and toxic effects, drug interactions and overdose, and physiological responses dependent on factors such as age and pre-existing conditions.

Since the completion of the Simulation Lecture Theatre, a wide range of new teaching sessions have been designed and integrated into undergraduate Pharmacy, Biomedical Sciences, Clinical Sciences, Chemistry, Radiography, Physiotherapy and postgraduate Archaeological Sciences. This has involved mapping and re-design of modules, continuous reflection and evaluation with input from students and staff to ensure that the sessions are engaging, appropriate and continuously developing in terms of best practice.

Specifically, using the Anatomage Table® we have facilitated large- and small-group teaching formal and informal sessions, studying a wide variety of anatomy and physiology alongside pathological examples, and are in the process of creating short Anatomage video demonstrations to enhance the provision of easily accessible learning materials.

The HPS is used to demonstrate the physiological responses of a human (healthy or pre-existing disorder) either untreated or with drugs/drug overdoses. The HPS is also replacing traditional pharmacology experiments which use animal tissue to demonstrate the effects of different drugs.

Undergraduate student projects have been carried out using the Anatomage Table and the HPS, in order to provide examples of less formal peer-assisted learning.

Benefits:
- Use of interactive and engaging technology in non-traditional settings;
- Comfortable (“safe”) environment;
- Avoids ethical and cost issues of using animal tissue or human subjects, including dissection rooms;
- Flexibility in teaching format;
- HPS software enables individual work in large-class practicals;
- Facilitates interprofessional learning across healthcare-related programmes;
- Visual and kinaesthetic methods of learning(4);
- Consistently receives very positive feedback from students and staff.

Drawbacks:
- Interactive elements are particularly suited to very small group teaching (repeated sessions mean increased staff time);
- Support needed by dedicated staff;
- Set-up, training and maintenance costs.

Summary:
The simulation facility in the Faculty of Life Sciences provides a safe and comfortable environment and equipment for highly effective teaching and learning, engaging students across Life Sciences programmes.

Phase II of the ILC development (taking place 2015) will include a new Anatomy and Pathology Resource Centre linked to the existing Simulation Lecture Theatre, strengthening links to the Digitised Diseases project(5), Digital Pathology and the new University of Bradford Digital Health Zone(6).

2. Anatomage (http://medical.anatomage.com/)
5. Digitised Diseases, University of Bradford (http://www.digitised-diseases.org/)
6. University of Bradford Digital Health Zone (http://www.bradford.ac.uk/dhez/)

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DNA, digesting the DNA with restriction enzymes, setting up ligation reactions, making *E. coli* cells competent, transforming ligation mixtures into competent cells, and testing transformants for successful cloning by colony PCR. One of the key challenges was that groups had to plan in advance what they were going to do in their next lab session, and had to fill in and submit order forms to allow technical staff to plan and prepare equipment and reagents for each session. Students also had to learn how to label their reagents appropriately so that they can be found again when required, sometimes weeks after they were initially generated. Practical sessions were mostly supervised by senior demonstrators with occasional help by academic staff.

Students were assessed by a written group project report in the style of a FEBS Letters paper (3), an individual abstract, and a poster which was aimed at informed lay people. Posters were displayed in dedicated poster sessions, peer-assessed and graded by staff.

The following challenges were identified during the first instalments of the project: protocols needed to be detailed and robust enough so that students can work through them independently. What appears like a clear procedure to an experienced experimentalist can seem entirely cryptic to undergraduate students, which can lead to unnecessary mistakes. Demonstrators needed to be familiar with all techniques and the theoretical background of the project so that they could help students not only technically, but also to make decisions and plan ahead. Logistics in the lab needed to be planned in detail, and technicians needed to know in advance what reagents and equipment were required in a particular session. In the first year, students on the whole were not able to build their planned device, therefore, a large number did not enjoy the project. However, there was also considerable positive feedback. In the second year of the project, several groups succeeded in cloning at least two of their planned biobricks, with two groups managing to build the device that they had initially planned.

One consequence of this very challenging project is that these cohorts of students are a lot more confident than previous year groups in practical projects during subsequent years, and we believe that students learn a great deal about molecular biology research, associated techniques, and how to plan a project.

http://parts.igem.org/Main_Page
http://igem.org/Main_Page
http://www.febsletters.org/

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**SA059**

Our experience of the use of an online Dynamic Laboratory Manual (eBiolabs) to enhance practical teaching in integrated professional programmes

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At Bristol we have developed an interactive Moodle-based web tool, eBiolabs1, that is available online and supports students in preparing for practicals, by providing introductory material including photos, videos, interactive animations and compulsory quizzes. eBiolabs also supports post-practical assessments for uploading experimental data, performing post-practical analyses and assessments. Students receive instant feedback (marks and automated comments) on all submissions.

eBiolabs was first introduced for BSc students in Biochemistry 2008 followed by Physiology in 2009, and has since been extended to the Veterinary, Medical and Dental programmes. Curriculum review of the veterinary programme at Bristol in 2013-14 led to the introduction of integrated first year units which were previously taught separately as Anatomy, Biochemistry and Physiology. To coincide with this we have developed new material for eBiolabs to include anatomy classes from 2013-14 and histology classes from 2014-15 to make a single platform for students to get similar experience in all aspects of their first and second year animal health science units. A similar approach is being also being employed in the Medical Curriculum.

Prior to a practical class, be it on anatomy, histology, physiology or biochemistry, students log on to eBiolabs either having reviewed printed material or to review the online pre practical information. They then complete a short pre practical quiz. The quiz is open for 5 days and closes at midnight the day before the class. The students then attend the practical class and complete all of the required elements recording their results in a lab book. Following the class they log back on to ebiolabs to complete a post lab assessment, the format of which varies depending on the class.

In evaluation surveys students consistently rate aspects of eBiolabs highly. For example in a survey of first year physiology students 87% agreed or strongly agreed to the following statement “Completing the pre-practical quizzes left me better prepared for the practical classes than if I was left to my own devices to prepare for practicals”. And 75% agreed or strongly agreed that “The post lab assessments were helpful in getting me to consider the mechanisms addressed in practicals”. Since the introduction of eBiolabs we have found that students gain a better understanding of the practicals, work more effectively in the lab and engage with the practical. Staff and demonstrators are readily able to access all teaching materials online, which improves both preparation for practicals and monitoring of student attendance and performance.


The new veterinary material on eBiolabs was been developed by colleagues from the University of Bristol in: The Centre for Comparative and Clinical Anatomy, School of Biochemistry and School of Physiology and Pharmacology. eBiolabs was initially funded principally by HEFCE via the Applied and Integrated Medical Sciences Centre for Excellence in Teaching and Learning, which adapted the Dynamic Laboratory Manual system developed by the Bristol ChemLabs CETL.

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**SA060**

The impact of DNA damage on aging and the effect of nutritional interventions

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Previously we have found that accumulation of DNA damage is a major driver of many features of aging, as revealed by a
Molecular mechanisms of aging-associated hypertension

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Cardiovascular disease (CVD) is the leading cause of the death worldwide and its prevalence greatly increases with advancing age. Vascular aging and hypertension (HTN) are independent risk factors for CVD. We have previously shown that mice with inducible smooth muscle cell-specific deletion of mineralocorticoid receptor (SMC-MR-KO) lack the aging-associated rise in blood pressure (BP) that occurs in MR-intact littermates, confirming a new role for SMC-MR in aging-associated HTN. Here, we sought to characterize changes in vascular and cardiac fibrosis with aging and to explore the role of SMC-MR in the molecular regulation of aging-associated HTN. All mice were anaesthetized under isoflurane for tissue harvests and ex vivo studies. Histological analyses of aortas, carotids, renal arteries and left ventricles reveal that vascular and cardiac fibrosis increase with aging and are attenuated in SMC-MR-KO mice (p<0.05 vs. MR-intact). Global gene expression profiling reveals a network of fibrosis-related genes that is down-regulated with aging in SMC-MR-KO mice, suggesting a possible mechanism for the reduced vascular fibrosis with aging observed in SMC-MR-KO mice. Next, we hypothesized that SMC-MR contributes to BP regulation with aging by regulating vascular L-type calcium channel (LTCC) expression and/or function. Patch clamp studies on mesenteric resistance vessel SMC from young (3-4 mo.) and aged (9-12 mo.) MR-intact and SMC-MR-KO mice reveal reduced LTCC current density in aged SMC-MR-KO (p<0.05 vs. MR-intact). Fura-2 photometry studies in mesenteric resistance vessels reveal decreased Ca\(^{2+}\) flux in aged SMC-MR-KO (p<0.05 vs. MR-intact) in response to Bayk8644 (LTCC agonist). Contraction to Bayk8644 is blunted in aged SMC-MR-KO mesenteric resistance vessels (p<0.05 vs. MR-intact). RNA expression of Cavi.2, the pore-forming subunit of LTCC, is reduced by 60% in aged SMC-MR-KO versus MR-intact mice (p<0.05). MicroRNA expression profiling identified mir-155 as being modulated by SMC-MR with aging. Mesenteric resistance vessel mir-155 expression is increased in aged SMC-MR-KO mice (p<0.05 vs. MR-intact). Ingenuity pathway analysis identified Cav1.2 as a potential target of mir-155. Overexpression of mir-155 in mouse mesenteric SMC reduced Cav1.2 expression by 45% (p<0.05 vs. ctrl). Together, these data suggest that SMC-MR contributes to aging-associated CVD via the regulation of vascular and cardiac fibrosis in addition to the regulation of vascular L-type calcium channels. These results not only enhance our basic understanding of vascular aging and BP control with aging, but also provide support for the identification of innovative therapeutic targets to treat aging-associated HTN and reduce the substantial burden of cardiovascular morbidity and mortality in the aging population.

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SA062

Vascular smooth muscle cell senescence and DNA damage promote atherosclerosis and features of plaque vulnerability

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Background: Although vascular smooth muscle cell (VSMC) proliferation is implicated in atherogenesis, VSMCs in advanced plaques and cultured from plaques show evidence of VSMC senescence and DNA damage. In particular plaque VSMCs show shortening of telomeres, which can directly...
The molecular biomarkers of atherosclerosis: Telomeres and cardiovascular aging

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Atherosclerotic cardiovascular disease is the dominant health problem in the western world. Major clinical manifestations of cardiovascular disease include myocardial infarction, coronary artery disease, stroke, peripheral artery disease and congestive heart failure. In most cases, these clinical conditions result from atherosclerosis, a progressive disease of the arterial wall, characterized by focal thickening and luminal obstruction. The old view, not more than 10 to 15 years ago, saw atherosclerosis as a simple ‘plumbing problem’ due to a gradual build-up of a plaque containing cholesterol and fatty material on the surface of the passive artery walls. Currently, new view recognizes atherosclerosis as a chronic inflammatory disease, involving many cell interactions at the level of the active vascular wall (1). The cellular and molecular mechanisms involved in atherosclerosis and its acute complications are being defined (2), but much is still unknown. Growing evidence indicates a critical role for telomere dysfunction as key causal events in the biological process of atherosclerosis and its complications.

Telomeres are special DNA regions located at the ends of eukaryotic chromosomes that prevent chromosomal fusions, offering genomic integrity and stability (3). The telomeric DNA is composed of noncoding double-stranded repeats of G-rich tandem DNA sequences (TTAGGG in humans), that are extended several thousand base pairs (10 to 15 kb in humans) and end in a 150 to 200 nucleotide 3’ single-stranded overhang (G-strand overhang). The synthesis of new telomeric DNA repeats in dividing cells requires the activity of telomerase, a ribonucleoprotein enzyme that is present in human cells at embryonic stages, in tumor cells, and in human germ cells, but is not detectable in normal adult somatic cells, which exhibit low or no telomerase activity. In contrast to adult somatic cells, germ, stem and tumor cells maintain high telomerase activity, long telomeres and high proliferative potential. Cells affected by critical telomere attrition accumulate chromosomal aberrations and succumb to replicative senescence or apoptosis (4).

During the last years, evidence for the presence of chromosomal instability in atherosclerosis has also been obtained from experimental and clinical investigations (5). Furthermore, an increasing body of evidence has established the critical role of the telomere in vascular cells (6). An age-dependent telomere shortening has been reported in ECs from iliac, thoracic, and coronary arteries (7,8). The loss of telomere function induces a senescent phenotype and endothelial dysfunction, that are observed in aged arteries, as well as functional alterations involved in atherogenesis, such as an increased expression of intercellular adhesion molecule-1 and diminished endothelial nitric oxide synthase activity. Interestingly, inhibition of telomere shortening suppressed both EC senescence and associated functional alterations (9). Shorter telomeres in coronary ECs from atherosclerotic coronary arteries compared with those at non-atherosclerotic specimens (9). Similarly, plaque VSMCs also show multiple markers of senescence, accelerated both in vivo and in vitro by oxidative stress-induced DNA damage, inhibition of telomerase, and marked telomere shortening (10).

Available observational data show an inverse association between telomere length in human blood cells and risk of...
age-associated vascular diseases (3,6). A pilot study found that patients with early-onset coronary artery disease with premature myocardial infarction had a shorter mean LTL compared with healthy subjects of the same age and gender (11). The West of Scotland Primary Prevention Study also demonstrated that the risk of developing CAD was highest in individuals with short telomeres and that this risk was substantially attenuated by treatment with pravastatin (12). However, a large prospective study of patients with stable CAD found that leukocyte telomere length is associated with mortality independently of chronological age, clinical factors, echocardiographic variables and no difference in mean telomere length between users and nonusers of statins (13).

In a large cohort of 620 CHF patients compared to 183 age- and gender-matched controls (14) Telomeres were shown to be related to the severity of heart failure as they were shorter in patients with higher New York Heart Association class (14). Furthermore, there are suggestions that telomere length is associated with reduced ejection fraction in the elderly (15). A recent meta-analysis indicates that telomere length is inversely associated with risk of coronary heart disease independently of conventional vascular risk factors (16). Whether telomere testing is clinically useful predictor of risk that can help guide treatment decisions, however, will require further evaluation in large prospective studies with long follow-up Furthermore, anti-aging therapies that modulate telomere length are expected to have an impact various age-related diseases.


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Angiogenesis is regulated, under both physiological and pathological conditions, by numerous "classic" factors, among which are vascular endothelial growth factor, fibroblast growth factor-2, transforming growth factors, angiopoietins, platelet-derived growth factor, thrombospondin-1, and angiostatin. In recent years, evidence has accumulated that, in addition to the classic factors, many other endogenous peptides play an important regulatory role in angiogenesis, especially under pathological conditions.

Solid tumor growth occurs by means of an avascular phase followed by a vascular phase. Assuming that such growth is dependent on angiogenesis and that this depends on the release of angiogenic factors, the acquisition of an angiogenic ability can be seen as an expression of progression from neoplastic transformation to tumor growth and metastasis. Practically all solid tumors, including those of the colon, lung, breast, cervix, bladder, prostate and pancreas, progress through these two phases. The role of angiogenesis in the growth and survival of leukemias and other hematological malignancies has only become evident since 1994, thanks to a series of studies demonstrating that progression in several forms is clearly related to their degree of angiogenesis.

Finally, the new possible therapeutic perspectives opened by investigations of angiogenic mechanisms will be discussed. The development of a clinical trial requires the identification and characterization of the physiological targets involved in angiostimulatory and angioinhibitory activities. Much research effort has been concentrated on the role of angiogenesis in cancer, and inhibition of angiogenesis is a major area of therapeutic development for the treatment of this disease. New patho-physiological concepts generated in the past few decades have given rise to the development of a large variety of new drugs to interfere with angiogenesis. The target of anti-angiogenic therapy is the vascular endothelial cell rather than the tumor cell. In fact, whereas conventional chemotherapy, radiotherapy, and immunotherapy are directed against tumor cells, anti-angiogenic therapy is aimed at the tumor vasculature and will either cause tumor regression or keep the tumor in a state of dormancy.

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of endothelial migration and endothelial tube morphogenesis, with the net acceleration of the angiogenesis processes and vice versa.

Angiogenesis inhibitors are relatively less toxic than conventional chemotherapy and represent a new class of anti-cancer agents. Several endogenous angiogenesis inhibitors have been discovered in the circulation and/or in the ECM acting at specific sites. Examples of endogenous angiogenesis inhibitors include: Soluble VEGFR-1 ( decoy receptor for VEGF-B and PIGF); Angiopoietin 2 (antagonist of angiopoietin 1); Thrombospondin; Angiostatin; Endostatin; Vasoactive Intestinal Polypeptide; Platelet Factor 4; Tissue Factor Pathway Inhibitor; Heparin and Kininogen fragments; and several other ECM fragments. Several endogenous angiogenesis inhibitors and pharmacological inhibitors of endogenous pro-angiogenesis switches have been developed and approved for clinical use alone and in combinations with other agents such as chemotherapy. Examples of such agents include bevacizumab, which was the first angiogenesis inhibitor shown to slow tumor growth and, more importantly, to extend the lives of patients with some cancers. The FDA has also approved other drugs that have anti-angiogenic activity, including sorafenib ( Nexavar® ), sunitinib ( Sutent® ), pazopanib ( Votrient® ), and everolimus ( Afinitor® ). Sorafenib is approved for hepatocellular carcinoma and kidney cancer, sunitinib and everolimus for both kidney cancer and neuroendocrine tumors, and pazopanib for kidney cancer.

The use of other novel angiogenesis inhibitors ( integrin antagonists, Nanotechnology-based targeted strategies ( NanotetraC ) to treat other types of cancer and others disorders that involve the development of abnormal blood vessel growth such as diabetic retinopathy, macular degeneration, and other vascular-associated disorders, are advancing into clinical development. 

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SA067

Endogenous resistance to pharmacological anti-angiogenesis

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Success with clinical application of anti-angiogenesis therapy to cancers has been modest. Tumor resistance to such therapy, for example, with bevacizumab, is attributed to tumor reliance on angiogenic pathways that are alternatives to VEGF, selection of hypoxia-tolerant, HIF-1α-producing tumor cells, contributions from stem cells or dormancy of tumor cells during anti-VEGF therapy. We have proposed that in cancer patients there are additional host factors with inherent pro-angiogenic properties that oppose therapeutic anti-angiogenesis. Examples of such factors are estrogen, androgen and thyroid hormone. Physiological concentrations of thyroid hormone— 3, 3’-triiodo-L-thyronine (T3) and L-thyroxine (T4)—initiate their multi-mechanism, pro-angiogenic activity via a cell surface receptor site on integrin αvβ3 on dividing endothelial cells and tumor cells. We will review here the mechanistic pathways in the pro-angiogenic action of thyroid hormone that may limit success of anti-angiogenesis treatment. Our recent results are obtained in the model of human ovarian cancer cells.

The treatment of ovarian cancer cells with T3 (1 nM) and T4 (100 nM) produced an αvβ3-mediated increase in cell viability, cell number, cell survival as well as ERK activation. The mRNA abundance and protein level of HIF-1α, a potent angiogenesis inducer, was upregulated following T3 and T4 treatments. We found that several anti-angiogenic genes are potently and quickly (1 h) inhibited in the presence of the hormones, particularly in ovarian cancer cells with high integrin expression; these genes include p16, GDF15 as well as IGFBP-6. The suppression of the latter gene correlated with a parallel increase in b-catenin, a protein which augments angiogenesis and was recently shown to potently inhibit the expression of IGFBP-6. By using HEK293 avb3 deficient or expressing cells, we have revealed that T4, but not T3, phosphorylates tyrosine 759 in the b3 subunit. This phosphorylation event not only controls outside-in signaling by the integrin, but also is the regulatory switch accommodating the proangiogenic cooperative binding interaction between VEGFR-2 and αvβ3. These new findings compliment results from our own and other laboratories showing enhancement by thyroid hormone of pro-angiogenic bFGF and VEGFA gene transcription, stimulatory crosstalk between the integrin and adjacent receptors for VEGF, bFGF, PDGF and EGF, transcription of MMP genes relevant to blood vessel formation, endothelial cell migration and release of vascular growth factors.

Thus, thyroid hormone is a potent endogenous pro-angiogenic factor which we propose contributes to clinical resistance to current antiangiogenic treatments. Disruption of the thyroid-hormone-integrin axis is anti-angiogenic and may support clinical actions of specific anti-angiogenic drugs in cancer.

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SA068

Modulation of angiogenesis and anti-angiogenesis at targets on integrin αvβ3

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Plasma membrane integrin αvβ3 is extensively involved in angiogenesis. Generously expressed on dividing endothelial cells and tumor cells, it mediates cell-cell interactions and interactions with angiogenesis-relevant extracellular matrix (ECM) proteins such as osteopontin, vitronectin and von Willebrand factor. avb3 also engages in crosstalk with adjacent vascular growth factor receptors. We have described small molecule ligands of the integrin, such as thyroid hormone, that exert extensive control over blood vessel formation. Acting at the integrin, L-thyroxine (T4) (< 10^{-10} M free hormone) and 3, 3’-S-triiodo-L-thyronine (T3) rapidly stimulate angiogenesis in the chick chorioallantoic membrane (CAM) model and Matrigel® microtubule formation system. Nanoparticulate T4 (agarose-T4) does not gain access to the cell interior and is equipotent angiogenically to unmodified T4. Thyroid hormone is angiogenically equipotent to VEGF and bFGF in the CAM model. The hormone receptor on αvβ3 mediates upregulation by T4 of a set of genes relevant to angiogenesis, including basic fibroblast growth factor (bFGF; FGFR2) and several matrix metalloproteinases (MMPs). T4 also supports endothelial cell migration towards a vitronectin cue. Tetraiodothyroacetic acid (tetrac), a deaminated derivative of T4, inhibits binding
of T₃ and T₄ to αvβ3 and, unmodified or reformulated as a poly(lactic-co-glycolic acid) nanoparticle (Nanotetracon), this agent antagonizes all of the pro-angiogenic actions of T₃ and T₄. Nanotetracon is anti-angiogenic by additional mechanisms unrelated to the binding of T₃ and T₄. These αvβ3-dependent actions include inhibition of the angiogenic actions of VEGF, bFGF and PGDF—reflecting, we propose, disruption of cross-talk between the integrin and nearby receptors for vascular growth factors—and stimulation of expression of the anti-angiogenic thrombospondin 1 (TSP1) gene in tumor cells. Also relevant to angiogenesis are the actions of Nanotetracon to decrease expression of VEGFA, EGF, MMP-2, MMP-9 genes and mir-21 abundance. The microRNA increases cellular HIF-1α and VEGF content. Finally, resveratrol and testosterone are other small molecule ligands of αvβ3 that modulate angiogenesis. The pro-angiogenic action of resveratrol has been shown to be expressed via the αvβ3 receptor site for stilbenes, but the possibility that the dihydrotestosterone effect on HIF-1α is αvβ3-requiring has not yet been examined. In summary, integrin αvβ3 has recently been appreciated to have small molecule ligands that regulate angiogenesis. A set of disparate thyroid hormone analogues is the best studied of such ligands, and multiple molecular mechanisms these analogues are pro- or anti-angiogenic.

Resveratrol is anti-angiogenic via the integrin (M Belleri, Mol Cancer Ther 2008), while the stilbene may have modest pro-angiogenic effects by other pathways.

Links to OPN (increases angio- via ERks, PI3K; J Dai Oncogene 2009)
Vitronectin induces VEGF-beta3 cross-activation/phosphorylation (EF Plow Cric Res 2007)
mir-21 and HIF-1alpha, VEGF (LZ Liu, PLoS One 2011)

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SA069
In injured nerves, c-jun controls the generation of repair (Bungner) Schwann cells, RAG expression in neurons and myelin clearance by myelinophagy
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Nerve injury activates transcriptional mechanisms that reprogram Schwann cells and neurons to generate cells, which are specialised to promote repair. Schwann cells abandon their normal functions relating to axonal ensheathment of and myelination and convert to repair Schwann cells tailored to support regeneration. Similarly, neurons activate regeneration associated genes (RAGs) and switch from signalling mode to growth mode. We find that activation of the transcription factor c-Jun in Schwann cells has an important role in both of these reprogramming events, and that Schwann cell c-Jun is therefore a central regulator of nerve regeneration. In the nerve distal to the injury, injury-induced expression of c-Jun in Schwann cells governs major aspects of Wallerian degeneration, the complex cellular events that allow cut axons to re-grow. c-Jun has two key functions in the response of Schwann cells to injury. First, and most importantly, it activ-
to cross the injury site can be very substantial. That results in further delays and even worse functional recovery. Therefore, there is a great need for novel treatment modalities to expedite nerve growth and functional return. Post-surgical electrical stimulation to the proximal nerve stump has been shown to accelerate functional recovery in animal models for several decades. However, the underlying mechanisms of this are only becoming better understood in the new millennium. Up-regulation of neurotrophic factors such as BDNF in Schwann cells, along with downstream intermediaries including cAMP play a key role in speeding up the extension of growth cones across the injury site. Indeed, in NGF 4/5 knockout mice and BDNF/TrkB deficient mice, the acceleration effect of electrical stimulation is abolished. Sensory and motor nerve fibres respond to ES differently. Although motor nerve fibres show positive response to a wide range of stimulation durations from as long as several weeks to as short as an hour, the optimum window of stimulation duration for sensory nerve fibres is much narrower. While robust acceleration occurs with 1 hour of electrical stimulation, the accelerated growth becomes increasingly attenuated with long periods of stimulation. This is likely due to intrinsic differences in motor and sensory nerve fibres. For example, polysialic acid was shown to be critical in determining motor axons’ capacity for accelerated regeneration after electrical stimulation. The beneficial effects of electrical stimulation of the proximal nerve stump only occur in motoneurons that are capable of up-regulating polysialic acid. Conversely, the benefits of stimulation were completely abolished if polysialic acid was removed from the regenerating axons. A second critical downstream effector in preferential motor nerve reinnervation is HNK-1 glycan that is exclusively expressed by motor axons. Enhancement of HNK-1 expression was not seen in TrkB deficient mice in whom preferential motor reinnervation does not occur. Electrical stimulation has also been shown to improve specific path finding for regenerating sensory nerve fibres. However, the precise mechanism for that has not been established.

With promising results shown in animal studies, the same brief post-surgical electrical stimulation paradigm has been applied clinically to patients with severe axon loss injury. These include severe compressive median neuropathy in carpal tunnel syndrome and compressive ulnar neuropathy at the elbow. In those studies, significantly greater motor nerve reinnervation and better functional outcomes were found in the treatment group that received 1 hour 20 Hz continuous electrical stimulation. The same benefits on sensory nerve regeneration were also recently shown in patients with digital nerve laceration. These clinical translational studies open the door to test the effects of brief electrical stimulation to devastating proximal peripheral nerve conditions such as injury to the brachial plexus that often carry poor outcomes with conventional treatments.

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SA071
Successful regeneration of the larval zebrafish spinal cord
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In contrast to mammals, adult zebrafish can regenerate their spinal cord. However, for observation purposes, a larval regeneration paradigm would be advantageous. We here describe the establishment of a lesion paradigm in larval zebrafish. Complete mechanical transection of the spinal cord at 3 days post-fertilisation leads to paralysis of the fish. Within 48 hours after injury, the lesion site closes and larvae show recovery of swimming activity. This is accompanied by a reaction of the immune system, involving influx of neutrophils, macrophages and microglia from 6 hours after lesion. In two different mutant lines of the innate immune system, locomotor recovery is significantly impaired, indicating an essential role of the immune response for functional recovery. We are currently analysing, which of the crucial events in spinal cord repair, such as wound closure, fusion of the spinal cord, axon regrowth and neurogenesis are affected in the mutants to determine the role of the innate immune system in successful repair.

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SA072
Therapeutic strategies to enhance axonal remyelination following spinal cord injury
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Spinal cord injury (SCI) results in degeneration of oligodendrocytes leading to axonal demyelination, white matter degeneration and consequently functional deficits. We and others have shown the potential of transplanting neural precursor cells (NPCs) or activating endogenous precursor cells for oligodendrocyte differentiation following SCI. Further studies, however, indicate the capacity of NPCs for replacement of myelinating oligodendrocytes is challenged by inhibitory alterations in the local microenvironment of post-SCI. Under profound modifications in the extracellular milieu of the injured spinal cord, resident or transplanted NSCs give rise predominantly to astrocytes at the expense of oligodendrocytes. Also challenging, the majority of NPC-derived oligodendrocyte precursor cells either undergo cell death or fail to fully mature into myelinating phenotype. Moreover, the myelin sheath that is formed by the newly formed oligodendrocytes lacks the normal thickness. As a result of these limitations, we find that many of the surviving axons either show evidence of abnormal myelination or undergo retrograde degeneration during the chronic phase of SCI. We have recently found that robust and chronic depletion of axonally localized Neuregulin-1 (Nrg-1) after SCI correlates closely with the poor replacement of oligodendrocytes. Nrg-1 is an essential factor for axons and
oligodendrocytes development and function. Interestingly, restoration of the deficient level of Nrg-1 after SCI by intrathecal infusion of recombinant Nrg-1 was able to promote oligodendrocyte differentiation of spinal cord derived NPCs. Additionally, our parallel studies demonstrate that reactive astrocyte driven increases in matrix chondroitin sulfate proteoglycans (CSPGs) inhibit the potential of NPCs for oligodendrocytes differentiation and survival in vitro and in SCI. We find that targeting CSPGs by their pharmacological removal from the injured spinal cord using chondroitinase ABC (ChABC) or inhibiting CSPGs receptors in NPCs allows better integration of NPCs and increase their ability for oligodendrocyte differentiation. This talk will discuss our recent research findings in these areas. Careful elucidation of molecules/processes contributing to impaired oligodendrocyte differentiation in the post-SCI milieu is essential for developing effective remyelination therapies for white matter repair following SCI or other demyelinating disorders.

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SA073

Efficacy of Schwann cell (SC) transplantation for spinal cord repair is improved with combinational strategies

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When cells (including SCs) of the rat PNS could be purified and expanded in number in tissue culture, Richard Bunge in 1975 envisioned that SCs could be introduced to repair the CNS. SCs are important candidates for spinal cord repair. They are known to be essential for regeneration of PNS axons following injury. They produce growth factors and extracellular matrix components important for axon growth and can myelinate axons in the CNS, restoring electrical activity. They can be prepared in large numbers in culture and, importantly, could be autologously transplanted into spinal cord injured persons after acquiring SCs from one of their peripheral nerves. SCs can be genetically engineered to produce factors to promote repair. Up to 1 million SCs may be obtained from a human adult sural nerve biopsy; these can be expanded to 100 million in 3 to 5 weeks. In 1980, a key study was reported by the Aguayo team showing that, when a piece of peripheral nerve was inserted into a complete gap in the spinal cord, axons regenerated into the implant. Thus, we decided to test the efficacy of transplanting purified populations of SCs. When a bridge of rat SCs was introduced into a complete transection gap in the spinal cord, Xu et al (1997) found that axons grew into the SC bridge from both spinal cord stumps. There were 2,000 SC-myelinated axons and eight times more non-myelinated axons in these bridges. A spinal cord injury (SCI) contusion model revealed that the transplanted SCs reduced cyst formation, protected spinal cord tissue from secondary damage, and supported axon growth into the SC implant in which 5,000 SC-myelinated axons were present after some weeks.

Strategies combining SC transplantation with methylprednisolone, neurtrophins, olfactory ensheathing cells, elevation of cyclic AMP or chondroitinase all led to increased repair and function. There were more myelinated axons in implants, more regenerated axons from neurons above the spinal cord, some exit of regenerated axons from the graft into the caudal spinal cord and improvement in hindlimb movement. When a neurotrophin mimicking the actions of brain derived neurotrophistic factor (BDNF) and neurotrophin-3 (NT3) was generated by SCs following insertion of the DNA for D15-A, there was a 5-fold increase in graft volume and SC number and an increase in SC-myelinated axons from 5,000 to 26,000 (Golden et al., 2007). If D15-A was further modified to reduce its affinity to p75NTR, after SCI SC survival was increased 10X and SC-myelinated axons were 6X more numerous (Enomoto et al 2013). Brainstem axons were increased in the graft. Kanno et al (2014) combined SCs with D15-A and/or chondroitinase. Animals receiving the full combination exhibited better outcomes than with either the growth factor or the enzyme. The transplants contained 10,000 more SC-myelinated axons. The borders of SC implants were better interdigitated with the host cord with chondroitinase. More neurons above the spinal cord responded to the full treatment. Walking scores were improved and there was lessened pain in the hindlimbs.

Other work has pointed to the importance of the state of astrocytes following injury. If the border between the SC implant and the spinal cord is sharp, there is little extension of axons into the implant. In contrast, when the interface border is irregular due to extension of astrocyte processes into the SC implant, axons regenerate into the implant; the higher the number of astrocyte processes in the implant, the more axons regenerated into the implant. Electro microscopy revealed that in the SC bridge there are tunnel-like structures containing astrocytes, SCs and axons, all encircled by basal lamina.

Studies by Guest et al (1997), demonstrated that human SCs are as robust as rat SCs in promoting axon regeneration into the SC bridge and myelination of the regenerated axons. Various types of axons were present in the bridge and some spinal and sensory axons were observed to leave the bridge and enter the spinal cord. Interestingly, interdigitated interfaces were observed, in agreement with our rat studies. Pre-clinical data from transplantation studies with rat and human SCs in multiple species, rats, pigs and primates, supported the feasibility of transplanting SCs into humans for spinal cord repair. These data contributed to obtaining approval from the FDA in July 2012 for an open label, unblinded, non-randomized, and non-placebo dose escalation clinical trial to assess safety of autologous human SC transplantation.

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SA074

Actions and interactions of H$_2$S with NO in the cardiovascular system

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Hydrogen sulfide (H$_2$S) is the latest addition to the gasotransmitter family, that also includes nitric oxide (NO) and carbon monoxide (CO). As with NO and CO, initially, the majority of publications on H$_2$S focused on the toxicological profile of this gasotransmitter, with the general bioscience community shown only a very limited interest in the biology of H$_2$S. Proof for endogenous production of H$_2$S in mammalian cells, along with observations that H$_2$S acts as a neuromodulator and vasorelaxing agent sparked the interest for this diatomic molecule. H$_2$S is now recognized as a signaling molecule, with important functions in cell metabolism, growth and apoptosis. Moreover, H$_2$S modulates inflammatory responses, regulates vascular smooth muscle tone, promotes angiogenesis and reduces blood pressure. The prototype gasotransmitter NO, also exhibits many of the above-mentioned properties. Although initially, ATP-sensitive K$^+$ channels were thought to be the main mechanism through which H$_2$S exerts its cardiovascular effects, cGMP and protein sulfhydration are increasingly being shown to contribute to the actions of H$_2$S. We and others have demonstrated that H$_2$S increases eNOS phosphorylation on the stimulatory residue Ser1177, leading to eNOS activation and increased NO production. eNOS activation along with the indirect and direct anti-oxidant properties of H$_2$S, facilitate the formation of increased amounts of cGMP. In addition, H$_2$S exhibits phosphodiesterase inhibitory activity leading to a further increase in cGMP levels. Thus, some of the actions of H$_2$S in the both the blood vessels and the heart are abolished by eNOS or cGMP-dependent protein kinase inhibition. During this presentation the cross-talk between H$_2$S and NO in the context of vasorelaxation, angiogenesis and ischemia-reperfusion injury in the heart will be reviewed.

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SA075

Carbon monoxide and mitochondria: modulation of cell metabolism and cell death

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The endogenously produced gasotransmitter carbon monoxide (CO) has been studied as a factor involved in cytoprotection, homeostasis and anti-inflammation. Several evidences show CO targeting mitochondria and small amounts of reactive oxygen species (ROS) are described as signaling factors in CO’s biological mode of action. Mitochondria are the main source of ROS and are also key organelles in orchestrating cell function: metabolism, cell death control and redox signaling. Mitochondria can act as calcium buffering organelles and respond to it by two distinct ways. Calcium entry in mitochondrial matrix stimulates ATP production by improving mitochondrial respiration and increasing pyruvate dehydrogenase activity. Nevertheless, when the capacity of mitochondria is exceeded, calcium overload promotes the opening of permeability transition pore (PTP), which is a high-conductance channel, leading to mitochondrial depolarization, permeabilization and ultimately cell death. Astrocytes are most abundant glial cells and essential for neuronal function, namely metabolic and physical support, expression of neurotransmitters and promotion of neuroprotection. Primary culture of astrocytes is the cellular model. Non-synaptic mitochondria isolated from rodent cortex are the cell-free system used for studying the direct effect of CO on mitochondria. Two CO sources are used: CO gas through PBS saturated solutions and CO-releasing molecule –A1 (CORM-A1). Low concentrations of CO partially inhibited oxidative stress-induced apoptosis in astrocytes, by preventing caspase-3 activation, mitochondrial potential depolarization and plasmatic membrane permeability. Furthermore, CO directly targets non-synaptic mitochondria and inhibits PTP opening by partially inhibiting (i) loss of potential, (ii) opening of a non specific pore through the inner membrane and (iii) mitochondrial swelling, which are induced by calcium or atracyloside (a ligand of adenine nucleotide translocator, ANT) 4,5. Thus, CO limits the release of cytochrome c from mitochondria into the cytosol, which limited caspase activation and triggering of apoptotic cascade in astrocytes. CO induced ROS generation, and their scavenging by β-carotene, decreased CO cytoprotection in intact cells, as well as in isolated mitochondria, revealing the key role of ROS. Additionally, CO promotes a slight increase on mitochondrial oxidized glutathione, which in turn modified ANT protein by glutathionylation. Glutathionylation of ANT increased the ATP/ADP exchange through mitochondrial inner membrane and limited the opening of PTP.

In addition, CO exposure enhanced ATP generation, which was accompanied by an increase on specific oxygen consumption, a decrease on lactate production and a reduction of glucose use, indicating an improvement of oxidative phosphorylation. Accordingly, CO increased cytochrome c oxidase specific enzyme activity and enhanced mitochondrial population. The CO-induced oxidative metabolic improvement is dependent on Bcl-2 expression, since silencing Bcl-2 expression with siRNA reverted cytoprotection and metabolic improvement. Dysfunctional mitochondrial can be eliminated by mitophagy, which is a crucial process for maintaining their good function and quality control. In astrocytes, CO promotes mitophagy at 1h of treatment, while following 24h mitochondrial population is back to basal levels, indicating that CO contributes to mitochondrial turnover. Furthermore, CO limits astrocytic cell death in an autophagic dependent manner. All these data suggest that CO modulates calcium entry into mitochondria. Thus, CO prevents astrocytic cell death and improves cell metabolism by targeting mitochondria, and some of the underlying molecular mechanism are disclosed.


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**SA076**

**Mitochondria-targeted hydrogen sulfide: A novel therapeutic opportunity?**

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Hydrogen sulfide (H$_2$S) has rapidly emerged as an additional ‘gasomediator’ important in health and disease and recently compounds which generate H$_2$S have been proposed as novel therapeutic agents. H$_2$S can be synthesised in mammalian cells from cysteine/homocysteine via the PPL-dependent enzymes cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE) in the cytoplasm. Mitochondria are also capable of generating H$_2$S from mercaptopyruvate via 3-mercaptopyruvate sulfurtransferase (3-MST) where H$_2$S is thought to stimulate mitochondrial respiration and ATP synthesis, and regulate cell death and inflammation. Oxidative stress results in the inhibition of mitochondrial H$_2$S synthesis and/or depletion of mitochondrial H$_2$S and the translocation of CSE and CBS to mitochondria resulting in mitochondrial H$_2$S synthesis, cellular respiration and cell survival. Cellular and mitochondrial H$_2$S levels are depleted, or H$_2$S synthesis is inhibited, in vascular disorders such as hypertension, COPD, heart failure and diabetes etc. Pharmacological inhibition or genetic removal of CSE exacerbates the detrimental effects of oxidative stress (e.g. mitochondrial dysfunction), on inflammation, heart and blood vessel damage in atherosclerosis, hypertension and pre-eclampsia. Conversely, ‘H$_2$S replacement’ with source of H$_2$S such as sulfide salts (e.g. NaSH) or donors (e.g. GYY4137) prevents/reverses mitochondrial damage in these models. These studies have suggested that mitochondrial generation of H$_2$S is crucial for cell survival and approaches to ‘replenish’ lost H$_2$S offer a novel therapeutic approach for the treatment of disease. However, neither NaSH nor GYY4137 deliver H$_2$S to mitochondria. We have therefore developed a series of novel mitochondria-targeted H$_2$S donors (mTH$_2$SD) containing a well characterised targeting moiety triphenylphosphonium, a variable length aliphatic linker and sulfide generating moieties e.g. hydroxythiobenzamide or anethole dithiolethione. *In vitro* experiments using mTH$_2$SD in a variety of cells showed potent (e.g. <100 nM) cytoprotection against biological oxidants (e.g. H$_2$O$_2$, 4-HNE, peroxynitrite, glucose oxidase, hyperglycaemia etc) and abrogation of mitochondrial and cellular ‘ROS’ production, mitochondrial and cytoplasmic protein and DNA damage, ATP synthesis and cellular bioenergetics (Seahorse). From these studies two lead compounds were identified, AP39 (containing ADT-OH) and AP123 (containing HTB). In each assay AP39 and AP123 were shown to confer greater cytoprotection, reduced oxidative stress and mitochondrial damage than GYY4137 (or NaSH) at substantially lower concentrations (e.g. 30-100 nM c.f. 200 μM GYY4137). In NO-deficient (L-NNAME) rats, AP39 lowered heart rate and systemic blood pressure and reversed arterial stiffness (180 μg/kg c.f. 25 mg/kg umol/kg GYY4137). Patch clamping experiments showed AP39 inhibited T-type Ca$^{2+}$ channels (< 300 nM) as well as RyR2 channels in isolated cardiac sarcoplasmic reticulum suggesting a mechanism of action that may include regulation of intracellular calcium levels. Recent data from *in vivo* studies using low doses of AP39 (e.g. 7 – 200 μg/kg) in models of cardiac arrest, myocardial and renal ischaemia-reperfusion will be discussed. These studies strongly suggest that targeting mitochondria with a source of H$_2$S may represent a novel therapeutic approach to treat human disease.

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**SA077**

**Physiological concentrations of hydrogen sulfide selectively inhibit Ca$_{3,2}$ T-type Ca$^{2+}$ channels**

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Hydrogen sulfide (H$_2$S) is emerging as an important regulator of ion channels$^1$, which may account for many of this gasotransmitter’s biological actions. The purpose of this study was to investigate its effects on T-type Ca$^{2+}$ channels. The three different isoforms of T-type Ca$^{2+}$ channels were separately overexpressed in HEK293 cells. Using patch-clamp electrophysiology, we demonstrated that the H$_2$S donor, NaHS (10μM-1mM) selectively inhibits Ca$_{3,2}$ T-type channels whilst Ca$_{3,3}$ channels are unaffected. This inhibitory effect is voltage-independent and concentration-dependent: 10μM NaHS was sufficient to evoke a significant reduction of Ca$_{3,2}$ current, and maximal inhibition (ca. 35%) was observed at 1mM NaHS.

Although H$_2$S-dependent inhibition of Ca$_{3,2}$ persisted (>5 min) after washing NaHS out, the reducing agent dithiothreitol (DTT) immediately reversed this inhibition, suggesting that this mechanism is redox modulated. Mutagenesis studies revealed that the redox-sensitive extracellular residue H191 is essential for H$_2$S sensitivity: currents evoked in mutant Ca$_{3,2}$ (H191Q) expressing cells were insensitive to NaHS, and the analogous reverse mutation in Ca$_{3,1}$ (Q172H) conferred sensitivity to NaHS on Cav3.1.

We also explored the ability of H$_2$S to inhibit native T-type current in rat aortic smooth muscle (A7r5), murine atrial cardiomyocytes (HL-1), rodent neuroblastoma x glioma hybrid (NG 108-15) cells, and primary cultures of rat dorsal root ganglia
modulating intracellular free calcium levels, H₂S inhibition correlated with expression of Ca³.² and not Ca³.¹ channels. Importantly, H₂S also inhibited native T-type (primarily Cav3.²) channels in DRG neurons, where inhibitions of ca. 20% were observed. Our data clearly indicate that H₂S selectively inhibits Ca³.² T-type Ca²⁺ channels in the micromolar concentration range and inhibition requires the presence of H191 (see also J. Elies et al. (2014). FASEB J. 28(12):5376-87). The results obtained from this study raise important issues regarding the mechanisms involved in the biological actions of H₂S in health and disease. 


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**SA078**

Hydrogen sulfide, EDHF, and calcium

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Hydrogen sulfide (H₂S) is a unique gasotransmitter that shares many common targets with NO and CO but acts via different molecular mechanisms [1]. In the blood vessels, H₂S is mostly generated in endothelial cells (ECs) and vascular smooth muscle cells (VSMCs), catalyzed by cystathionine gamma-laye (CSE). EC-generated H₂S stimulates the opening of IKCa channels and causes VSMC hyperpolarization, fulfilling the role of endothelium-derived hyperpolarizing factor (EDHF) [2,3,4]. VSMC-generated H₂S stimulates KCa channels and also causes VSMC hyperpolarization [5]. Consequently, voltage-dependent calcium channels in VSMCs are inactivated and intracellular free calcium level drops. The interaction of H₂S with IKCa as well as KCa channels is accomplished via post-translational modification of selective cysteine residues of respective ion channel proteins [6]. Whether H₂S directly acts on voltage-dependent calcium channels in VSMCs is uncertain and future study in this direction is warranted. By modulating intracellular free calcium levels, H₂S actively participates in the regulation of numerous cellular events, including migration, growth, proliferation, senescence, apoptosis, contraction and secretion.


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**SA079**

Secretion of glucagon-like peptide-1 (GLP-1) from intestinal L-cells

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Glucagon-like peptide-1 (GLP-1) is an incretin, boosting proprandial insulin secretion, and anorexie hormone released from so called L-cells found scattered in the intestinal epithelium. GLP-1 mimetics and inhibitors of dipeptidy-peptidase-4, which prolong the plasma half-life of incretins are now widely used in the treatment of diabetes. An alternative strategy is the recruitment of endogenous GLP-1 “reserves”, as L-cells increase in number in the distal intestine and increased GLP-1 secretion has been correlated with improved glucose homeostasis after gastric bypass surgery. We used transgenic mouse models to identify and study L-cells and GLP-1 receptor expressing target cells. Interestingly, proximal and distal L-cells differ in their expression profiles, with duodenaljejunal L-cells co-expressing glucose-dependent insulinotropic polypeptide and cholecytokinin, while colonic L-cells co-express the anorexigene peptide peptideYY and, with increasing frequency towards the rectum, orexigene insulin-like peptide-5. Intestinal L-cells are open-type enteroendocrine cells with apical microvilli making direct contact with luminal contents. While duodenaljejunal L-cells are likely to be exposed to primary nutrients such as glucose, dipeptides/amino acids and long-chain fatty acids, more distally located colonic L-cells would more likely encounter bacterial fermentation products, such as short chain fatty acids and indole, a breakdown-product of tryptophan. We found the response of L-cells to glucose and dipeptides to involve electrogenic sodium/proton coupled nutrient uptake, resulting in increased action potential frequency and elevation of cytosolic calcium. While indole widened action potentials through inhibition of voltage-gated potassium channels, it also reduced cytosolic ATP levels, thereby inhibiting GLP-1 secretion over longer exposure times. Short-chain fatty acids recruited the Gq-coupled receptor FFAR2, leading to acute stimulation of GLP-1 secretion from colonic cultures. Other luminal factors, such as allyl isothiocyanate, the pungent component of mustard, and bile acids can stimulate L-cells through activation of the TrpA1 cation channel and the predominantly Gs coupled receptor GPBAR1-1, respectively; while it was thought that GPBAR1 directly monitors luminal bile acids I will present evidence for a basolateral location of GPBAR-1. Characterising the pathways and receptors underlying L-cell stimulation is hoped to identify new therapeutic opportunities for the treatment of diabetes and obesity.

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SA080
Gastric bypass reverses the effects of diet-induced obesity to inhibit the responsiveness of central vagal neurones to GLP-1
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Gastric bypass surgery is the most effective treatment for obesity and related comorbidities, with Roux-en-Y gastric bypass (RYGB) being one of the most common procedures. While mechanically restricting the size of the stomach clearly contributes to altered satiety signaling and reduced food intake, several studies have shown that changes in the secretion and actions of gastrointestinal (GI) neurohormones may also play significant roles. Vagally-dependent reflexes are critical to the control, regulation and organization of appropriate GI functions, including early satiety. Visceral sensory information from the GI tract is transduced, encoded and relayed centrally via the afferent vagus nerve, the central terminals of which enter the brainstem via the tractus solitarius and terminate within the nucleus of the tractus solitarius (NTS). NTS neurones integrate this vast volume of sensory information with hormonal and metabolic signals as well as neural inputs from other brainstem and higher central nuclei involved in autonomic homeostasis. The integrated neural response from NTS neurones is then relayed to the adjacent dorsal motor nucleus of the vagus (DMV) which contains the preganglionic parasympathetic motoneurones that supply the motor output to the GI tract via the efferent vagus nerve.

Studies from several laboratories have shown that the behavior, responsiveness and activity of vagal sensory neurones is compromised by diet-induced obesity, yet very attention has been paid to the effects of diet or obesity on central brainstem neurones and even less information is available regarding the effects of bariatric surgeries on these neurones. The aim of the present study was to use electrophysiological techniques to determine the effects of diet-induced obesity on the biophysical, morphological and pharmacological properties of vagal efferent motoneurones and whether these effects were reversed by RYGB-induced weight loss.

Male Sprague-Dawley rats were fed a high fat diet (HFD; 60% kcal from fat; D12491, Research Diets Inc) from 4 weeks of age for 12 weeks. One group of rats (n=17) underwent RYGB surgery, one group underwent ‘sham’ surgery (n=7) while the remaining rats (n=30) were maintained on high fat diet but did not undergo any surgical intervention. An additional group of rats were fed control diet (13.5% kcal from fat; Purina Mills) from 4 weeks of age for 12-14 weeks (n=19). Whole cell patch clamp recordings were made subsequently from DMV neurones in thin (300um) brainstem slices.

There were no differences between HFD and sham surgery rats; these results were therefore grouped. DMV neurones from rats exposed to a HFD for 12-14 weeks were less excitable with a decreased membrane input resistance (290±15MΩ vs 314±22MΩ; P<0.05) and decreased ability to fire action potentials in response to current injection (2.6±0.3 action potentials vs 3.6±0.4 action potentials in response to 30pA current injection; P<0.05 and 4.3±0.4 action potentials vs 5.3±0.6 action potentials in response to 270pA current injection; P<0.05). DMV neurones from HFD rats were also less responsive to superfusion of satiety neuropeptides such as glucagon-like peptide 1 (GLP-1; 100nM). Specifically, only 3 of 21 HFD neurones tested responded to GLP-1 with an increase in action potential firing, whereas 4 of 7 control neurones tested increased action potential firing in response to GLP-1 (P<0.05). RYGB reversed all of these effects (input resistance 400±37MΩ; P<0.05 vs HFD; P>0.05 vs control; 5.5±0.5 and 9.3±0.7 action potentials in response to 30 and 270pA current injection, respectively, P<0.05 vs HFD and control for both; GLP-1 increased the action potential firing rate of 8 of 10 neurones tested; P<0.05 vs RYGB; P>0.05 vs control) suggesting these effects may be due to obesity, rather than diet. Diet-induced obesity also altered the morphological properties of DMV neurones, increasing the soma size and dendritic arborization, although these morphological alterations were not reversed by RYGB, suggesting these effects may be due to diet, rather than obesity.

These studies demonstrate that diet-induced obesity also affects the properties of central autonomic neurons, reducing their excitability and responsiveness to neuropeptides. Furthermore, these results represent the first direct evidence for the plausible effects of RYGB to improve vagal neuronal ‘health’ by reversing some of the effects of a chronic HFD. Vago-vagal neurocircuits appear to remain open to modulation and adaptation throughout life, and understanding the mechanisms of these effects may help in the development of novel therapeutic interventions to alleviate environmental (i.e., dietary) ailments.

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SA081
Role of endogenous peripheral GLP-1 in energy homeostasis and glycemic control
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Enteroendocrine L-cells release GLP-1 in response to luminal nutrient (primarily carbohydrate and fat) stimulation. Peripheral and central administration of GLP-1 inhibits eating, and peripheral GLP-1 inhibits gastric emptying (GE) and enhances glucose-induced insulin release. GLP-1 receptors (GLP-1R) are expressed in the periphery and in several brain areas implicated in eating and metabolic control, but the sites and mechanism through which intestinal endogenous GLP-1 exerts its effects are uncertain. Previous studies from our laboratory demonstrated that intact vagal afferent neurones (VAN) are required for the satiating effect of intraperitoneally (IP) administered GLP-1. To address the role of VAN GLP-1R, we knocked down the expression of VAN GLP-1R by bilateral nodose ganglion injection of lentiviral GLP-1R shRNA in male Sprague-Dawley rats. GLP-1R KD in chow-fed rats did not affect the body weight and 24h cumulative food intake compared to control animals, but it increased meal size, decreased meal frequency, enhanced GE, and postprandial glycemia, indicating that these effects are due to a paracrine effect of endogenous GLP-1 on abdominal VAN. Moreover, GLP-1R KD blunted the satiating and GE inhibitory effects of GLP-1 after IP, but not ICV (4th ventricle) administration. Surprisingly, GLP-1R KD rats fed a high-fat diet (HFD) showed decreased body weight gain, improved glycemic control and increased energy expenditure compared to control animals. This appeared to be related to an increased brown adipose tissue (BAT) UCP1 expression and
enhanced sympathetic input to interscapular BAT. Finally, whereas GLP-1R KD decreased nucleus tractus solitarii (NTS) pre-pro-glucagon (PPG) expression in chow-fed animals, it prevented the decrease in NTS PPG expression observed in HFD-fed control rats. Our findings demonstrate that intact VAN GLP-1R signaling is required for the full expression of the satiating, GE inhibiting and glycemia controlling effects of endogenous GLP-1 in chow-fed animals. In addition, VAN GLP-1R signaling appears to contribute to some of the hallmarks of HFD-induced obesity. Further studies are necessary to identify the exact mechanism(s) of the beneficial effects of GLP-1R KD with chronic HFD feeding. SNSF MHV grant PMPD3_151360 (SJL), ETH Research Grant 47 12-2 (WL and SJL).

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SA083

Sex differences in the human vasculature: Impacts of fitness and exercise training
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This talk will review the historical evidence for the purported impacts of male and female sex hormones on the vasculature in humans, including effects on macro- and micro-vascular function and health. Direct comparisons between the sexes, in terms of in vivo measures of arterial size and function, will be provided from a large sample of 950 asymptomatic and healthy, pre- and post-pubertal children (6-18 yrs). These data will be compared to sex differences apparent in 180 adults in whom the modulating impacts of fitness, BMI and cardiovascular risk factors are also be addressed. Finally, the results of two recent laboratory studies from our team, in which younger men and women were recruited and directly compared to older cohorts, will be presented. Although based on small sample sizes, these studies suggest that differences exist between men and women in terms of the impact of age on arterial function (flow-mediated dilation FMD and glyceryl trinitrate GTN) and structure (arterial diameter, wall thickness and wall:lumen ratio) and that cardiorespiratory fitness modulates these differences. The impact of exercise training in older sedentary men and women will also be considered, with an emphasis on the degree to which age-related changes can be ameliorated in both sexes. Ultimately, this talk will highlight the paucity of high quality and compelling evidence regarding the fundamental impact, in humans, of sex differences on arterial function and structure as people age.

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SA084

Sex differences in muscle metabolism: impact on exercise and nutritional strategies to optimize health and exercise performance in women
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Sex differences in substrate metabolism during moderate-intensity endurance exercise have been well established. Specifi-
ically, at the whole body level women have a lower respiratory exchange ratio (RER) during moderate-intensity endurance exercise as compared with men (1), indicative of a lesser reliance on whole body carbohydrate (CHO) oxidation to support exercise fuel needs. In fact, as compared with men, women have a lower reliance on both liver (decreased glucose rate of appearance (Ra), rate of disappearance (Rd) and metabolic clearance rate (MCR)) and muscle glycogen stores during moderate-intensity endurance exercise (1). Furthermore, while the effects of sex on intramyocellular lipid (IMCL) utilization during endurance exercise are controversial, women have a greater depot of IMCL available to support exercise fuel needs and a greater percentage of IMCL in contact with mitochondria following a bout of moderate-intensity endurance exercise as compared with men (2), suggestive of a greater capacity to utilize IMCL. These sex differences in metabolism during endurance exercise are known to be mediated by estrogen. Indeed, estrogen supplementation to young men decreases RER and liver glycogen utilization, with no effect on muscle glycogen utilization during moderate-intensity endurance exercise (3, 4). However, while not impacting muscle glycogen utilization, estrogen supplementation did lower muscle glycogen stores prior to exercise (3), which may preclude changes in muscle glycogen utilization should estrogen supplementation continue, as is seen when comparing men to women. Given that estrogen concentrations fluctuate across the menstrual cycle it is not surprising that substrate metabolism during moderate-intensity endurance exercise also varies across the menstrual cycle. In the luteal phase when estrogen levels are high, women have a lower liver and muscle glycogen utilization as compared with women in the follicular phase (1). Taken together, these findings support substantial differences in muscle metabolism during moderate-intensity endurance exercise between men and women and within women across the menstrual cycle, which may impact the response to exercise and nutritional strategies aimed at improving health and performance in women.

Despite these well-recognized differences in substrate metabolism during endurance exercise between men and women and across the menstrual cycle, there is a paucity of research examining the effects of exercise and nutritional regimes aimed to enhance performance and/or health in women. Furthermore, the evidence that does exist is suggestive of discordance in the effectiveness of nutritional and exercise regimes between the sexes. While we have reached the era of personalized sport nutrition, we are without a sufficient body of evidence to truly optimize sport nutrition in women athletes. It is not just sport performance where the benefits of exercise and nutrition in women are unknown, the effects of specific exercise and nutritional regimes to optimize health in women are also not completely understood. The focus of this talk will be to provide an overview of the well-established sex differences in metabolism during endurance exercise and how they relate to the existing data showing sex differences in response to nutritional (i.e. carbohydrate loading, creatine, protein) and exercise strategies (i.e. high intensity interval training, resistance exercise) intended to improve exercise performance and/or health.


Sex differences in skeletal muscle fatigue

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Men and women differ in anatomy and physiology resulting in marked sex differences in neuromuscular performance and fatigability of skeletal muscle. Muscle fatigability is an acute activity-induced reduction of force or power of a muscle: it is typically quantified as the relative reduction in maximal strength and power, and the time to failure of a submaximal task (Gandevia, 2001). Multiple mechanisms are responsible for fatigability in men and women and may include activation of the motoneurone pool from cortical and subcortical regions, synaptic inputs to the motor neuron pool via activation of metabolically-sensitive small afferent fibers in the muscle, muscle perfusion, skeletal muscle metabolism and altered crossbridge dynamics in the muscle fibers. The mechanisms of fatigability are specific to the details of the task because different neuromuscular sites will be stressed when the requirements of the task are altered (Enoka & Duchateau, 2008). There are sex differences in muscle fatigability which are specific to the requirements of the task. This means that the magnitude of the sex difference in fatigability and the contributing mechanisms to this difference can alter as the task varies (Hunter, 2014). Typically, studies show that for upper and lower limb muscles, women are usually less fatigable than men for similar intensity isometric fatiguing contractions due to contractile mechanisms and muscle perfusion. Less is known however, about the sex differences in fatigability of dynamic contractions and the responsible mechanisms. Data from my laboratory supports findings that for slow-to-moderate velocity contractions, women are less fatigable than men (Hunter, 2014). However, this sex difference in fatigability of dynamic contractions varies with the type of dynamic contraction (shortening or lengthening), intensity and speed of the contraction and the muscle group assessed.

For muscle shortening tasks, when men and women were asked to contract as quickly as possible with a load at 20% of maximum, the decline in power for both the elbow flexor and knee extensor muscles was similar for men and women. However, the reduction in maximum voluntary isometric force (MVIC) measured immediately after the dynamic contraction was greater for men than women but only for the knee extensor muscles (Seneffel et al., 2013). Additional experiments using transcranial magnetic stimulation, showed that supraspinal fatigue (fatigue from inadequate activation of the motor cortex) increased but was similar for men and women after the dynamic fatiguing contraction. Rather, contractile properties measured from electrically evoked contractions,
sloved more dramatically for men than women. Hence, the contractile mechanisms were responsible for a sex difference in fatigability after the dynamic contraction when assessed with a MVIC. These findings are consistent with muscle biopsy studies that indicate the whole skeletal muscles of women possess a greater proportional area of oxidative and fatigue resistant fibers than men (Hunter, 2014). Furthermore, these studies highlight a task specific interaction between the type of fatiguing task and the maximal strength measurements typically utilized to quantify sex-based differences in fatigability.

In contrast to shortening contractions, the sex difference in fatigability for lengthening contractions may be reversed because women appear to experience greater fatigability within the muscle than men (Power et al., 2013). More studies are needed because our understanding of sex differences in response to lengthening contractions is very limited, despite their importance to daily tasks, training and rehabilitation. Finally, non-physiological factors contribute to a profound understanding of sex-based differences in muscle fatigability. There is a historical and current sex bias of studying more males than females in human and animal experiments in both physiology and fatigability (Beery & Zucker, 2011). There is also the false assumption that men and women respond similarly to fatiguing exercise. The field is ripe with opportunities. Knowledge of the underlying mechanisms of sex-based differences in fatigability during the different types of dynamic contractions will clarify the benefits and limitations that skeletal muscle fatigability can exert during daily tasks, exercise performance, and training in both men and women.


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Innsbruck, Austria and clinically used to treat hypertension (1). In the brain only the members of the L-type calcium-channel family of calcium-dependent signaling processes in electrically excited cells. The voltage-gated calcium-channels are key molecular regulators of calcium signaling. More recently, we (3) and others (4) discovered somatic mutations in the pore-forming α1-subunit of Cav1.3 (CACNA1D) which induce a strong increase in Cav1.3 channel function and cause excessive aldosterone production in aldosterone-producing adrenals. When present germline, two of these de novo mutations cause a severe congenital syndrome with primary aldosteronism, but also with neurodevelopmental deficits and seizures at early age (PASNA, 4). Moreover, we have recently found that two very similar de novo mutations (G407R, A749G) identified in two patients with autism and intellectual disability also cause a strong gain-of-channel function (5). Together these data strongly suggest that altered channel gating of Cav1.2 and Cav1.3 channels can lead to neuropsychiatric disease.

To directly address this question we have generated mice expressing Cav1.3 α1 subunits with a hemagglutinin antibody (HA-) tag inserted in the C-terminal tail (Cav1.3HA/HA). This HA-tag disrupts a C-terminal modulatory domain which, as shown in heterologous expression systems (1), moderates channel function in long (but not short) splice variants of the channel which comprise about 50% of the Cav1.3 channels in the brain (1). Using these mice we demonstrate that, while the long splice variant is still expressed at unchanged levels in the brain of homozygous mutants, disruption of its C-terminal modulatory domain also alters Cav1.3 channel gating in their native cellular environment. Behavioral analysis revealed an increased anxiety-like behavior in Cav1.3HA/HA mice. These data in mice further support the hypothesis that Cav1.3 dysregulation can lead to CNS dysfunction. Based on the finding that enhanced LTCC activity contributes to neuropsychiatric disease risk, already existing or novel (e.g. Cav1.3-selective) LTCC blockers should be considered as potential therapeutics in individuals with increased genetic risk.

**References**

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9. Support: Austrian Science Fund (FWF F44020; F44010)

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SA090

Interaction between the Fragile X Mental Retardation Protein (FMRP) and the N-type Calcium Channel Ca\textsubscript{v}2.2 to modulate synaptic transmission

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Fragile X syndrome (FXS), the most common heritable form of mental retardation, is characterized by synaptic dysfunction. FXS results from the partial or complete loss of Fragile X mental retardation protein (FMRP) expression and function. FMRP is an RNA binding protein, involved in the control of local translation, which has pleiotropic effects, particularly on synaptic function. Synaptic transmission depends critically on presynaptic calcium entry via voltage-gated calcium (Ca\textsubscript{v}) channels. Here we show that the functional expression of neuronal N-type Ca\textsubscript{v} channels (Ca\textsubscript{v}2.2) is regulated by fragile X mental retardation protein (FMRP). We find that FMRP knockdown in dorsal root ganglion neurons increases Ca\textsubscript{v} channel density in somata and in presynaptic terminals. We then show that FMRP controls Ca\textsubscript{v}2.2 surface expression by targeting the channels to the proteasome for degradation. The interaction between FMRP and Ca\textsubscript{v}2.2 occurs between the C-terminal domain of FMRP and domains of Ca\textsubscript{v}2.2 known to interact with the neurotransmitter release machinery. Finally, we show that FMRP controls synaptic exocytosis via Ca\textsubscript{v}2.2 channels. Our data indicate that FMRP is a potent regulator of presynaptic activity, and its loss is likely to contribute to synaptic dysfunction in FXS.

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SA091

L-type calcium channels in anxiety disorders and drug-taking behaviours

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Intronic single nucleotide polymorphisms (SNPs) in the CACNA1C gene, which encodes for the Ca\textsubscript{v}1.2 protein, have been identified as risk factors for a number of neuropsychiatric disorders including bipolar disorder, major depressive disorder, and schizophrenia (1). Recent studies have revealed that these intronic SNPs can lead to both higher and lower levels of CACNA1C mRNA (2, 3) suggesting that a gain or loss of Ca\textsubscript{v}1.2 function can contribute to neuropsychiatric conditions.

Anxiety is a common core underlying symptom across all neuropsychiatric conditions in which CACNA1C has been implicated. Additionally, altered brain reward circuitry and addictive behavior is a common, co-morbid condition often observed in patients with anxiety disorders. Many studies have revealed overlapping anatomical pathways and molecular mechanisms involved in mood disorders and addiction (4). Recently, human carriers of the bipolar disorder and schizophrenia-associated SNP rs1006737 were found to be associated with altered reward responsiveness compared to non-carriers (5), suggesting that variants in CACNA1C may predispose individuals to anxiety disorders and addiction. Thus, to better understand the role of CACNA1C in mediating neuropsychiatric-related behavioral phenotypes we have utilized a combination of cacna1c (Ca\textsubscript{v}1.2) genetic knockout mice, viral vector-mediated focal knockouts and behavioral assays to map the Ca\textsubscript{v}1.2 brain circuitry in anxiety- and addiction-related behaviors. Our studies have revealed that Ca\textsubscript{v}1.2 channels within glutamatergic neurons of the forebrain regulate anxiety-related behaviors in mice as measured by the open field, light-dark, and elevated plus maze tests (6). Stereotoxic delivery of adenosinated viral vector (AAV) expressing Cre recombinase under the control of the CaMK2 promoter in Ca\textsubscript{v}1.2 floxed mice, has further identified glutamatergic neurons within the prefrontal cortex (PFC) in regulating anxiety-like behaviors. This is consistent with human and rodent studies that have established the PFC as a critical brain center involved in emotional regulation.

As carriers of CACNA1C SNPs have been shown to have altered brain reward responsivity (5), we tested cacna1c knockout mice in cocaine conditioned place preference (CPP), a behavioral test used to measure cocaine reward. We find that cacna1c knockout mice that exhibit high anxiety have higher preference for cocaine than control wildtype mice. AAV-Cre-mediated conditional knockout of Ca\textsubscript{v}1.2 further demonstrates that loss of Ca\textsubscript{v}1.2 in the hippocampus (HPC) results in a long-lasting maintenance of cocaine preference compared to control mice. Taken together our data using mouse models suggests that loss of Ca\textsubscript{v}1.2 channel function within key brain regions contributes to anxiety and addiction-related behaviors and that similarly, SNPs in CACNA1C may predispose humans to anxiety disorders and addiction.


A. S. Lee et al., Forebrain elimination of cacna1c mediates anxiety-like behavior in mice. \textit{Mol Psychiatry} \textbf{17}, 1054-1055 (2012).

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SA092

Voltage gated calcium channels in the physiology of heart automaticity

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Pacemaker activity of sino-atrial myocytes permanently controls the heart rate. Heart automaticity is modulated by the autonomic nervous system, which regulates the activity of ion
channels of the plasma membrane in automatic cells. Several classes of ion channels contribute to pacemaking. The functional role of voltage-dependent calcium channels (VGCCs) in heart automaticity and impulse conduction has been matter of debate for 30 years. Here we present and discuss recent evidence showing that VGCCs are important mechanisms underlying pacemaker activity and impulse conduction. Indeed, studies performed in genetically modified mice lacking L-type Cav1.3 (Cav1.3<sup>-/-</sup>) or T-type Cav3.1 (Cav3.1<sup>-/-</sup>) channels demonstrate that ablation of these channels severely impairs pacemaker activity. In sino-atrial myocytes, VGCCs activate at negative voltages at the beginning of the diastolic depolarization and importantly contribute to this phase by supplying inward current. Ablation or loss-of-function of these channels induces also heart block. Furthermore, inactivation of Cav1.3 channels promotes also tachy-brady syndromes in knockout mice indicating that these channels are important in stabilisation of atrial rhythm. Genomic analysis demonstrated that Cav1.3 and Cav3.1 channels are widely expressed in pacemaker tissue of mice, rabbits and humans. Importantly, human diseases of automaticity such as congenital bradycardia and heart block have been attributed to loss-of-function of Cav1.3 and Cav3.1 channels.

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**SA093**

*T and L-type calcium channels in the control of aldosterone production and low-renin hypertensive disorders*

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Calcium current carried by low voltage-activated, T-type calcium channels is the pacemaker current in a variety of electrically excitable cells. Zona glomerulosa cells (ZG) clustered in rosettes within the outer cortex of the adrenal gland have robust Cav3.2 currents in all recorded species (rat, bovine, mouse, human) including man. We and others have championed the putative role of Cav3.2 window currents (channel activation without full inactivation) to explain how this largely transiently activating calcium channel could provide a tonic current that is sufficient to sustain the production of aldosterone that lasts minutes to hours. Yet, the critical privileged role played by these channels in driving aldosterone production from an electrically quiescent cell that maintains a baseline voltage of ~−85 mV has never been fully reconciled. We now provide new insight into the functional organization of the ZG tissue layer, and the behavior of ZG cells retained within rosettes. We find that in situ ZG cells behave as electrical oscillators and exhibit coordinated activity within the tissue layer. This updated view of calcium signaling in ZG cells provides a satisfying mechanism to explain how Cav3.2 channels generate a significant and recurring calcium signal. It also provides the foundation for recent human genetic studies that have identified somatic gain-of-function mutations in Cav1.3 channels in zona glomerulosa-like aldosterone producing adenomas. Specifically, the large amplitude of voltage oscillations in ZG cells implicates potential roles for high-voltage-activated conductances in the control of aldosterone production, expanding the scope of ionic regulation and the recognition of low renin hypertension as a channelopathy.

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**SA094**

*Structural dynamics and timing of the CFTR pore opening conformational transition*

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The CFTR chloride channel is regulated by phosphorylation of its intracellular regulatory (R) domain and ATP binding/hydrolysis at two conserved cytosolic nucleotide binding domains (NBDDs). Once phosphorylated by protein kinase A (PKA), gating of the CFTR channel pore in the presence of MgATP displays a bursting pattern. This bursting process reflects an irreversible functional cycle: initiation of a burst coincides with formation of an intramolecular NBD1/NBD2 heterodimer stabilized by two molecules of ATP sandwiched at the dimer interface, while termination of a burst coincides with disruption of this NBD dimer, normally following hydrolysis of one of the two bound nucleotides (Nature 433:876-880). The overall cycle is rate limited by the high enthalpy of the transition state for the pore opening step (J Gen Physiol 128:523-533), but the molecular details of this unstable short-lived structure, and the relative timing of molecular motions that lead from the open-dimer/closed-pore to the tight-dimer/open-pore conformation, are unknown. We present here a thermodynamic study of the opening conformational transition using the rate-equilibrium free energy relationship (REFER) approach. Because REFER analysis provides no information on non-equilibrium systems (J Gen Physiol 134:129-136), we use a CFTR background construct in which mutation of the NBD2 Walker B aspartate prevents ATP hydrolysis, and thus reduces channel gating to a simple equilibrium process. Our results provide direct information on the relative timing of gating motions along the longitudinal axis of the channel protein structure, and on conformational details of the opening transition state.

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**SA095**

*Structural basis of interdomain communication in a heterodimeric ABC exporter*

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Many ABC exporters are heterodimers encompassing asymmetric ATP binding sites. In these transport proteins, one ATP binding site – called the degenerate site – entails a non-canonical catalytic dyad and is impaired in ATP hydrolysis but not in nucleotide binding. It is therefore at the second ATP binding site – the so-called consensus site – where ATP is predominantly if not exclusively consumed. Recently, we have solved the crystal structure of TM287/288 from the thermophilic bacterium *Thermotoga maritima*, which is the first structural representative of an ABC exporter containing asymmetric ATP binding sites. The transporter was crystallized in its apo state.
The cystic fibrosis transmembrane conductance regulator (CFTR) regulates the flow of water and anions through the apical membrane of epithelia. CFTR, although functioning as an ion channel, is a member of the ATP binding cassette (ABC) transporter superfamily and shares a common mechanism with ABC transporters, in which conformational changes during gating are driven by ATP binding and hydrolysis. CFTR features a structural core common among ABC transporters: two transmembrane domains (TMDs) form the pore for anion flow, while two conserved nucleotide binding domains (NBDs) provide the binding sites for two ATP molecules. Mutations in CFTR affect the proper folding, trafficking and function of the protein, resulting in the genetic disease cystic fibrosis. The lack of experimental structures of the full length CFTR hampers a global understanding of CFTR mechanism, and thus the development of approaches directly targeting dysfunctional CFTR.

In this study, we aimed at investigating possible conformational states visited by CFTR during the gating cycle. By means of homology modeling techniques, molecular models of CFTR were built using, as templates, the structures of four homologous ABC transporters, namely TM287-288, ABC-B10, McjD and Sav1866. Comparisons with published experimental data suggest that the TM287-288-based CFTR model could represent a closed-state channel, bearing a large opening between the intracellular sides of the TMDs while maintaining some contacts at the NBD interface. In this model the extracellular mouth of the pore is closed, not allowing passage of water molecules during simulations. In contrast, the McjD-based CFTR model, with tightly dimerized NBDs, provides features of an open-state channel, with openings on the upper TMDs that allow water to flow across the pore. Here, we present a detailed overview of the models, and discuss how they could help understand the role of specific residues in the gating cycle of CFTR, as well as the conformational transition from closed to open channels.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Electrophysiological aspects of ABCC6-mediated cellular ATP release

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Pseudoxanthoma elasticum (PXE) is an autosomal recessive disease characterized by progressive ectopic mineralization of the skin, eyes and arteries, for which no effective treatment exists. PXE is caused by inactivating mutations in the gene encoding the hepatic efflux transporter ABCC6. Elegant experiments performed by Uitto and co-workers have demonstrated that PXE is a metabolic disease caused by the absence of an unknown factor in the circulation, the presence of which depends on ABCC6 in the liver[1,2]. Why absence of this factor results in PXE has for long remained a mystery. We have recently shown that medium from HEK293 cells overexpressing ABCC6 potently inhibits mineralization in vitro, whereas medium of HEK293 control cells does not. Untargeted metabolomics revealed that cells expressing ABCC6 excrete large amounts of nucleoside triphosphates, predominantly ATP. Extracellularly, ectonucleotidases hydrolysed the excreted amounts of nucleoside triphosphates, predominantly ATP. The medium of HEK293 control cells does not. Untargeted metabolomics revealed that cells expressing ABCC6 excrete large amounts of nucleoside triphosphates, predominantly ATP. Extracellularly, ectonucleotidases hydrolysed the excreted amounts of nucleoside triphosphates, predominantly ATP. The medium of HEK293 control cells does not. Untargeted metabolomics revealed that cells expressing ABCC6 excrete large amounts of nucleoside triphosphates, predominantly ATP. Extracellularly, ectonucleotidases hydrolysed the excreted amounts of nucleoside triphosphates, predominantly ATP. The medium of HEK293 control cells does not. Untargeted metabolomics revealed that cells expressing ABCC6 excrete large amounts of nucleoside triphosphates, predominantly ATP. Extracellularly, ectonucleotidases hydrolysed the excreted amounts of nucleoside triphosphates, predominantly ATP. 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A high resolution analysis of matrix vesicle-derived destabilizing microcalcifications in thinning atherosclerotic caps

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Despite evidence linking arterial calcification to acute cardiovascular event risk, the physicochemical mechanisms underlying mineral nucleation and growth in atherosclerotic plaques remain unknown. Prospective clinical data show that the risk of cardiovascular events inversely correlates with the density of calcification present within arterial plaques. Finite element modeling of stress distribution within plaques indicates that subcellular microcalcifications in a plaque’s fibrous cap promote material failure of the plaque, causing myocardial infarction and stroke. In contrast, large calcifications may stabilize the plaque, but mechanisms that give rise to these two morphologies are unclear. The formation of both large calcifications and microcalcifications in the atherosclerotic plaque may involve extracellular vesicles (EVs) released by cells within the plaque that serve as nucleating foci for mineralization. The lack of certainty in this process exists due to the inability to visualize calcification nucleation and growth in vivo. Using advanced high-resolution microscopic and spectroscopic analyses of calcified human atherosclerotic plaques and a three-dimensional synthetic collagen hydrogel system to mimic the plaques, we provide a crucial link between plaque collagen content and calcification morphology—the two determinants of atherosclerotic plaque stability—thus offering novel insight into the mechanisms of plaque integrity. Confocal images revealed small microcalcifications abundantly present throughout the fibrous cap of human carotid plaques obtained from endarterectomy or autopsy. The microcalcifications appeared to form in gaps between collagen fibers and are composed of discrete spheres less than 1
µm in diameter. Pearson correlation analysis revealed a significant inverse relationship between microcalcification area and the collagen content within the analyzed area (n = 9 plaques, r = -0.29, p = 0.01). Microcalcifications were also found directly adjacent to large calcific regions, where the microcalcifications appeared to merge, forming the larger calcifications. Density-dependent color scanning electron microscopy (DDC-SEM) revealed structural heterogeneity within the large calcific regions with small calcium phosphate-rich microcalcification spheres less than 1 µm in diameter aggregating to form the larger calcific region.

We developed a three-dimensional in vitro preparation to visualize these processes to test our hypothesis that nucleation and growth of calcific mineral depends on calcification and aggregation of cell-derived EVs. Human coronary artery smooth muscle cells (SMCs) from primary donors were cultured in control or calcifying media for 14 days, a sufficient time for the SMCs to release specialized calcifying EVs. The EVs were collected and added to collagen hydrogels, mimicking the fibrous cap of atherosclerotic plaques. After 72 h incubation at 37 °C, the calcifying samples exhibited structures observed by confocal reflection microscopy in gaps between collagen fibers that contained regions of calcification as detected by a molecular imaging calcium tracer. DDC-SEM revealed aggregation of EVs from the calcifying media samples between collagen fibers, forming dense structures resembling those observed within calcified human plaques. Structured illumination super-resolution microscopy of fluorescently labeled EVs provided an optical approach to visualize EV aggregation and calcification. This technique allowed visualization of objects the size of individual EVs aggregating (approximately 200 nm in diameter) within the collagen hydrogels. EVs appeared to aggregate to produce spherical microcalcifications. Increasing the collagen concentration within the hydrogels from 0 to 1 mg/ml reduced the average aggregate size by 90% compared to the samples without collagen (n=4, ANOVA, p < 0.001). We provide direct visualization of vascular calcific mineral nucleation and growth using multimodal imaging and spectroscopic techniques. We show that calcification growth and maturation results from a series of aggregation processes that is controlled by collagen concentration. We found an inverse relationship between collagen content in the fibrous cap of human atherosclerotic plaques and the size of observed microcalcifications. Further, by increasing collagen concentration in three-dimensional hydrogels, we showed a decrease in the size of EV aggregates that form within the hydrogel and a concomitant decrease in the maturity of the resulting calcific mineral. These observations suggest that formation of microcalcifications accompanies thinning of the fibrous cap. We propose that inflammation leading to collagen degradation within the cap allows localized calcifying EV aggregation and microcalcification formation, thus unifying two prominent theories of plaque instability.

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Mechanisms of exosome secretion by vascular smooth muscle cells and their coagulant properties

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Vascular smooth muscle cells (VSMCs) play a key role in regulating the circulation of blood in the vasculature by providing vascular wall structural integrity and regulating arterial tone and systemic blood pressure. Numerous stress factors including inflammation and mechanical stress induce loss of the contractile VSMC phenotype and stimulate their migration and proliferation. This can lead to impaired vascular function and is associated with high cardiovascular mortality and morbidity. We recently showed that VSMCs secrete exosomes which form the nidus for calcification (Kapustin et al., Circ Res, 2015). Notably, we found that exosome release is a unique feature of actively proliferating VSMCs undergoing phenotypic conversion. Treatment of the cells with pro-inflammatory PDGF-BB or TNF-alpha in vitro enhanced exosome secretion and this was mediated by up-regulation of sPLA2iiA and sHSP70, proteins annexins and PT as well as thrombin inhibitors thus counteracting their pro-coagulant activity.

Together our data shows that exosome secretion by VSMCs is associated with thrombosis formation in the vascular lumen which is caused by exposure of coagulation factor binding sites, namely phosphatidylserine (PS), on the surface of apoptotic cells. We have also identified PS on VSMC-derived exosomes suggesting that exosomes can stimulate coagulation. Addition of VSMC exosomes to normal plasma stimulated thrombin generation in vitro whilst annexin A5, proposed to play a role in the inhibition of blood coagulation, abrogated this effect indicating that it is mediated by PS exposed on exosomes. We found that coagulation factor II, prothrombin (PT) is uptaken by VSMCs and loaded onto the exosome surface. Unexpectedly, PT recycled in exosomes was not susceptible for conversion to thrombin by prothrombinase complex and the blocking of PS sites by inactive PT reduced exosome thrombotic activity.

Rupture of the fibrous cap in atherosclerotic lesions is often associated with thrombus formation in the vascular lumen which is caused by exposure of coagulation factor binding sites, namely phosphatidylserine (PS), on the surface of apoptotic cells. We have also identified PS on VSMC-derived exosomes suggesting that exosomes can stimulate coagulation. Addition of VSMC exosomes to normal plasma stimulated thrombin generation in vitro whilst annexin A5, proposed to play a role in the inhibition of blood coagulation, abrogated this effect indicating that it is mediated by PS exposed on exosomes. We found that coagulation factor II, prothrombin (PT) is uptaken by VSMCs and loaded onto the exosome surface. Unexpectedly, PT recycled in exosomes was not susceptible for conversion to thrombin by prothrombinase complex and the blocking of PS sites by inactive PT reduced exosome thrombotic activity. Further studies showed that exosomes are enriched with specific thrombin inhibitors, alpha-macroglobulin and protease nexin-1 which inhibit activation of PT and reduce exosome pro-coagulant activities.

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The role of microvesicles in cardiovascular calcification

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Cardiovascular calcification, a growing burden in Westernized countries, is not only a risk factor for cardiovascular events, but may itself contribute to cardiovascular risk. Research into treatment of cardiovascular calcification is lacking, as shown by clinical trials that have failed to demonstrate the reduction of calcific aortic stenosis. Hence the need to elucidate the pathways that contribute to cardiovascular calcification and to develop new therapeutic strategies to prevent or reverse calcification has driven our research investigations.

We previously showed that early calcification/microcalcification associates with macrophage accumulation in vulnerable atherosclerotic plaques. Chronic renal disease (CRD) accelerates calcification and the subsequent release of matrix vesicles (MVs) — precursors of microcalcifications. We tested the hypothesis that macrophage-derived MVs contribute directly to microcalcifications. We showed that macrophages associated with regions of calcified vesicular structures in human carotid plaque samples (n=136 patients). In vitro, macrophages released MVs with high calcification potential. MVs expressed exosomal markers (CD9 and TSG101), and contained S100A9 and annexin V (Anx5). Silencing S100A9 and genetic deficiency in S100A9+ mice reduced MV calcification, while stimulation with S100A9 increased calcification potential. Externalization of phosphatidylserine (PS) after Ca/P stimulation, and interaction of S100A9 and Anx5, indicated that a PS–Anx5–S100A9 membrane complex facilitates hydroxypatite nucleation within the macrophage-derived MV membrane. These results supported the novel concept that macrophages release calcifying MVs, which contribute to accelerated formation of microcalcification, thus providing an alternative mechanism of calcification as opposed to osteogenic differentiation.


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New findings on proteolysis in muscle wasting

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Skeletal muscle adapts its mass as consequence of physical activity, metabolism and hormones. Catabolic conditions or inactivity induce signaling pathways that regulate the process of muscle loss. Muscle atrophy in adult tissue occurs when protein degradation rates exceed protein synthesis. Two major protein degradation pathways, the ubiquitin-proteasome and the autophagy-lysosomes systems, are activated during muscle atrophy and variably contribute to the loss of muscle mass. These degradation systems are controlled by transcription dependent programs that modulate the expression of rate-limiting enzymes of these proteolytic systems. The transcription factors FoxO, which are negatively regulated by Insulin-Akt pathway, and NF-kB, which is activated by inflammatory cytokines, were the first to be indentified as critical for the atrophy process. In the last years a variety of pathways and transcription factors have been found to be involved in regulation of atrophy. I’ll summarise the last findings on protein synthesis and breakdown regulation and their implications in clinical practice to counteract muscle wasting.

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Strategies to improve muscle protein synthesis in wasting conditions

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While new molecular targets and pharmacological therapeutics (e.g. SARMS, myostatin inhibitors) to enhance muscle protein synthesis (MPS) and muscle mass have arisen over past years, the most proven, safe intervention to mitigate muscle wasting remains exercise-induced musculoskeletal loading i.e. resistance exercise training (RET). That said, we have shown that acute RET-induced increases in MPS and chronic muscle hypertrophy are blunted in older (O) vs. younger (Y) individuals. Moreover, while the etiology of sarcopenia and age-related “exercise resistance” (NB. also seen in some young people) remain poorly defined, it could be conjectured chronic deficits in MPS and associated imbalances in anabolic/catabolic factors (i.e. testostereone/IGF-1/myostatin/mTORc1-signalling) may drive these phenotypes. We hypothesized blunted hypertrophic responses to RET would be associated with impaired chronic MPS (using our newly developed heavy water (D2O) methods) and that this would be accompanied by hormonal and anabolic signalling deficits. Ten young (Y: 23±1y) and ten older (O: 69±3 y) men undertook 6-wks supervised progressive unilateral RET (i.e. one-leg knee-extensor: 6x8 reps, 75%-1RM 3 wk-1). In both legs: Vastus Lateralis muscle thickness, architecture, maximal voluntary contraction (MVC) and 1-repetition maximum (1-RM) were assessed at intervals with DXA at baseline (0-wks) and completion (6-wks). After baseline testing, subjects consumed 150ml D2O (70-Atom%) with a further 50ml wk-1; bi-lateral biopsies and blood samples were taken ~90 min post exercise at 0/3/6-wk to temporally quantify rested and exercised anabolic signalling (mTORc1) and MPS via GC-Pyrolysis-IRMS: MPS=(%·d-1)=Ln((1-ΑPEΔΑM/ ΑPEΔEj))/t)×100. We also collected blood samples at baseline and following the first bout of unilateral RET, to quantify systemic hormones (i.e. myostatin, IGF-1, testosterone; ELISA). Physical activity and food-intake were monitored via accelerometry and diet diaries, respectively. After 6-wks RET, 1-repetition maximum (RM) had increased 35±4% P<0.01 in Y and 25±3% in O P<0.01, while maximal voluntary contraction (MVC) increased at various joint angles in Y (e.g. 70° 29±6% P<0.01) but not O (8±3% P=N.S.). Similarly, quadriceps mass assessed by DXA increased only in Y (Y: 4±1% P<0.01 vs. O: 1±0.3% P=0.3). This was also consistent with blunted increases in muscle thickness (Y: 8±1 and 11±2%, P<0.01 vs. O: 2.6±1 and 3.5±2%, P=0.07 at 3 and 6-wks, respectively). Basal MPS was not different between groups (Y: 1.35±0.08%.d-1 vs. O: 1.38±0.09%.d-1). In contrast, reflecting early hypertrophy, MPS increased in Y but not O after 3-wks RET (Y: 1.61±0.1%.d-1 P<0.01 vs. O: 1.53±0.09%.d-1 P=0.1). Again, matching hypertrophic responses, MPS was not enhanced [over basal] 3-6wks in either group (Y: 1.29±0.11%.d-1 and O: 1.39±0.15%.d-1). Basal concentrations of myostatin did not differ with ageing (Y: 4563±403 pg/ml vs. O: 3781±373 pg/ml), while Y presented with greater testosterone (Y: 3.6±0.2 ng/ml vs. O: 2.6±0.2 ng/ml P<0.05) and IGF-1 (Y: 155.1± 16 ng/ml vs. O: 84.2±8 ng/ml P<0.01) concentrations. Following the first bout of RET, serum testosterone increased but this occurred only in Y (post-RET: 3.93±0.2 ng/ml P<0.05). During the study, neither protein (Y: 1.7±0.1 g (kg.FFM.d)-1 vs. O: 1.4±0.1 g (kg.FFM.d)-1 P=0.1) nor caloric (Y: 44±5 Kcal (kg.FFM.d)-1 vs. O: 35±6 Kcal (kg.FFM.d)-1 P=0.1) intake significantly differed with age. However, Y subjects were more habitually active: 7657±7521 vs. 5031±6996 activity counts.d-1 (P<0.05). As such, hypertrophic responses to RET in Y predominated in early stages of RET i.e. ~3-wks, underpinned by sustained increases in MPS; in contrast hypertrophic adaptations are blunted in older age as a result of chronic deficits in MPS. Moreover, in agreement with previous work we observed lower testosterone and IGF-1 in O, and speculate this contributes to blunted hypertrophy, and by extension, sarcopenia; in contrast, myostatin was unaltered by age. Further, we have shown D2O is a powerful tool to acquire long-term measures of MPS with potential to study multiple substrates (myofibrillar, mitochondria, satellite cells). Coupled to the relative ease in the application and minimal invasiveness, D2O holds promise for unraveling the role of muscle substrate synthesis in muscle wasting and for investigating the efficacy of anabolic/anti-catabolic interventions. In future, it is crucial we embrace heterogeneous mechanisms of human muscle wasting (e.g. in ageing, ICU, sarcopenia, cachexia syndromes etc) and that one-size will not fit all for treatments. Finally, there are big challenges associated physical therapies, beyond exercise resistance i.e. adherence, application to some populations (e.g. ICU, severe illness, disability) rendering pursuit of adjuvant therapies crucial.


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Current and prospective therapies to limit muscle wasting in cancer cachexia
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Loss of skeletal muscle is the main feature of cachexia that predicts risk of physical impairment, post-operative complications, chemotherapy toxicity, and mortality in patients with cancer. Studies in animal models provide evidence in principle that such muscle loss and these risks can be reversed. In the experimental setting cancer-associated muscle loss has been shown to have distinct pathophysiology involving specific catabolic pro-inflammatory signals (such as MIC-1/Growth and Differentiation Factor (GDF)-15, Interleukin-6, eicosanoids), tumor derived catabolic actors (e.g. parathyroid hormone related peptide, PTHrP) and antiproliferative factors (e.g.myostatin) or loss/reduced activity of anabolic effectors (e.g. androgens). Muscle loss can be prevented as well as reversed after it is already well established, by agents targeting these signals in experimental systems. A key finding is that the muscle wasting can be dissociated from the progression of the cancer itself. Zhou et al [1] provide one example: the activin IIb receptor (ActRIIB) mediates antiproliferative and catabolic effects on muscle by myostatin and Activin and pharmacological blockade of these actions by a decoy receptor resulted in muscle hypertrophy, enhanced strength, and extended survival. Tumor growth and tumor-associated inflammation were not affected by this treatment, suggesting that the extension of survival was associated with the specific gain of muscle mass. Such findings define the rationale for placing muscle as the therapeutic target and gain of muscle mass as the first primary outcome of new cachexia treatments currently in clinical trials.

In moving forward with clinical trials in this area, a central question is whether patients with advanced cancer possess the anabolic potential for reversal of muscle wasting as has been shown repeatedly in animal models. Od age, poor nutritional status, deconditioning, inflammation, cancer, and comorbid conditions are features of the patients typically affected by cancer, making this clinical entity considerably more complex than the controlled animal models. Patients with cancer are typically older (the median age of diagnosis is 63 y) and prone to lose muscle mass and function rapidly during periods of inactivity. Further catabolic losses of muscle are induced by many types of cancer therapy either because they cause malnutrition or because of their mechanisms of action to inhibit proliferation and anabolism which is directed at the tumor but may occur off-target in the muscle.

The constrained mentioned above notwithstanding, several findings support the suggestion that clinically significant gains in muscle mass are possible in patients with cancer. Recent reports demonstrate quantitatively significant lean tissue / muscle mass gains in patients with advanced cancer receiving anticachexia therapeutics. Some recently published findings include results of phase 2 trials of anamorelin (an orally bioavailable, small-molecule ghrelin mimetic with appetite-stimulating and anabolic activities) [2]. This agent stimulates the growth hormone secretagogue receptor centrally, mimicking the appetite-stimulating and growth hormone-releasing effects of ghrelin. A phase 2 study of the orally active selective androgen receptor modulator enobosarm induced gain of lean body mass of median 15 kg (range –21 to 126, p=00012) in a group patients of various cancers, whereas the placebo-treated group lost lean mass [3]. In a phase 2 study of patients with cholangiocarcinoma, selumetinib, a MEK kinase inhibitor (associated with systemic reduction in interleukin-6 levels) induced gain of skeletal muscle detected by CT scans [4]. The mean overall gain of total lumbar muscle cross-sectional area was 136 cm² (SD 119; or –23 kg of muscle on a whole-body basis). Muscle protein synthesis is clearly not shut down in patients with cancer. Several studies suggest that protein synthesis in muscle is unimpaired and responsive to the systemic availability of amino acids, albeit somewhat higher quantity than in young and healthy individuals [5].

Future priorities in therapeutic interventions for cancer associated muscle loss include matching of the most highly specific mechanism-based therapeutics to those patients likely to most benefit and the optimization of the muscle anabolism by the strategic combination of anti-catabolic and anabolic modalities of treatment, aligned with nutrient mixtures with levels and proportions of amino acids and other nutrients essential for muscle anabolism.


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Regulation of muscle metabolism during exercise
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Exercise results in a large and immediate increase in muscle energy demand that requires a coordinated integration of muscle fuel utilisation, which in non-pathological states is usually dictated by the availability of fuel substrate and the magnitude of increase in the energy demand of exercise. This lecture will focus on the regulation of muscle fuel utilisation in the rest to steady-state exercise transition period and during steady-state exercise. Furthermore, given that the pattern of muscle fuel use during exercise will influence fuel selection during recovery from exercise, this lecture will also hopefully set the scene for lectures to follow focused on the post-exercise recovery period.

Specifically, this lecture will focus on the integration of oxygen dependent (mitochondrial) and oxygen independent (cytosolic) energy production during the non-steady state period at the onset of exercise, and the rate limiting steps
Importance of AMPKα2 in substrate selection during post-exercise recovery

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Several studies have shown a substantial increase in fatty acid (FA) oxidation in the post-exercise period compared to the resting state. Even with an increased dietary intake of carbohydrates post-exercise, where a high carbohydrate oxidation would be expected, the contribution of FA for oxidation is high. It appears that muscle glycogen resynthesis after exercise has such a high metabolic priority that utilisation of lipids is elevated to cover the energy expenditure in muscle cells. The mechanisms regulating the selection of energy substrate towards oxidation/resynthesis in muscle during recovery from exercise remain unsolved. To investigate whether 5'-AMP activated protein kinase (AMPK) plays a role in this regulation, we measured substrate oxidation in metabolic chambers in AMPKα2 knockout (KO) and wild type (WT) mice during six hours, following a prolonged exercise bout, in which period the mice had free access to food and water. While relative substrate oxidation was similar during exercise performed at the same relative intensity between genotypes, the AMPKα2 KO during post exercise recovery displayed a lower (p<0.05) FA oxidation (respiratory exchange ratio (RER)=0.84±0.02) than WT mice (RER=0.79±0.01). This could not be explained by the AMPK-ACC-malonyl-CoA axis in the muscle. Instead, data revealed a role of AMPK in the regulation of the PDH complex in the skeletal muscle, which can explain the difference in RER between AMPKα2 KO and WT mice during post exercise recovery. Thus, while AMPKα2 seems not to be of importance for regulation of lipid utilisation in skeletal muscle during exercise, AMPKα2 seems to play an important role for substrate selection during post exercise recovery by inhibiting the activity of PDH complex, in turn increasing lipid oxidation and thus directing carbohydrates to muscle glycogen resynthesis.

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Regulation of substrate choice in recovery from exercise

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Following exercise, the metabolic rate remains elevated for several hours (up to 24 hours). This rise in metabolic rate is dependent on a variety of factors that include exercise intensity and duration. The main goals of exercise recovery are to re-establish metabolic homeostasis and to contribute to exercise adaptations. Whereas skeletal muscle tends to rely on carbohydrate oxidation for ATP production during exercise, energy provision during the post-exercise period tends to rely on lipid oxidation. This fuel shift is optimal because it allows for the replenishment of carbohydrate stores and the metabolic cost associated with protein synthesis (myofibrillar and/or mitochondrial protein synthesis depending on the exercise protocol). Regulation of the metabolic processes favored during exercise recovery has not been systematically studied; however, the roles of several signaling cascades have been investigated. Evidence suggests that several exercise-responsive signaling cascades are implicated in the regulation of metabolism and gene expression following exercise. It is well accepted that exercise activates AMP-activated protein kinase (AMPK), protein kinase B (PKB/Akt), several branches of the stress and/or mitogen-activated protein kinases (MAPK) as well as calcium/calmodulin-dependent protein kinases such as CaMKII. However, a direct link between activation of any of these signaling intermediates during exercise and alterations in substrate utilization following exercise has not been clearly established. In this presentation, I will summarize data investigating the importance of the AMPK-ACC pathway and the Akt-mTOR pathway in the regulation of muscle metabolism and gene expression during exercise recovery. Coordination of these signaling pathways and their respective metabolic goals will be addressed. The presentation will also address the influence of post-exercise nutritional strategies on cellular signaling and on the eventual return of metabolic homeostasis.

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Molecular signalling and insulin sensitivity of human skeletal muscle during glycogen supercompensation

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A single bout of prolonged exercise followed by intake of carbohydrate can increase muscle glycogen content far above basal levels. The molecular mechanisms leading to this glycogen supercompensation are still incompletely understood. To investigate this, nine healthy male subjects were studied 3 times during 5 days following knee-extensor exercise of one
long exercise at 70-75 % of VO2max until exhaustion activates GS phosphorylation and glucose storage. The apparent glycogen synthase (GS) on day 1 (p<0.001). This enhanced effect of exercise on GS was preserved the subsequent day. Hexokinase II, but not GLUT 4, protein content was increased (~ + 30%, p<0.01) in the prior exercised muscle throughout the supercompensation regime.

We hypothesize that the mechanisms leading to muscle supercompensation involve enhanced insulin stimulated glucose uptake mediated by enhanced capacity for glucose-transport, -phosphorylation and glucose storage. The apparent glycogen independent regulation of these events is novel and questions current thoughts on the regulatory role of glycogen in human muscle insulin action.

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The metabolic translation from exercise to recovery is decidedly regulated by dietary intake

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The intensity and duration of exercise are major regulators of metabolism, and carbohydrate oxidation, in particular skeletal muscles glycogen, becomes increasingly important as the exercise intensity rises above 60 % of maximal oxygen uptake (VO2max). During maximal effort exercise, like 30 seconds of “all-out” cycling (Wingate test), about 25 % of the skeletal muscles glycogen stores can be degraded, mainly anaerobically. Cycling at intensities above 80 % of VO2max causes fatigue before the glycogen stores are depleted in the active muscles. The largest glycogen depletion occurs at intensities of about 70-75 % of VO2max, where about 75-85 % of skeletal muscle glycogen is utilized. The translation from exercise to recovery also depends on the nutritional state prior to exercise as well as intensity and duration of the exercise. The glycogen at the end of the exercise also influences the switch from exercise to recovery, and influences the signalling mechanism activated when translation from exercise to recovery occurs. Short-term high-intensity exercise has minimal effect on AMPK activation, but ACC phosphorylation is increased which may contribute to increased fat oxidation and glycogen storage. Prolonged exercise at 70-75 % of VO2max until exhaustion activates AMPK, glycogen synthase (GS) and a number of other signalling pathways. In the recovery period, fat oxidation is completely dominating during the first 2 h after exhausting exercise unless carbohydrate is ingested. We also see that GS phosphorylation is reduced (activating GS) after exhausting exercise, which prepares muscles for repletion of glycogen stores. Interestingly, the exercise-induced activation of GS seems to remain dephosphorylated 5 h after exercise despite a major part of glycogen is resynthesised and to some degree to disintegrate the close relationship between glycogen content and GS fractional activity, which may contribute stimulate glycogen synthesis above normal level. Recovery of performance requires also protein synthesis. Intake of protein immediately after endurance exercise stimulates protein synthesis and increases resting metabolic rate. Exhaustive exercise also stimulates substantial amount of nitrogen excretion. Protein additional to carbohydrate immediately after exhaustive exercise regulates the mechanisms acting in the translation from exhaustive exercise to recovery.

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Basic focus: Lessons from the laboratory; integrated regulation of cerebral blood flow during hypoxia

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Understanding the influence of oxygen (O2) availability on cerebral blood flow (CBF) and brain metabolism is an essential step toward a better understanding of brain energy homeostasis and has obvious clinical implications. In the context of the influence of O2 availability on human CBF regulation, the following three key theses are highlighted: 1) in every form of hypoxemia (e.g., prolonged apnea, and conditions of acute and chronic exposure to normobaric and hypobaric hypoxia) in healthy humans, elevations in CBF are intimately matched to compensate for reductions in oxygen content in order to precisely maintain cerebral oxygen delivery; 2) studies of hemodilution, and those in patients with anemia and polycythemia, all support the notion that oxygen content has an independent influence on CBF, whereby stable cerebral oxygen delivery is maintained; and 3) the molecular mechanisms underpinning the regulation of CBF during changes in O2 are multifactorial but chiefly involve prostaglandin and adenosine activity, and activation of ATP-sensitive potassium channels to compensate for reductions in oxygen content in order to precisely maintain cerebral oxygen delivery: 2) studies of hemodilution, and those in patients with anemia and polycythemia, all support the notion that oxygen content has an independent influence on CBF, whereby stable cerebral oxygen delivery is maintained; and 3) the molecular mechanisms underpinning the regulation of CBF during changes in O2 are multifactorial but chiefly involve prostaglandin and adenosine activity, and activation of ATP-sensitive potassium channels to compensate for reductions in oxygen content in order to precisely maintain cerebral oxygen delivery: 2) studies of hemodilution, and those in patients with anemia and polycythemia, all support the notion that oxygen content has an independent influence on CBF, whereby stable cerebral oxygen delivery is maintained; and 3) the molecular mechanisms underpinning the regulation of CBF during changes in O2 are multifactorial but chiefly involve prostaglandin and adenosine activity, and activation of ATP-sensitive potassium channels to compensate for reductions in oxygen content in order to precisely maintain cerebral oxygen delivery. The emerging picture supports the role of oxygen delivery as a biological regulator of CBF in order to tightly regulate tissue oxygenation of the brain during changes in oxygen content originating from alterations in O2 tension, hemodilution, and anemia.

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Optimising exercise strategies for the brain

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Exercise is a uniquely effective and pluripotent medicine against several non-communicable diseases of westernised lifestyles, including protection against neurodegenerative disorders. High-intensity interval exercise training (HIT) is emerging as an effective alternative to current health-related exercise guidelines. Compared to traditional moderate-intensity continuous exercise training, HIT confers equivalent if not indeed superior metabolic, cardiac and systemic vascular adaptation. Consequently, HIT is being promoted as a more time-efficient and practical approach to optimise health thereby reducing the burden of disease associated with physical inactivity. However, no studies to date have examined the impact of HIT on the cerebrovasculature and corresponding implications for cognitive function. This talk will critique the implications of HIT for cerebrovascular function, with a focus on the mechanisms and translational impact for patient health and well-being. It also introduces similarly novel interventions currently under investigation as alternative means of accelerating exercise-induced cerebrovascular adaptation. The objective of this talk is to highlight a need for studies of the mechanisms and thereby also the optimal dose-response strategies to guide exercise prescription, and for studies to explore alternative approaches to optimise exercise outcomes in brain-related health and disease prevention. From a clinical perspective, interventions that selectively target the ageing brain have the potential to prevent stroke and associated neurovascular diseases.

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Lessons from Nature: Brain tolerance to hypoxia in vertebrates

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While the mammalian brain exhibits an exquisite sensitivity to low oxygen levels, some vertebrate animals are able to survive extended periods of hypoxia (low oxygen), while others are able to live for hours to days in the complete absence of oxygen (anoxia). Many vertebrates encounter low oxygen tensions either acutely (exercise, diving), or chronically (hibernation, burrowing, high altitudes), but a few extremophiles have developed elaborate cerebral defense mechanisms to survive complete anoxia. The most anoxia tolerant vertebrates include certain species of North American pond turtles (Chrysemys picta and Trachemys scripta), and the crucian carp (Carassius carassius) of northern Europe, which can go for days without oxygen at room temperature and live weeks to months at 3°C. Survival is the result of complex physiological and molecular adaptations that defend the turtle against the stress of oxygen deprivation, with the suppression of pathological processes and the robust expression of protective mechanisms. These changes occur in three phases in the turtle: an initial inhibition to a highly depressed metabolic state, the long term maintenance of brain function in the absence of oxygen, and recovery in the face of potential oxidative stress. Metabolic changes occur against a backdrop of “constitutive preconditioning”; many protective mechanisms are seen even in normoxia, providing ready protection in the face of low oxygen. Early anoxia is characterized by increases in adenosine and cerebral blood flow, and the suppression of energy intensive processes such as excitatory neurotransmitter release, RNA transcription, and protein synthesis. Decreased ion permeability (channel arrest), and the suppression of action potentials (spike arrest) also decrease energy requirements; together, the reductions in ion flow and neurotransmitter release result in a reversible “coma”. This abrogated electrical activity is accompanied by increases in protective MAPK and heat shock proteins (HSPs) that tilt the balance in favor of cell survival and away from apoptosis. The maintenance of long term anoxia, interestingly, allows for the continued release and reuptake of neurotransmitters, albeit at greatly reduced rates. Many neuroprotective pathways remain activated, and antioxidant levels may even increase. In contrast to mammalian survival mechanisms, cellular protection does not appear to be related to a strong HIF response.

The crucian carp follows a similar strategy of reducing energy demand to meet the decreased energy supplied by anaerobic glycolysis. However, the carp remains mildly active during anoxia; channel arrest and GABA levels increase to a lesser extent than in the turtle, and HSPs vary by organ and temperature. In the carp, HSP increases with cold temperatures may continue to increase during anoxia, and the suppression of ROS formation linked in part to Hsp72. One potential antioxidant mechanism in T. scripta is the Methionine Sulfoxide Reductase system (MsrA and MsrB), which is upregulated in the turtle brain during anoxia/reoxygenation. Methionine (Met) is one of the most readily oxidized amino acids and Msr may restore the activity of damaged proteins by the reduction of oxidized Met. Msr has been shown in mammalian models to catalytically scavenge ROS before they damage cellular constituents by the reversible oxidation/reduction of readily available protein Met, and increased cell death and ROS damage occur when Msr levels are reduced. The turtle is the first vertebrate model in which Msr transcript and protein levels are induced by low oxygen concentration, which makes T. scripta radically different from other animal models, and provides a unique opportunity to investigate the function and regulation of this peptide which may play a critical role in protection against oxidative damage. We are currently investigating the mechanism of Msr regulation in response to oxygen levels, which may involve the FOXO3a transcription factor; in C. elegans DAF-16/FOXO3a affects oxidative stress resistance and longevity, and activates the human MrSA promoter in cell culture.

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Lessons from sport; pushing the limits of human endurance performance with oxygen and the brain

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Brain function requires oxygen and maintenance of brain oxygenation and substrate delivery is important for normal function. A steady supply of oxygen maintains oxidation of carbohydrates fulfilling the high resting cerebral metabolic rate. The primary metabolic substrates for cerebral metabolism are glucose and lactate. While maybe not a universal observation, during a number of different circumstances exercise becomes associated with central fatigue i.e. an inability of the central nervous system (CNS) to recruit the full motor pool. We have shown that at the same time as the occurrence of central fatigue, marked perturbations in cerebral oxygenation and metabolism occur such as reductions of the cerebral oxygen-to-carbohydrate index (OCI) and the cerebral mitochondrial oxygen tension (PmitO2). While glucose remains the primary cerebral fuel, lactate’s role in cerebral metabolism during exercise, particularly in hypoxia and at high altitude, is poorly understood. Lactate’s relation to cerebral metabolism has evolved from one of a metabolic waste product to a role in neuro-energetics and control of blood flow. Elevations in systemic arterial lactate during exercise leads to an increased extraction of lactate by the brain, partially fulfilling the glycolytic demands of the citric acid cycle in both normoxia and hypoxia; possibly as a glucose sparing mechanism. Additionally, the brain can both produce and release a substantial amount of lactate in hypoxia, demonstrating not only a shuttling ‘per se’ of lactate between the muscles and brain, but possibly between cerebral neurons and astrocytes. These chances in cerebral metabolic state is associated with reductions maximal handgrip strength. In addition, compared to low intensity cycling exercise without signs of central fatigue or marked cerebral metabolic deviations, exercise in hypoxia reduce PmitO2 while rating of perceived exertion increased in concert with reduced MVC and voluntary activation. Thus, both exhaustive exercise as well as exercise in hypoxia provokes changes in cerebral oxygenation and metabolism that are similar to those established during exercise in hypoxia with similar indices of central fatigue. Perturbation in cerebral oxygenation and metabolism may therefore play a key role in central fatigue and thereby modulates exercise capacity. Increases in cerebral blood flow during hypoxic exercise, however, fails to maintain adequate cerebral substrate delivery, as the brain appears to fatigue, possibly contributing to a drop in hypoxic exercise performance. Interestingly, attempts to further elevate cerebral blood flow, and consequently substrate delivery via hypercapnia have proven unsuccessful at attenuating the decline in hypoxic exercise performance and the link, if any, between cerebral metabolism and fatigue remains elusive.


Lessons from altitude: Cerebral perfusion insights and their clinical significance

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Rapid ascent to altitude can result in high altitude headache, acute mountain sickness and on occasion high altitude cerebral and pulmonary oedema. The exact mechanisms by which these clinical syndromes develop have yet to be fully determined. The brain is particularly sensitive to a lack of oxygen and several adaptive mechanisms are thought to maintain adequate oxygen delivery.

It is thought that the underlying mechanism behind the development of the high altitude cerebral syndromes relates to an increase in intracranial pressure (ICP). Cytotoxic (intracellular) and vasogenic (extracellular water accumulation due to increased permeability of the blood–brain barrier) oedema have been postulated as the mechanisms that underlie HACE. However, the rise in parenchymal volume or decrease in CSF volume could also be due to a rise in the cerebral blood flow and/or efferent restriction of cerebral venous outflow, thus increasing the ICP. Headache burden has been shown to be related to the retinal vein distension and individuals with relatively narrow transverse sinuses were more prone to hypoxia induced headaches.

Preliminary evidence to supporting a unification hypothesis combining raised arterial inflow (with cytotoxic and vasogenic oedema) and restricted venous outflow theories will be discussed.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Shear stress activates monovalent cation channel TRPM4 via type 2 inositol 1,4,5-trisphosphate receptor-mediated Ca\(^{2+}\) release in atrial myocytes

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Atrial myocytes are subjected to shear stress during the cardiac cycle under physiological or pathological conditions. The ionic currents regulated by shear stress remain poorly understood. We report the characteristics, molecular identity and activation mechanism of the shear stress-sensitive current (I\(_{\text{shear}}\)) in rat atrial myocytes. Atrial myocytes were enzymatically isolated from male Sprague-Dawley rats (230–300 g) and from wild-type and type 2 inositol 1,4,5-trisphosphate receptor (IP3R) knock-out mice (C57/B6, 24–28 g) (anesthesia: pentobarbital sodium, 150 mg kg\(^{-1}\), i.p.). Shear stress of \(16\) dyn cm\(^{-2}\) was applied to single myocytes using a pressurized microflow system, and the current was measured by whole-cell patch clamp. Values are means ± S.E.M., compared by student t test. In symmetrical CsCl solutions with minimal concentrations of internal EGTA, I\(_{\text{shear}}\) showed an outwardly rectifying current–voltage relationship (reversal at approximately \(-2.1±1.66\) mV, \(n=62\)) and was well sustained. The current was conducted primarily (approximately 80%) by monovalent cations, but not Ca\(^{2+}\). It was suppressed by intracellular dialysis with 15 mM EGTA (p<0.0001 vs. 0.5 mM EGTA), selective inhibitors of transient receptor potential melastatin subfamily 4 (TRPM4) (p<0.01, 10 mM 9-phenanthrol vs. control; p<0.001, 100 mM 9-phenanthrol vs. control; p<0.01, 10 mM flufenamic acid vs. control), intracellular introduction of TRPM4 antibodies (p<0.001 vs. antibodies plus blocking peptides), or knock-down of TRPM4 expression (p<0.01 vs. wild-type), suggesting that TRPM4 carries most of this current. A notable reduction in I\(_{\text{shear}}\) occurred upon inhibition of Ca\(^{2+}\) release through the ryanodine receptors (p<0.05, 20 mM ryanodine vs. control; p<0.001, 50 mM ryanodine vs. control) or inositol 1,4,5-trisphosphate receptors (IP3R) (p<0.01, 2 mM 2-APB vs. control; p<0.05, 3 mM kemptoscinogen vs. control) and upon depletion of sarcoplasmic reticulum Ca\(^{2+}\) (p<0.01, 10 mM cyclopiazonic acid vs. control). In type 2 IP3R (IP3R2) knock-out atrial myocytes, I\(_{\text{shear}}\) was 10–20% of that in wild-type myocytes (p<0.01, wild-type vs. knock-out). Inhibition of protein kinase C, another protein activated by phospholipase C signaling, eliminated the sustained I\(_{\text{shear}}\) (p<0.01, control vs. 2 mM chelerythrine at 5-min shear), with no effect on initial I\(_{\text{shear}}\) (p>0.05, control vs. 2 mM chelerythrine at 30-min shear). Immunocytochemistry revealed that TRPM4 and IP3R2 were expressed at peripheral junctional sites with considerable co-localization. Our data suggest that shear stress activates TRPM4 current by triggering Ca\(^{2+}\) release from the IP3R2 in peripheral domains of atrial myocytes.

We thank Dr. Ju Chen for type 2 IP3 receptor knock-out mice. **Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.**

Late Na\(^{+}\) Current Contributes to Arrhythmogenic Diastolic Ca\(^{2+}\) Release in Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)

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Arrhythmias caused by abnormal impulse formation including CPVT are associated with aberrant diastolic Ca\(^{2+}\) release (DCR) from the sarcoplasmic reticulum. Despite high response of CPVT to agents directly affecting Ca\(^{2+}\) cycling, the incidence of refractory cases is still significant. Surprisingly, these patients often respond to treatment with Na\(^{+}\) channel blockers. However, the relationship between Na\(^{+}\) influx and DCR in CPVT remains elusive. To address this issue we used a murine model of cardiac calsequestrin-associated CPVT. Mice (male, 25-32g; n=12) were anaesthetised with isoflurane (5%), and once a deep level of anaesthesia was reached, the heart was rapidly removed and cardiomyocytes enzymatically isolated. We performed confocal microscopy in isolated ventricular myocytes from 6 mice to assess Ca\(^{2+}\) handling during various pharmacological interventions. Immunocytochemistry experiments (n=3) were performed to determine the structural underpinnings of aberrant Na\(^{+}\)/Ca\(^{2+}\) signaling. Late Na\(^{+}\) current (I\(_{\text{nNa}}\)) was assessed in whole cell patch-clamp mode (n=3). To monitor arrhythmic activity in vivo surface electrocardiograms were performed in mice anaesthetized with isoflurane, at minimum effective concentration (1–1.5%) before and after intraperitoneal injection of epinephrine (1.5mg/kg) and caffeine (120mg/kg). Values are means ± S.E.M., compared by student t-test or Mantel-Haenszel. Immunocytochemistry experiments revealed that neuronal Na\(^{+}\) channels (nNa\(_{\text{v}}\)) colocalize with the ryanodine receptors (RyR2) Ca\(^{2+}\) release channels. Isoproterenol (ISO; 100nM) induced late I\(_{\text{nNa}}\) (0.02±0.55 nA/Pf; p<0.05) in isolated CPVT myocytes which was sensitive to 100nM tetrodotoxin (TTX; -18.2±9.9 AmsF\(^{-1}\), p<0.05 relative to ISO). Furthermore, nNa\(_{\text{v}}\) blockade with either TTX or riluzole (10\(\mu\)M) reduced DCR frequency (0.30±0.06 vs. 0.13±0.03 and 0.10±0.04 events/Hz, p<0.05, respectively). This antiarrhythmic effect on cellular level translated in decreased ventricular tachycardia (VT) incidence in vivo (7/35 vs 0/18, p<0.05). On the other hand, nNa\(_{\text{v}}\) augmentation with β-Pompidilotoxin (β-PMTX, 40μM) increased late I\(_{\text{nNa}}\) (0.02±0.05 nA/Pf; p<0.05), DCR frequency (0.30±0.06 vs. 0.62±0.11 events/Hz, p<0.05) and VT (7/35 vs 13/14, p<0.05) all of which were reversed by nNa\(_{\text{v}}\) blockade with riluzole (10μM; late I\(_{\text{nNa}}\) 0.03±0.05 vs. -18.2±0.02 nA/Pf; p<0.05). These data suggest that nNa\(_{\text{v}}\)-mediated late I\(_{\text{nNa}}\) contributes to the generation of arrhythmias in CPVT and can therefore serve as an antiarrhythmic target. This finding provides a major conceptual shift in arrhythmogenesis and could impact antiarrhythmic therapies, as current type I antiarrhythmic drugs are all blockers of the cardiac isofrom of Na\(^{+}\) channel rather than the neuronal isofrom.

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C04

Flecainide, a class Ic anti-arrhythmic, has emerged as an effective therapy for catecholaminergic polymorphic ventricular tachycardia (CPVT) \(^1\); a malignant arrhythmia characterized by dysfunctional sarcoplasmic reticulum (SR) \(Ca^{2+}\) release \(^2\).

It has been proposed that the clinical efficacy of flecainide in CPVT is due to the combined actions of direct blockade of cardiac ryanodine receptors (RyR2) and Na\(^+\) channel inhibition \(^3\). However, there is presently no direct evidence to support the notion that flecainide blocks RyR2 \(Ca^{2+}\) flux in the physiologically relevant (luminal to cytoplasmic) direction. In this study, we examined in detail the effect of flecainide on the human RyR2 channel to establish whether direct blockade of physiologically relevant RyR2 ion flow by the drug contributes to its therapeutic efficacy in the clinical management of CPVT.

We have investigated the interactions of flecainide with individual recombiant RyR2 channels reconstituted into planar phospholipid bilayers under voltage clamp conditions. Data are given as means±SEM, compared using t-tests. Consistent with earlier reports \(^1\), we show that flecainide blocked cat ion movement through the channel in the non-physiological direction. The probability of block is dependent upon the concentration of cytosolic flecainide, with 50% of maximal occurrence of block at 13.1±1.9 \(\mu\)M (n=6).

Crucially though, flecainide, even at supra-physiological concentrations (50 \(\mu\)M), had no effect on the physiologically relevant SR to cytosol calcium flux through RyR2, producing no noticeable blocking events. This was found to be the case when using K\(^+\) as the charge carrying species to maximize the resolution of RyR2 gating and block (n=6) and under physiological salt conditions when Ca\(^{2+}\) was the permeant ion (n=3). Moreover, flecainide did not alter RyR2 gating with open probability and mean open and closed durations remaining unaltered (n=4-8). Flecainide (50 \(\mu\)M) had a negligible effect on the mechanisms responsible for the SR charge-compensating counter current; being unable to block the SR potassium channel (n=5) and only partially reducing the charge compensating K\(^+\) counter current through RyR2 (by 16.2±3.8\%, n=6). Using permeabilised rat cardiac myocytes to eliminate any contribution of plasmemmal Na\(^+\) channels to the observed actions of the drug, flecainide did not inhibit RyR2-dependent SR Ca\(^{2+}\) release, producing no significant changes in Ca\(^{2+}\) spark and wave frequency or amplitude (n=19). We conclude that the principal action of flecainide in CPVT is via Na\(^+\) channel dependent modulation of intracellular Ca\(^{2+}\) cycling and not by a direct interaction with RyR2. Our data do not negate the clinical use of flecainide but serve to highlight that class ic compounds should not be considered as prototypical RyR2 blockers.


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C03

The \(\beta\)-adrenergic receptor blocker metoprolol improves survival and partially restores \(Ca^{2+}\) handling abnormalities in rat pulmonary artery hypertension

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\(\beta\)-blockers are not routinely prescribed in pulmonary artery hypertension (PAH) and right ventricle (RV) failure, however recent small scale clinical studies suggest they may be safe or beneficial \(^1,2\). We show chronic administration of the \(\beta\)_1 blocker, metoprolol, in a rat model of PAH prolongs survival and partially restores abnormal \(Ca^{2+}\) handling and contractility to RV myocytes.

Male Wistar rats were injected with 60 mg/kg monocrotaline (MCT) to induce PAH; metoprolol or placebo treatment was initiated 15 days after MCT injection. Experiments were performed when placebo-treated MCT rats developed symptoms of heart failure (FAIL n=13) such as weight loss or dyspnoea; metoprolol treated MCT (MCT+BB n=12) and saline-injected control (CON n=16) rats were used on the median survival day (±1) of FAIL rats to compare RV function at temporally equivalent points. Rats were humanely killed by stunning and cervical dislocation. The heart was removed and single RV myocytes isolated. Cells were stimulated to contract between 1-7 Hz. T-tubules were analysed in di-8-ANPEPS (5\(\mu\)M) loaded cells using a fast Fourier transform (FFT). Intracellular \(Ca^{2+}\) transients were monitored using Fura-4F-AM (2\(\mu\)M) or Fluo-4-AM (6\(\mu\)M). Experiments were performed at 37±1 or 22±1°C. Data are presented as mean±SEM. One-way ANOVA or two-way repeated measures ANOVA were used as appropriate. P<0.05 was considered significant.

The median time for FAIL rats to develop symptoms of heart failure was 24 days; a group of metoprolol-treated MCT rats (n=7) were monitored until symptoms of heart failure developed (median day 31, P<0.05 vs FAIL, Mantel-Cox test). The T-tubule network was disrupted in FAIL cells (reduced amplitude of the FFT first harmonic), but restored to control levels in MCT+BB cells (FAIL 17.5±2.4 a.u., MCT+BB 88.0±13.6 a.u., CON 63.8±9.2 a.u., n=7-30 cells, P<0.001). Spatiotemporal homogeneity of systolic \(Ca^{2+}\) release was measured in confocal linescan mode as the coefficient of variation (CV%) in Fluo-4 fluorescence at the beginning of a \(Ca^{2+}\) transient; \(Ca^{2+}\) release CV% increased in FAIL cells indicating reduced homogeneity, but was restored to control levels in MCT+BB cells (FAIL 27.2±0.9%, MCT+BB 21.2±1.0%, CON 23.3±0.9%, n=43-50 cells, P<0.05). FAIL cell fractional shortening decreased steeply as pacing frequency increased from 1-7Hz, whereas CON cell shortening increased and MCT+BB cells were intermediate between CON and FAIL; simultaneous recording of \(Ca^{2+}\) transient amplitude showed a similar relationship (n=28-30 cells per group).

Improved \(Ca^{2+}\) handling in RV myocytes from PAH rats treated with metoprolol may delay the onset of heart failure through preservation of RV function. \(\beta\)-blockers are a potential treatment for the failing RV in PAH.


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Calcineurin and protein phosphatase 2A regulate Cx40 and Cx43 phosphorylation in guinea-pig left atrium


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Action potential conduction velocity in myocardium is a function of gap junction (GJ) electrical conductance [1]. GJ conductance (Gj) itself depends on the phosphorylation state of GJ proteins, connexins (Cx). We have shown that during elevated intracellular Ca²⁺ concentration ([Ca²⁺]) phosphatases mediate a decrease of Gj in the left atrium (LA). Consequently we investigated the role of serine threonine protein phosphatases (PPs) on Cx phosphorylation, in particular the Ca²⁺-dependent PP, calcineurin, and the Ca²⁺-independent PPs, PP1, PP2A, Male, Dunkin-Hartley guinea pigs were euthanised and the LA dissected. Freshly isolated atrial cardiomyocytes were used to measure [Ca²⁺], using Fura-2 AM in control (Na=149.4 mM) or low-Na solution (Na=29.4 mM). Samples used for western blots were perfused in control or low-Na solution in the absence/presence of inhibitors for: calcineurin (cyclosporin-A (CsA); 50 μM, n=4) or calcineurin auto-inhibitory peptide (CAIP; 50 μM, n=4)), PP1 (tautomycin (TTM; 5 μM; n=4)) and PP2A (fostriecin; FST; 100 nM; n=4). Western blots were used to assess protein expression of total-Cx43 (T-Cx43), T-Cx40 and Cx43 phosphorylation at Ser368 (Cx43-pSer368) and Ser365 (Cx43-pSer365). Immunoprecipitated T-Cx40 was also probed for serine and threonine phosphorylation. Values are integrated density normalised to GAPDH or T-Cx and shown as mean±SEM. Differences between sets were tested by ANOVA; the null hypothesis was rejected at p<0.05.

Low-Na solution significantly and reversibly increased [Ca²⁺]_j. T-Cx43 and T-Cx40 were unchanged throughout all interventions. During low-Na exposure, phosphorylation at Cx43-pSer368 was significantly increased (control 0.05±0.01 vs low-Na 1.09±0.04; p<0.001); this was reduced by inhibition of calcineurin (low-Na+CAIP 0.03±0.01; p<0.001) and PP2A (low-Na+FST 0.58±0.05; p<0.001), but not PP1 (low-Na+TTM 1.01±0.03). In parallel, Cx43-pSer365, which is phosphorylated under control, was diminished during low-Na solution; this was attenuated during FST treatment (control 0.50±0.02 vs low-Na 0.38±0.02 vs low-Na+FST 0.47±0.03; n=3; p<0.05). Phosphorylation of T-Cx40 at serine and threonine residues was significantly increased in low-Na solution (control 0.09±0.04 vs low-Na 1.06±0.06; p<0.001). This was partially reduced by CsA (0.47±0.04; p<0.01) and FST (0.61±0.04; p<0.01) and returned to control levels during exposure to CAIP (0.11±0.03; p<0.01) but TTM had no effect (1.08±0.02). This study showed that Cx43-Ser368 phosphorylation by protein kinase C is mediated by calcineurin and PP2A via initial dephosphorylation of Cx43-Ser365. Furthermore we showed that during raised [Ca²⁺], Cx40 phosphorylation is increased at serine and threonine residues and this is also mediated solely by calcineurin with partial involvement of PP2A. There was no evidence of a role for PP1 in phosphorylating Cx40 in the (LA).


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

DII4/Notch signaling promotes macrophage activation and vein graft disease


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Background Vein graft failure is a global health burden with no effective medical solutions. Saphenous vein grafts for peripheral artery disease are widely used because they remain patent longer than artificial conduits. Approximately 50% of vein grafts for lower extremity arterial disease, however, become occluded or narrowed within a year, leading to devastating limb amputation or expensive angioplasty or surgical revascularization. Although many mechanisms for arterial diseases have been established, the pathogenesis of vein graft failure remains incompletely understood. We previously demonstrated that Notch signaling triggered by its ligand Delta-like 4 (DII4) induces macrophage activation (1,2). We tested the hypothesis that macrophage DII4 promotes the development of vein graft disease. Methods Vein graft surgery was performed in high-fat fed LDL receptor-deficient (Ldlr−/−) mice by implanting donor inferior vena cava into recipient right carotid arteries under general anesthesia (ketamine 90 mg/kg, xylazine 10 mg/kg, i.p.). We used clinically relevant biotherapeutics: 1) DII4 blocking antibody; and 2) macrophage-targeted DII4 siRNA. Statistical analysis of differences was performed by Student’s t test. Results 1) Antibody DII4 antibody or control IgG (250 mg; n=9-10) was administered for 28 days. DII4 antibody treatment inhibited lesion development (Figure 1) and macrophage accumulation (Mac3 immunostain, Figure 2) in vein grafts. DII4 blockade suppressed macrophage expression of genes typical of pro-inflammatory “M1” macrophages (e.g., IL-1β, TNFα). In vivo molecular imaging of lesional macrophages demonstrated that DII4 antibody suppressed the activity of MMPs, matrix-degrading enzymes responsible for plaque vulnerability. DII4 blockade attenuated collagen thinning, suggesting the lesion stabilization. 2) siRNA To address the relative contribution of macrophages to DII4-mediated vein graft disease in vivo, we delivered DII4 siRNA or control siRNA (0.5 mg/kg; n=8-9) encapsulated in macrophage-targeted lipid nanoparticles. In vivo DII4 silencing in macrophages reduced lesion size (p<0.05) and macrophage burden (p<0.05) in vein grafts of Ldlr−/− mice to a similar extent as those of DII4 antibody therapy. 3) In vitro studies In gain-of-function and loss-of-function experiments, DII4 promoted pro-inflammatory molecules (e.g., “M1” genes) in macrophages. Macrophage DII4 stimulated smooth muscle cell proliferation and migration and suppressed their differentiation. Conclusion These results suggest the novel mechanism that macrophage DII4 promotes vein graft lesion development by exacerbating inflammation and crosstalk between macrophages and smooth muscle cells, supporting the DII4-Notch axis as a potential therapeutic target.

Oral Communications

This work is supported by the British Heart Foundation. Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
and spring (treatment, Commonwealth University during the fall (control, 

an anonymous cardiorespiratory evaluation was distributed to two groups of undergraduate students from Virginia 

application of the material covered. To compare performance, 

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Formative assessment: A tool to improve learning of 

Crystal West. 

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

requirements. 

C07 

Formative assessment: 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 voluntary active learning opportunities would improve student understanding and application of the material covered. To compare performance, an anonymous cardiorespiratory evaluation was distributed to two groups of undergraduate students from Virginia Commonwealth University 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 voluntary participate in two additional active learning exercises: 1) a small group discussion, 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 [$\chi^2$ (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. 

C08 

Students’ use of supplementary resources in advance of lecture sessions 

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The traditional format of university lectures can be challenging for international students (Ryan, 2005). In a diverse student population, the provision of information in multiple formats could be important, particularly for students whose first language is not English. This study investigated the ways in which students used supplementary resources as an adjunct to traditional lectures. The study focused on a preliminary year (level 3) Physiology lecture and was completed in accordance with ethical guidelines (BERA, 2011). All students were provided with supplementary resources containing the same information in three formats (an online video, an online textbook chapter and the hard copy of the textbook) given 14 days before the lecture session; viewing the material in advance of the lecture was recommended. Students’ use of the resources was assessed by anonymous questionnaire after the lecture. There was a clear difference in the use of the resources by students whose first language was not English (‘international’ students), compared to those with English as a first language (‘home’ students). International students were more likely to access the resources in advance of the lecture. 74% (17/23) of international students viewed the video, with 52% (12/23) accessing the online textbook and 43% (10/23) also reading the hard copy; 43% (10/23) viewed all three resources. In contrast, only 45% (5/11) of ‘home’ students accessed any of these resources in advance and all but one used a single information source. International students were also more likely to review the entire content. 59% (10/17) watched 75-100% of the video and 88% (14/16) feedback evaluations were positive. In contrast, only 18% (2/11) of the home students accessed the video;
these students viewed <50% of the material and gave it the most negative evaluation. Half (6/12) of those international students who accessed the book in either form read at least 75% of the text, but a minority of home students (4/11) read any of the book chapter, with just two individuals reading more than 75%.

The results showed that students who were not studying in their first language were more likely to access and engage with support material in preparation for formal teaching sessions. These students perceived direct benefits from these resources, suggesting that they appreciated access to the same information in different formats. International students, in particular, may benefit from having control over the pace of delivery of information. ‘Home’ students appear less likely to prepare for lectures by reviewing the course material in advance and are, therefore, more dependent on the lecture as their primary source of information. These students may not have the same drive to engage with the material in advance because the live presentation is less likely to present a barrier to learning.


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C09

Introducing an active learning element to lectures and tutorials in pre-clinical medical physiology and pathology lectures

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The General Medical Council directs medical schools to facilitate lifelong learning skills among students. Trainee Doctors are encouraged to “Reflect, learn and teach others, acquire, assess, apply and integrate new knowledge, learn to adapt to changing circumstance and establish the foundations for lifelong learning” (General Medical Council, 2009). It is essential, that for learning to be effective in achieving these aims, it must be a process in which the student actively participates. With such active learning, any knowledge gained is more likely to be retained by students, who will thus learn to apply it to different contexts. Rather than merely inculcating students with facts, active learning strategies aim to “upskill” them (Svinicki, 1998, Michael, 2006).

In our first and second year medical physiology, pathology and microscopic anatomy courses we gently introduce students to “active learning” strategies. This gentle phased introduction is necessary as our medical student cohort is composed of 80% school leavers used to secondary level didactic teaching. Strategies used include “gapped” lecture guides replete with tasks to be completed, as detailed by Richardson (2008), and case based tutorials with no definitive answers given (Wood, 2003).

We surveyed student attitudes to these strategies in module review documentation distributed to students at the conclusion of each module. Modules surveyed were the first year “cells tissues and organs” (CTO) module and the second year “physiologic basis of clinical practice” (PBCP) module. In each of these modules, students were invited to make free-form comments on what they liked about the module and what they disliked. Comments in favour of the “active learning” strategies (gapped lecture guides with tasks and case based tutorials) were counted and compared with those against the strategies. A total of 546 student comments were collated for the study. There were 13 comments in favour of the “active learning” strategies against 27 against them. The vast majority of positive comments on teaching in these modules praised clear didactic instruction from lecturers, with considerable resistance to anything beyond passive transmission of clear information in classes.

Our findings reflect anecdotal data from student consultative fora where active learning techniques are regularly challenged by student representatives. This furthers pressure on academics to accede to student requests for more didactic instruction, given the importance of popularity in the National Student Survey (NSS) in this age of tuition fees and competition for student numbers. It raises the debate about whether we, as academics, should continue to pursue educational best practice but risk poor student satisfaction.

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C10

Glucocorticoids affect the diurnal rhythm of the thiazide-sensitive NaCl co-transporter

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Reabsorbing ~7% of the sodium load, the distal convoluted tubule plays key roles in blood pressure (BP) homeostasis. Here, NaCl co-transport (NCC) is the major route for apical Na entry making thiazide diuretics (NCC inhibitors) a mainstay hypertension treatment. Predictive adaptations of sodium excretory rhythms are supported by an intrinsic renal clock which regulates transporter activity according to physiological need. Peripheral clocks can be influenced by glucocorticoids which also have a circadian rhythm. We therefore hypothesized that NCC’s diurnal rhythm is regulated by glucocorticoids.

Male C57BL6 mice were kept on a 12h light cycle with subjective day starting at 7am. Urine was collected in 12h periods and kidneys harvested at 1am (night) and 1pm (day). Statistics used were t-tests or twoway ANOVA with post hoc Sidak tests. Scl12a3 (NCC encoding gene) mRNA and NCC protein abundance were similar between day and night (1.0±0.4 vs
C11

Store Operated Calcium Entry controls intracellular calcium waves in Xenopus oocytes

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Intracellular Ca\textsuperscript{2+} oscillations are made of highly spatially and timely controlled cytoplasmic waves of Ca\textsuperscript{2+} fueled by two main Ca\textsuperscript{2+} sources: the extracellular compartment and the endoplasmic reticulum (ER). Specific and highly regulated ion channels and pumps located at the plasma and ER membranes control the cytoplasmic Ca\textsuperscript{2+} levels as well as ER store refilling. When ER Ca\textsuperscript{2+} stores are depleted a mechanism termed Store-Operated Calcium Entry (SOCE) drives Ca\textsuperscript{2+} entry. In the oocyte of the frog Xenopus laevis intracellular Ca\textsuperscript{2+} variations are converted into chloride currents by Ca\textsuperscript{2+}-Activated Chloride Channels (CACC) allowing real time monitoring of sub-plasma membrane Ca\textsuperscript{2+} levels. We recently showed that SOCE and IP\textsubscript{3} receptors (IP\textsubscript{3}R) define a functional complex creating mid-range Ca\textsuperscript{2+} signaling to CACC (COURJARET and Machaca, 2014). Here we aimed at understanding how SOCE influences intracellular Ca\textsuperscript{2+} release by IP\textsubscript{3}R and Ca\textsuperscript{2+} oscillations. Injecting a non-hydrolysable IP\textsubscript{3} analog induced transient Ca\textsuperscript{2+} oscillation (~20 min) and stimulated SOCE. When Ca\textsuperscript{2+} influx through SOCE was increased by hyperpolarizing pulses or reduced by removing extracellular Ca\textsuperscript{2+} the duration of the Ca\textsuperscript{2+} oscillations was respectively reduced or increased. In addition, removing extracellular Ca\textsuperscript{2+} more than 30 minutes after the end of the oscillation restored the Ca\textsuperscript{2+} release, indicating that the emptying of the ER stores cannot account for the inhibition of Ca\textsuperscript{2+} oscillations. It was also possible to trigger Ca\textsuperscript{2+} release events after the end of the Ca\textsuperscript{2+} oscillations by inducing a large SOCE. Those events precisely depend on the amplitude and timing of SOCE. Imaging of intracellular Ca\textsuperscript{2+} also revealed that they only occur at the animal pole of the oocyte, where SOCE is larger. This indicates that a strong reloading of the ER Ca\textsuperscript{2+} store could overcome the inability of IP\textsubscript{3}R receptors to release Ca\textsuperscript{2+} and suggest a luminal regulation of the receptor. To further understand how SOCE was modulating Ca\textsuperscript{2+} release we monitored luminal ER Ca\textsuperscript{2+} levels by expressing the FRET sensor D1ER (Palmer et al., 2004). After injection of IP\textsubscript{3}, D1ER imaging revealed the depletion/reloading of the ER stores by SOCE as a function of membrane potential and time. During the long hyperpolarizing voltage jumps (SOCE) that are able to trigger a Ca\textsuperscript{2+} wave, the D1ER signal revealed that the high Ca\textsuperscript{2+} in the ER outlasts the cytoplasmic Ca\textsuperscript{2+} event leaving the IP\textsubscript{3}R facing low cytoplasmic Ca\textsuperscript{2+} concentration and high luminal Ca\textsuperscript{2+}. Together these findings shed new light on the complex regulation of the IP\textsubscript{3}R by SOCE through modulation of both cytoplasmic and luminal Ca\textsuperscript{2+} levels.


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

C12

The role of the intracellular glutamate gradient in driving organic anion transporter function

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The organic anion transporter (OAT) and organic anion transporter polypeptide (OATP) families mediate exchange of xenobiotics, hormones and drugs, including statins, antibiotics and anti-cancer drugs, whereby uptake of substrates is coupled to the efflux of counter-ions. To accumulate substrates in the cell by exchange, the counter-ion must have a higher concentration in the cell compared to the substrate. The gradients which drive OAT/OATP function are unknown but using Xenopus laevis efflux studies, we have demonstrated that OAT4 (SLC22A11) and OATP2B1 (SLCO2B1) transport glutamate and that in the placenta, glutamate is exchanged for organic anions. Previously OAT2 (SLC22A8) and OAT10 (SLC22A13)
have been shown to transport glutamate and this raises questions about other members of these families. This study uses a screening approach to investigate the other members of the OAT/OATP families’ affinity for glutamate. *Xenopus laevis* oocytes were microinjected with water or with 20 μg human cRNA of either: OAT3, OAT5, OAT7, OATP2A1, OATP2B1 or OATP4A1 and incubated for 48 hours. Individual oocytes were placed into wells of a 96 well plate and incubated with the prototypical substrate, 3H-estrone-sulphate (11 μM), for 10 min with and without 2.5 mM glutamate or 2.5 mM estrone-sulphate (n=10 oocytes per condition). Uptake presented as mean (SEM), n=3 experiments. Data were analysed using a one-way ANOVA with a Dunnett’s post hoc test and significance was assumed when p<0.05. Estrone-sulphate inhibited 3H-estrone-sulphate uptake in OAT3, OAT7, OATP2A1, OATP2B1 and OATP4A1 injected oocytes (p<0.05) indicating that the transporters were functioning as expected. Glutamate inhibited 3H-estrone-sulphate uptake in OATP2B1 and OATP4A1 oocytes (p<0.001) but did not alter uptake in OAT3, OAT5, OAT7 or OATP2A1 injected oocytes.

We have used a screening method to assess OAT/OATP glutamate affinity, which we have used to study transporters simultaneously. In addition to OAT2, OAT4, OAT10 and OATP2B1, we propose that glutamate is transported by or inhibits OATP4A1, which will now be investigated further. OAT/OATP transporters have important biological roles throughout the body, particularly in the kidneys, liver and placenta. Understanding what drives the activity of these transporters may thus have implications for pharmacokinetic modelling of drug transport throughout the body.


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isolation of alleles in the general population and 42% of this cohort. System b⁰⁺⁰ activity was measured by [³H]arginine (2.5uCi/ml, 10uM) uptake (60min, 22-24°C) in cRNA-injected oocytes (1-6 days post-injection). [³H]Arginine uptake was similar in oocytes injected with either rBAT or M618I. In contrast, there was a marked reduction in [³H]arginine uptake in Y579D-injected oocytes under most conditions. On day 1 post-injection, there was a significant (p<0.01) increase in uptake in rBAT-injected (50ng) oocytes, uptake being 5.7-fold greater than control (water-injected oocytes), whereas in Y579D-injected oocytes uptake was not significantly (p>0.05) different from control. [³H]Arginine uptake in Y579D-injected oocytes was dependent upon the quantity of cRNA injected and the number of days post-injection. Thus recovery of Y579D function was observed at day 6 post-injection but only in oocytes injected with 50ng cRNA. Immunoblotting and immunocytochemical measurements indicate that Y579D protein is synthesized within the oocytes but less is expressed at the plasma membrane suggesting that the decrease in function observed with Y579D is due to less efficient processing and trafficking of System b⁰⁺⁰. Similar observations have been made with the most common SLC3A1 missense mutation, M467T, which causes misfolding, abnormal trafficking, and reduced membrane expression of System b⁰⁺⁰ [2]. In the long term, the identification of the molecular cause and functional consequences of monogenic disorders such as cystinuria should aid in the development of personalised patient treatment.


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C15

FXR-mediated up-regulation of miR-29a-3p: implications for therapy of inflammatory bowel disease

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Inflammatory bowel disease (IBD) is a group of intestinal disorders that results in chronic intestinal inflammation. Although the etiology of IBD is not yet fully understood, it is thought to arise due to an inappropriate mucosal immune response in genetically susceptible individuals. miRNAs are small, single stranded RNA molecules that regulate gene expression. The colon epithelium of IBD patients has a unique miRNA expression profile, indicating the involvement of these molecules in disease pathogenesis. Farnesoid X receptor, FXR, is a nuclear bile acid receptor, activation of which has been shown to be protective in animal models of colitis by preserving integrity of the epithelial barrier (Gadaleta et al., 2011). Since the role of miRNAs in mediating FXR-mediated responses is not yet known, the aim of this study was to investigate the effect of the FXR agonist, GW4064 on miRNA expression in colonic epithelial cells.

Isolated human colonic crypts and T54 colonic epithelial cells, were treated with GW4064 [5 mM] for 6 hrs. Use of human tissue was approved by the Beaumont Hospital Ethics Committee. RNA was extracted and miRNA profiling was performed by Nanostring Technologies. nCount software was used to detect miRNAs that were up-or down-regulated 1.5 fold or more compared to controls. TargetScan was used to identify miRNA targets.

Validation of the miRNA array results, revealed that FXR activation increased expression of miR-29a-3p by 3.9 ± 0.8 fold (n = 4; p < 0.05). This increase in miR-29a-3p was mimicked by the natural FXR agonist, ursofloxacin acid (UDCA) [200 mM] and deoxycholic acid (DCA) [10 and 200 mM], miR-29a-3p [5K1] is predicted to target PTEN with a 92% context score as ascertained by TargetScan. Inhibition of PTEN has been well established to prevent epithelial cell apoptosis (Deevi et al., 2011). We found that treatment of colonic epithelial monolayers with GW4064 resulted in decreased PTEN mRNA (n = 8; p < 0.05) and decreased PTEN protein levels (n = 3; p < 0.05), [5K2] By virtue of their ability to increase expression of epithelial miR-29a-3p and reduce expression of its target PTEN, we hypothesise that FXR agonists may help to preserve intestinal barrier function by inhibiting apoptosis. Given the established importance of dysregulated barrier function in pathogenesis of IBD, our data suggest that FXR agonists represent a novel avenue for the development of new therapies for IBD.


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C16

Sex differences in neurovascular control during rhythmic forearm exercise in healthy humans

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Sympathetic vasoconstrictor activity increases with exercise. Previously we demonstrated sex differences in modulation of vasomotor sympathetic outflow during isometric forearm exercise in healthy young humans (Jarvis et al., 2011). The purpose of this study was to determine whether sympathetic activation during rhythmic forearm exercise differed in women compared with men. To accomplish this, responses to 6 minutes of non-fatiguing dynamic hand grip (DHG) were compared in supine healthy women (n=8, age: 26 [21, 32] years; mean [95% CI]) and men (n=9, 30 [26, 34] years). Exercise was performed at 40% of the maximal voluntary contraction (MVC) using the dominant hand with a cycle of 2 seconds of contraction, 2 seconds of release. Beat-beat heart rate (HR), blood pressures (BP), and muscle sympathetic nerve activity (MSNA) were determined using electrocardiography, finger photoplethysmography and microeurography respectively. Perceived exertion was assessed upon completion of the exercise task using the Borg (6-20) scale. Baseline heart

Oral Communications
rate and blood pressures were similar for both sexes (Table 1). However, baseline MSNA was lower in women compared with men. As expected, heart rate, blood pressures and MSNA increased during forearm exercise for both women and men. The responses of heart rate and blood pressures were similar in magnitude. However, the increase in MSNA was greater for women compared with men. Rating of perceived exertion was not different (13 [12, 15] for women versus 12 [11, 14] for men). These findings suggest sex differences in sympathetic activation during rhythmic forearm exercise. Young women experience a greater increase of vasomotor outflow during dynamic exercise compared with young men. This probably reflects the difference in baseline resting sympathetic vasoconstrictor activity.

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Change from baseline</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>Men</td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>66 [54, 74]</td>
<td>58 [50, 67]</td>
</tr>
<tr>
<td>Finger SBP, mmHg</td>
<td>122 [103, 140]</td>
<td>138 [121, 154]</td>
</tr>
<tr>
<td>Finger DBP, mmHg</td>
<td>66 [50, 74]</td>
<td>65 [57, 71]</td>
</tr>
</tbody>
</table>

Table 1. Cardiovascular and vasomotor sympathetic outflow at Baseline and Change from baseline during rhythmic forearm exercise. SBP, systolic blood pressure, DBP, Diastolic blood pressure. All values are means [95% Confidence Intervals]. 1 Difference from men, P < 0.05, unpaired t-test.

C17

Effects of blood donation and nitrate ingestion on the physiological response to moderate-intensity and incremental exercise

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Introduction - Nitrate-rich beetroot juice (BR) can reduce the oxygen (O2) cost of moderate-intensity exercise and enhance tolerance to severe-intensity exercise (Bailey et al., 2009). A derivative of nitrate (NO3), nitric oxide, plays a significant role in the regulation of skeletal muscle blood flow, contraction and efficiency. A reduction in blood O2 carrying capacity, as a result of blood donation, reduces the tolerance to severe-intensity exercise (Burnley et al., 2006). The aim of this study was to determine whether BR supplementation alters the haemodynamic response, efficiency and tolerance to cycling exercise post blood donation.

Methods - In a randomised and double blind experimental design, 22 recreationally active volunteers performed moderate-intensity and ramp incremental cycle exercise tests before and after blood donation. Blood donation, all subjects (~0.04 mmol NO3 per 70 mL) as a placebo (PL) in the 48 h preceding the exercise tests. Immediately after blood donation and during the 48 h prior to exercise, subjects consumed 7 shots of either BR (~6.2 mmol NO3 per 70 mL) or PL (n=11). Blood pressure (BP), plasma NO3 and nitrite (NO2) concentrations, haemoglobin concentration ([Hb]), haematocrit (Hct) and pulmonary VO2 responses to exercise were measured during each visit to the laboratory.

Results - BR supplementation resulted in an increased plasma [NO3] (PL: 50±14 vs. BR: 845±350 µM; P<0.05) and [NO2] (PL: 72±21 vs. BR: 619±363 mM; P<0.05) post blood donation. Systemic BP was reduced in BR post blood donation when compared with baseline. [Hb] and Hct decreased significantly from pre to post blood withdrawal, however, no difference was noted between PL and BR. Compared with pre donation, the steady state VO2 during moderate-intensity exercise was ~4% lower post donation in BR only (P<0.05). The ramp test peak power decreased from pre donation (PL: 341±70 vs. BR: 331±68 W) to post donation (PL: 324±69 vs. BR: 322±66 W) in both groups (P<0.05). However, the decrement in performance was less in BR compared with PL (P<0.05).

Discussion - Nitrate supplementation reduces the O2 cost of moderate-intensity exercise and lessens the decline in ramp incremental performance after blood donation. The results from this study may have implications for improving functional capacity in conditions where normal blood O2 carrying capacity is impaired.


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C18

Differential gender-specific muscle-tendon in vivo adaptations to resistance training are not modulated by circulating TGF-β1 and IGF-1 levels

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Mechanical, metabolic, hormonal and proteomic gender-specific differences at rest and in response to exercise/training of the muscle-tendon complex (MTC) have been identified. Previous studies suggest TGF-β1(1,2) and IGF-1(3) exert a potent stimulus on, amongst other factors, Tendon cell proliferation, differentiation and Type I collagen synthesis. Whether a link exists between gender specific changes in MTC properties following resistance training and circulating TGF-β1 and IGF-I levels is unknown.

Twenty-eight young participants were assigned to a training group and subdivided by gender (T males [TM] aged 20±1 year, n=8, T females [TF] aged 19±3 year, n=8) whilst 6 males and 6 females were assigned to control groups (CON). The training group completed 8 weeks of resistance training (RT) of the lower limbs. MTC properties (Vastus Lateralis, VL) pCSA and strength, patella tendon stiffness [K], Young’s modulus [E], volume [Tvol], cross-sectional area [Tcsa], and length [Tl] were assessed at baseline (week 0) and post RT (week 8) using ultrasonography, DEXA scanning, EMG & dynamometry. Circulating levels of TGF-β1 and IGF-I were assessed at baseline and post RT using the ELISA technique. ANCOVAs, using baseline measures as covariates, were used to compare relative changes in muscle-tendon complex parameters between
genders. Significant effect alpha was set at 0.05, with trends accepted at $p<0.01$. There were no significant differences in circulating TGFβ-1 and IGF-I levels between genders at baseline. Interestingly pooled population data showed that TGFβ-1 correlated with $K$ at baseline ($p=0.026$, $r=0.554$). IGF-I did not correlate with any of the monitored structural or functional measures at baseline. At week 8, there was a significant increase in the mechanical and morphological properties of the muscle-tendon unit in both TM & TF, compared to CON ($p<0.0001$). However, there were no significant gender-specific differences ($p>0.05$) in changes in VL pCSA and KEMVC, or PT K, Tvol, E, IGF-I, TGFβ-1 (see Figure 1). Interestingly, there were significant ($p<0.05$) gender differences in $\Delta K$ at 10,20,90 & 100% MVC, with females exhibiting greater changes than males at lower force levels, and the opposite effect seen at higher force levels. The results suggest, greater resting TGFβ-1 levels may indicate superior tendon mechanical properties, whereas gender-specific resistance training-induced changes in properties do not appear to be modulated by circulating TGFβ-1. Resistance training appears to impact different areas of the PT force-elongation curve in males and females, which suggest different loading patterns may be needed to maximise resistance training adaptations in each gender, in agreement with previous work (1). The potential for TGFβ-1 to modulate tendon properties may have therapeutic importance.

**Figure 1. Circulating IGF-I (lower panel) and TGFβ-1 (upper panel) levels pre and post-training in males (black bars) and females (white bars).** †Significantly different ($p<0.05$) compared to pre-training values.


McMahon G, Morse CL, Burden A, Winwood K and Onambélé GL, 2013. The manipulation of strain, when stress is controlled, modulates in vivo tendon mechanical properties but not systemic TGF-β1 levels. Physiological Reports. DOI: 10.1002/phy2.91


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**C19**

**Total body skeletal muscle mass: estimation by creatine (methyl-d3) dilution in athletes**

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Introduction: The maintenance of muscle mass is essential for both athletic and clinical populations. Creatine (methyl-d3) dilution ($D_3$-creatinine) is a novel technique for the estimation of muscle mass in humans and has recently been reported (Clark et al, 2014). The method uses a dose of deuterium-labelled creatine to determine total skeletal muscle mass via estimation of total body creatine pool size. This study is the first to apply this method in an athletic population. The aim of this study was to compare estimates of total body skeletal muscle mass from $D_3$-creatinine dilution method and whole body Magnetic Resonance Imaging (MRI) in an athletic population.

Methods: Fifteen male and five female national level kayakers (stature: 182.0±13.1 and 170.0±9.0 cm; body mass: 80.6±9.9 and 66.4±6.0 kg; VO2max: 56.5±7.0 and 49.6±4.4 ml·kg⁻¹·min⁻¹ for males and females, respectively) underwent assessment at the start of pre-season training. Muscle mass was determined using two methods: MRI and $D_3$-creatinine. The $D_3$-creatinine technique required ingestion of two 30mg capsules and total urine was collected at 0-4 and 4-24 hour intervals over the next four days. Urine aliquots were analysed for creatine, creatinine, $D_3$-creatinine and $D_3$-creatinine using liquid chromatography/mass spectroscopy (LC/MS/MS). Total creatine pool size was directly measured by enrichment of urinary $D_3$-creatinine on day three, with a subsequent calculation for estimated total skeletal muscle mass. A Pearson’s correlation coefficient, using R statistical programme (Version 3.1.2, R), was used to analyse the association between creatine pool size and MRI methods. Results: The preliminary data demonstrates creatine pool sizes of 207.9±36.0 g (mean ± S.D) and 144.0±12.6 g for male and female athletes. Once the conversion factor for creatine pool size to muscle mass was implemented, an estimate of 43.6±13.1 kg (57.41±8.5 % of total body mass) was obtained. Muscle mass measured by MRI was on average 36.3±6.2 kg (47.4±4.3 % of total body mass). Estimated muscle mass was 7.3 kg (20%) higher on average using the $D_3$-creatinine technique than observed with MRI. Pearson’s correlation coefficient showed a strong correlation between $D_3$-creatinine and MRI ($r=0.953$, $p<0.001$).

Discussion: This study is the first to apply the novel $D_3$-creatinine technique in an athletic population, for whom a convenient technique in an athletic population, for whom a convenient measure of muscle mass is required. The creatine pool sizes were larger than previously reported for young males by Clark and colleagues (187.6±56.3 g vs 158.6±26.9 g). $D_3$-creatinine over-estimated muscle mass in relation to MRI (8% - 28%). Possible explanations could include inexact MRI muscle mass estimates or inaccurate predicted concentration of creatine in whole wet muscle mass (4.3 g/kg; Kreisberg, 1970), required for muscle mass estimation from creatine pool size. Further
Fast-twitch muscle from old dystrophic mice contain complex branched fibres which are susceptible to contractile damage

S.I. Head, L. Kiriaev, J. Morley, S. Kueh and S. Chan

Duchenne muscular dystrophy (DMD) is characterized by progressive wasting of skeletal muscle. Previous work from our laboratory, using the extensor digitorum longus (EDL) muscle from adult (>6 mth) mdx mice, suggests that the branched fibres formed during repeated bouts of regeneration could be responsible for the terminal phase of muscle wasting [1-3]. To further test this hypothesis, fast-twitch EDL and slow-twitch soleus (SOL) muscles from mdx mice and littermate controls (6-9 wks, "young"; 17-28 months, "old") were attached to a force transducer. After contractile analysis single muscle fibres were enzymatically isolated and suspended in a relaxing solution in order to view individual fibres. In old controls, branching was found in <7% of the 426 fibres counted. In young mdx EDL muscles, 59% of 669 fibres counted were branched, while in old mdx EDL muscles, 98% of 672 fibres counted were branched. In young mdx EDL muscles, 63% of the branched fibres had only one branch, while in old mdx EDL muscles, 66% of the branched fibres contained 4 or more branches. In young mdx SOL muscles, 40% of 172 fibres counted were branched, while in old mdx SOL muscles, 79% of 462 fibres counted were branched. In young mdx SOL muscles, 73% of the branched fibres had only one branch, and in old mdx SOL muscles, only 17% of the branched fibres contained 4 or more branches. Muscles were subjected to a series of 10 isometric contractions (1-2 s with 1 min rest). For the EDL muscles from young control and young mdx mice lost <5% of force over the 10 contractions. In old mice, the force loss was 10.3 ± 1.0% (n = 15) for control and 33.8 ± 4.1% (n = 11) for mdx (p < 0.0001). For the SOL, force loss was negligible in control and mdx muscles of both age groups. Muscles were subjected to a series of eccentric contractions. In old mdx EDL muscles, the first contraction caused so much force loss that subsequent contractions resembled passive stretch. The first contraction at 10% strain produced a force loss of 5.5 ± 1.6% (n = 5) in young controls and 14.4 ± 1.5% (n = 6) in young mdx (p < 0.01). In old mice, the force loss was 1.7 ± 1.7% (n = 11) in controls and 63.1 ± 2.1% (n = 5) in mdx (p < 0.0001). For the age-genotype interaction, p < 0.0001. In SOL muscles, eccentric contractions produced negligible force losses in mdx and controls. We have shown that the marked aberrations in cytoarchitecture present in virtually all EDL fibres of very old mdx mice have a distinct association with the increased vulnerability to isometric and eccentric damage of their muscles. The association between degree of branching and degree of damage is further confirmed by the internal control of the SOL muscle, which shows much less complex branching than the EDL and virtually no damage from isometric and eccentric contractions.


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Mitosetempo (25 μM) both significantly reduced (P<0.05) spark frequency in statin fibres but were without effect in controls. Together these data show that increased SR Ca$^{2+}$ leak seen in intact muscle fibres from statin-treated rats is linked to an increase in NO and ROS. We propose that caveolin-regulated NO and Ca$^{2+}$-dependent mitochondrial ROS production modify the RyR to effect this leak. Defining the cellular processes that underlie statin induced myopathy is the first step in the development of co-therapies to improve statin compliance. 


Sponsored by the BHF

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Impact of LGI diet and exercise during experimental obese pregnancy on maternal and offspring cardiometabolic disorders


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The establishment of developmental programming has identified the intrauterine environment as a target for intervention to reduce the adverse effects of maternal obesity and the risk for childhood obesity and cardiometabolic disease in later life. We assessed the impact of low glycaemic index diet (LGI) and physical activity (phys) in obese pregnant rats on maternal and offspring physiological and biochemical parameters. At gestational day (GD) 7, control and high-fat fed obese rats were assigned either to Con (n=10), Ob (n=9) or Ob-phys-LGI (n=8) groups. Exercise was kept until GD 17 and LGI diet until day 14 of lactation. Offspring (Off) were followed until 3 months. Glucose tolerance test was performed and systolic (SBP) and mean arterial (MAP) pressure were measured until 3 months. Glucose tolerance test was performed and LGI diet until day 14 of lactation. Offspring (Off) were followed until 3 months. Glucose tolerance test was performed and systolic (SBP) and mean arterial (MAP) pressure were measured by radiotelemetry in isoflurane anesthetized rats. Blood was collected from the tail or by cardiac puncture in CO$_2$ sacrificed animals. Values represented as mean±SEM, using ANOVA or Mann-Whitney. Dams in Ob-phys-LGI group showed a reduction in gestational weight gain compared to Ob dams (46±19.2 vs 78±11.9;p<0.001). At the end of pregnancy, Ob dams were insulin resistant (HOMA:72±22.3 vs 23±2.6;p<0.001) and had enhanced levels of serum leptin (11850±1743 vs 4235±698.7pg/mL;p<0.001) and adipose tissue (10.9±0.9 vs 4.2±0.8; p<0.001) compared to Con dams. Interventions led to reversal of fat pad mass (4.9±0.7;p<0.001) and serum leptin (5004±845.2pg/mL;p<0.001). Maternal body weight (b.w.) gain during lactation in Ob dams was decreased when compared to Con group (-28±13 vs 33±7.4; p<0.001). This decrease was reversed by the intervention (31±7.8;p<0.001). From weaning until 3 months, the b.w. gain of OffOb showed a trend to increase when compared to OffCon (males:441±11 vs 414±7.8, females:229±4.2 vs 218±4.3). The increase in b.w. was less pronounced in OffOb-phys-LGI, without reaching statistical significance (males:419±6.5, females:218±6.6). Female OffOb had increased retroperitoneal fat (3.4±0.3 vs 2.3±0.3; p<0.05) and impaired glucose tolerance (AUC:1166±44.9 vs 1046±33.1; p<0.05) when compared to OffCon, with a trend to be less pronounced in female OffOb-phys-LGI (2.5±0.3g and 1075±27.0;p<0.05). With regard to cardiovascular parameters, female OffOb displayed an increase of MAP and SBP compared to female OffCon (MAP:104.2±1.2 vs 99.5±1.4mmHg, SBP:122.6±2.0 vs 114.9±2.5mmHg;p<0.05). Male OffOb showed an increase in MAP in comparison with male OffCon (107.6±1.6 vs 102.2±1.7mmHg) which reached statistical significance in the active (nocturnal) phase (p<0.05). No significant differences were observed in the intervention group. These data show that obese pregnant rats develop insulin resistance with increased fat mass and serum leptin levels. LGI diet combined with exercise improved the phenotype observed in obese pregnant rats but without a significant effect on adiposity or cardiovascular parameters in 3-month offspring of obese dams.

TommyCharity, EU-7-EarlyNutrition

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A ketone ester drink reduces appetite compared to an isocaloric carbohydrate drink

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Ketone bodies (KB) are produced during prolonged fasting or carbohydrate restriction and can act as oxidative fuel sources and metabolic signals. Increased KB concentrations reduces food intake in rodents [1] and in humans [2], although the mechanisms are poorly understood. Ketones may influence appetite via changes in circulating gut hormones [2] and hypothalamic AMPK phosphorylation [3]. We utilized a novel ketone ester (KE) to investigate the effect of ketosis on appetite, insulin and glucose and in non-obese, healthy volunteers. Methods: Following favorable ethical review, volunteers (n = 15) completed a randomized, blinded, cross-over study. Following an overnight fast subjects consumed 395mg/kg KE, or isocaloric dextrose (CHO), as citrus flavored drinks. Drinks were taste, tonicity and colour matched. Blood samples were obtained at regular intervals via an intravenous catheter. Appetite was assessed at baseline (BL) and at identical intervals to blood sampling after drink ingestion. Appetite was assessed as the mean of a 3 measure visual analogue scale (desire to eat, hunger, fullness) and expressed as a percentage. Blood samples were analysed for β-hydroxybutyrate (BHB), glucose and insulin. Values are means ±SEM. Two way repeated measures ANOVA with Sidak Post Hoc corrections were performed. Significance was considered as p < 0.05.

Results: Blood BHB increased from 0.16 mM (± 0.02 mM) at BL to 3.16 mM (± 0.14 mM) 1h post-KE. BHB concentration remained below 0.3 mM in CHO. Appetite fell after both drinks and rose slowly over the course of the study. Appetite was significantly lower (greater satiety) between 1-3h on KE vs. CHO (p<0.05), with a maximal difference of 16.2 % (p<0.05), with a maximal difference of 16.2 % (p<0.05). With regard to carbohydrates, ketone ester and KE consumption led to a trend to increased ketone excretion between 2.5h and 3h (p=0.08). Total insulin area under curve (AUC) was significantly higher following CHO (1972±142±18 mU/L min$^{-1}$ vs. KE = 1010±142±18 mU/L min$^{-1}$). Blood glucose was unchanged between conditions.

Conclusion: Blood glucose and insulin play a pivotal a role in energy balance and appetite [4, 5]. Insulin would normally correlate with decreased appetite [4]. Despite lower insulin following KE, appetite was significantly lower vs. CHO, suggesting an insulin independent mechanism for the changes in appetite during ketosis. This suggests that nutritional ketosis may provide a novel metabolic strategy to modulate appetite

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Meal feeding reduces preference for high fat food in male rats
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Recent pre-clinical (1) and epidemiological (2) evidence suggests that temporal feeding patterns may influence metabolic outcome and contribute to obesity (3). We have demonstrated that grazing and meal feeding with standard rodent chow exert small differential effects on growth and adiposity in rats and mice (4), but the potential impact of these feeding patterns on food choice is unknown.

Male Sprague-Dawley rats (4 weeks old; Charles River, UK) were singly housed in our automated feeding station and fed a standard low fat chow (Harlan Teklad RM3) either ad libitum (AL; n=6), or in a grazing (GR; consumption of 1/24th of the total food intake of the AL-fed rats every 30mins during the dark phase; n=6) or meal-fed (MF; 3x 1h period of AL feeding at 06:00h). On day 12 access to standard chow was replaced by water available AL and a 12h light:12h dark cycle (lights on at 18:00h, 23:30h and 05:00h; n=6) or meal-fed (MF; 3x 1h period of AL feeding at 06:00h). On day 16, but with LFD and HFD hoppers (HFD; P=0.0107). Food choice profiling (day 16) revealed that, following the initial meal consumed during the first 1h of the test by all three treatment groups (in which neither the total nor the proportion of HFD consumed were significantly different), AL, GR and MF rats consumed 6.2±0.8g (62±9% HFD vs 38±9% HFD P=0.2491), 9.2±1.1g (58±13% HFD vs 42±13% HFD; P=0.5529) and 7.1±0.4g (88±2% LFD vs 12±2% HFD; P<0.0001) respectively.

Thus, our data demonstrate that temporal feeding patterns influence food selection, meal feeding reducing preference for HFD. When seen in conjunction with evidence that meal feeding prevents HFD-induced obesity (1), our study indicates that grazing behaviour may represent a contributory factor in developing obesity.


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Identification of GLP-1 receptor expressing cells in the brain using a transgenic mouse model

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Glucagon-like peptide 1 (GLP-1) is emerging as a key regulator of energy metabolism and food intake acting in the brain. As a neuropeptide, GLP-1 is released from a discrete population of neurons in the brainstem which target key nuclei involved in metabolic control and reward throughout the brain. Once released, GLP-1 binds to GLP-1 receptors (GLP-1R), however the precise expression pattern of these receptors in the mouse brain is currently unknown.

Here we use a novel transgenic mouse model (GLP-1R-Cre) expressing Cre-recombinase under the control of the glp1r promoter with a ROSA26-EYFP or ROSA26-tdRFP reporter to map GLP-1R expressing cells throughout the murine brain. For immunofluorescence studies GLP-1R-Cre mice were terminally anaesthetized with 1.5g/kg urethane i.p. and transcardially perfused with 0.1M phosphate buffer followed by 4% paraformaldehyde. Brains were removed and 30μm thick coronal sections were cut from the caudal olfactory bulb to the thalamic paraventricular nucleus (PVT) and ventral tegmental area (VTA). These regions correlate with areas shown to receive many axons from brainstem preproglucagon (PPG) neurons. Furthermore a proportion of EYFP-IR neurons in the NTS, RVLm, PVN, DMH but not VTA or SN were found to contain tyrosine hydroxylase IR, but not parvalbumin IR. Also, glial fibrillary acidic protein IR was not co-localised with EYFP. Interestingly, EYFP-IR neurons were also found in some areas devoid of PPG-neuron projections, such as the hippocampus and cortex, raising the question whether these areas may respond to GLP-1 of non-neuronal origin.

Application of 100 nM GLP-1 in whole-cell recordings from RFP fluorescent cells in BNST and PVN elicited a reversible inward current or depolarization in all cells tested, thus confirming that these cells indeed express GLP-1R. Similarly, stereotaxic injection of a flex-switch AAV unilaterally into the PVN, demonstrated that red fluorescent cells express cre-recombinase and thus produce virally mediated GFP expression. This study comprises a comprehensive description of GLP-1R expression in the mouse CNS and provides information about the phenotype of GLP-1R expressing cells. The use of Cre-recombinase in cells expressing GLP-1R provides a novel molecular handle on this cell population enabling future investigation of their physiological role in vivo.

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Investigating the cellular properties of GLP-1 producing neurons using the genetically encoded calcium indicator GCaMP3

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Glucagon-like peptide-1 (GLP-1) acts in the brain to affect homeostatic processes, such as thermoregulation, cardiovascular control and food intake. GLP-1 is produced in the lower brainstem by preproglucagon (PPG) neurons in the nucleus of the solitary tract (NTS). Whilst these neurons are morphologically well described in several species, little is known about their physiology. Most evidence for their role in energy homeostasis comes from studies using cFos as a marker of neuronal activity. Additionally, patch-clamp recordings in mouse brain slices have revealed electrical responses to satiety hormones such as leptin and cholecystokinin (CCK). Here, we characterise functional properties of the PPG neurons using a genetically-encoded calcium indicator, GCaMP3. PPG-Cre/ROSA26-GCaMP3 mice express GCaMP3 from the ROSA26 locus in a Cre recombinase-dependent manner. As Cre recombinase is expressed under the control of the glucagon promoter, GCaMP3 fluorescence only occurs in pancreatic α-cells, intestinal L-cells and PPG neurons. Experiments were performed on 200 μm thick in vitro brainstem slices from PPG-Cre/ROSA26-GCaMP3 mice cut on a vibratome. Intracellular Ca2+ concentration [Ca2+]i was recorded optically (excitation
Acyl-ghrelin and Ghsr link calorie restriction with hippocampal plasticity, neurogenesis and memory

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The beneficial effects of calorie restriction (CR) have been described at organisal and cellular levels in multiple organs, including the brain1. However, our understanding of the causal mediators is relatively poorly understood, particularly in the context of improved memory. We have shown that the orexigenic gut hormone, acyl-ghrelin (AG)2, increases adult hippocampal neurogenesis (AHN) and improves memory in adult rats3. Here, we have studied the role of AG and it’s receptor, the growth hormone secretagogue receptor (Ghsr), as possible causal factors in mediating the beneficial effects of CR on AHN and memory.

Using the Ghsr-eGFP reporter mouse we found that Ghsr expression was enriched in the dentate gyrus (DG) of the hippocampus, where it was expressed in mature granule neurons in apposition to type II neural stem (NSC) cells. Ghsr expression was not observed in Sox2- or nestin+ NSCs, Ki67+ proliferating cells, DCX+ immature neurons or in S100B+ astrocytes. These data suggest that Ghsr may facilitate AHN within the DG.

Treatment of wild-type and Ghsr+/− mice with AG (7 days i.v. 48μg/day, n=3 per group) induced expression of hippocampal gene transcripts that support cognition and neurogenesis in a Ghsr dependent manner. Genome-wide microarray identified 1,106 AG/Ghsr-regulated transcripts (P<0.05 and >1.5 fold change). RT-qPCR validated the AG mediated up-regulation of transcripts associated with neurogenesis (Gadd45b, P = 0.0314) and plasticity (c-Fos, P = 0.0213; analysed by unpaired two-tailed t test, vs saline).

Next, we analysed whether AG and CR were able to similarly induce expression of the immediate early genes, Egr1 and c-Fos in Ghsr-eGFP+ and Ghsr-eGFP− neurons within the DG. We raised AG levels directly via injection (10μg/kg i.p.), indirectly via CR (16h overnight fast), or with both injection and CR (n=4-5 per group). Expression of Egr1 was increased in the DG after CR (P<0.05), direct AG injection (P<0.05), and after the combination of CR and exogenous treatment with AG (P<0.001). Notably, there was an increase in Ghsr-eGFP+ cells expressing c-Fos in the DG following AG treatment (P<0.05; analysed by 1-way ANOVA with Tukey post hoc testing; vs saline).

Finally, given the CR mediated increase in proneurogenic Egr1 we analysed the impact of 14-days of CR on AHN and hippocampal dependent memory in wild-type and Ghsr−/− mice (n=12 per group). Our data show that CR (70% of ad-lib controls) increased AHN in wild-type (P = 0.048) but not Ghsr−/− mice (P = 0.888). Furthermore, CR wild-type (P = 0.0121) but not Ghsr−/− mice (P = 0.1837) displayed improved contextual fear memory (analysed by 2-way ANOVA with Tukey post hoc testing; vs ad-libitum).

Together, these findings demonstrate a previously unknown function for Ghsr in mediating the beneficial effects of CR on enhancing AHN and memory.


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Acyl-ghrelin and Ghsr link calorie restriction with hippocampal plasticity, neurogenesis and memory

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Electrophysiological investigation of changes of nervous conduction in the spinal cord of transgenic mice with a changed metabolism of nerve fibres

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Excitability and conduction of nerve fibres in mice gained increasing interest, since transgenic mice have been generated with alterations of nerve and myelin properties, partly resembling the clinic of human diseases. The conduction velocity by itself may not be the crucially changed parameter, e.g. if there are metabolic deficits of the myelin function. Therefore, we now investigated the excitability and conduction of central nerve fibres in mice with metabolic myelin-
axon deficits. The Experiments were performed in fully anaesthetised mice (initially pentobarbital sodium 70 mg/kg i.p., continuance with methohexital sodium 60-70 mg/kg i.v.). A tracheotomy was performed for artificial ventilation after paralyisation (paralyisation with pancuronium bromide 800 μg/kg/h intraperitoneally, artificial ventilation with a gas mixture of O2 (47.5%), CO2 (2.5%), and N2 (50%) at about 120 strokes/min). ECG, heart rate, core body temperature and blood O2 saturation were permanently monitored and used to control the anaesthetic state. The lumbar spinal cord and dorsal roots were exposed by a laminectomy. Stimulation was performed at the dorsal root L4 with 100 Hz and a sub-maximal stimulus strength for 10 min. Recovery was tested with 0.1 Hz. The induced compound action potential (CAP) was recorded 7 to 10 mm cranially to the stimulation electrode at the dorsal column (fasciculus gracilis) of the spinal cord. The results from wild type mice were compared with results from two different types of transgenic mice: 1.) a model of metabolic support deficit in conditional NR1-KO mice (lacking NMDA-receptors in oligodendrocytes); 2.) a model of a classical neurodegenerative disease ALS, SOD1G93A mice (a deficit of oligodendroglial support of axons has been shown in a similar model of ALS, Lee et al. 2012). In both types of models, the decrement during the high-frequency stimulation period was significantly more pronounced in the transgenic mice compared to wild-type littermates. Time course of recovery was correspondingly delayed in the transgenic mice. The results suggest a deficit of the axon-glial metabolism with regard to energy support from oligodendroglia towards axons during tetanic high-frequency stimulation. The experimental approach is of interest for the functional characterisation of nerve fibres with metabolic deficits with or without structural abnormalities, also in classical models of neurodegenerative diseases.


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Functional modulation of astrocytic GABA<sub>G</sub> receptors by P2Y purinoceptors

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Astrocytes, the most abundant cell type in the CNS, are accepted to play essential roles in brain function by supporting neuronal viability and vascular integrity. Whilst astrocytes are not electrically active, their properties are subject to regulation via dynamic changes in intracellular Ca<sup>2+</sup> signalling, events that are believed to play a critical role in coordination of astrocyte communication and gliotransmission. GABA<sub>G</sub> receptors (GABA<sub>G</sub>Rs) are heterodimeric G-protein-coupled receptors, which mediate slow synaptic inhibition in the brain. Emerging evidence suggests astrocytes express GABA<sub>G</sub>Rs, however the role GABA<sub>G</sub>Rs play in regulating astrocyte activity remains largely speculative. To determine whether astrocytic GABA<sub>G</sub>Rs modulate Ca<sup>2+</sup> signalling, cultures were loaded with Fluo-4/AM and dynamic changes in cytosolic Ca<sup>2+</sup> levels were analysed using time-lapse confocal microscopy (Terunuma et al., 2015). Exposure of astrocytes to baclofen did not lead to any significant changes in intracellular Ca<sup>2+</sup> levels. In contrast, subsequent exposure of astrocytes to the P2 purinoceptor (P2R) agonist ATP rapidly increased intracellular Ca<sup>2+</sup> levels, consistent with published studies (Fischer et al., 2009). Importantly, after exposure to ATP, evidence of small baclofen induced Ca<sup>2+</sup> transients was observed. To assess whether GABA<sub>G</sub>Rs signalling is facilitated by pre-exposure to ATP, we used the GABA<sub>G</sub>R antagonist CGP54626. Pre-treatment of astrocytes with CGP54626 abolished baclofen-evoked Ca<sup>2+</sup> transients, however, CGP54626 alone did not have any effect on ATP-dependent increases in Ca<sup>2+</sup>. Collectively, these studies strongly suggest the ability of GABA<sub>G</sub>Rs to modulate astrocytic Ca<sup>2+</sup> signalling is facilitated by ATP.

To analyse the mechanisms by which ATP regulates GABA<sub>G</sub>R activity, we examined its effects on the phosphorylation of S783 and S892 within the GABA<sub>G</sub>R2 subunit, accepted substrates of AMPK and PKA, respectively (Couve et al., 2002; Terunuma et al., 2010). To do so, we used phospho-specific antibodies against these residues, and the ratio of p-S783/R2 and p-S892/R2 was then compared between treatments. Exposure of astrocytes to ATP stimulation significantly increased S783 and S892 phosphorylation in a time-dependent manner. ATP-induced phosphorylation of both residues was prevented by pre-application of the P2R antagonists, 30μM Suramin and 100μM PPADS (p-S783: p<0.037; p-S892: p<0.018 compared to ATP alone). Therefore, these results demonstrate that in astrocytes, P2Rs regulate the phosphorylation of S783 and S892 in GABA<sub>G</sub>R2.

In conclusion, our study suggests GABA<sub>G</sub>R signalling in astrocytes is critically dependent upon the activation of P2Rs leading to the phosphorylation of key residues in the GABA<sub>G</sub>R2. This synergistic interaction between P2Rs and GABA<sub>G</sub>Rs may act as a co-incidence detector to allow the fine-tuning of astrocytic Ca<sup>2+</sup> signalling.


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Optogenetic acidification of synaptic vesicles and lysosomes

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Optogenetics allows for spatiotemporal control of cellular processes with light. In neuroscience, microbial rhodopsins such as light-activated ion pumps and channelrhodopsins are commonly applied to alter the neuronal membrane potential, thereby tuning cellular excitability. Up to now, microbial rhodopsins have been targeted to a number of different neuronal compartments, including the postsynaptic density, dendrite and axon initial segments, thus allowing modulation of the local membrane potential. In contrast, optogenetic tools to change ion and voltage gradients along intracellular membranes have not been developed to date. We here report on
a new class of optogenetic actuators that enables light-activated acidification of intracellular compartments such as synaptic vesicles and lysosomes. Moreover, these tools incorporate fluorescent pH reporters to follow light-induced pH changes in the respective organelles.

For synaptic targeting we introduced the green-light activated proton pump Arch3 from *Halorobrum sodomense* (1) together with the red-fluorescent protein mKate2 (cytosolic side) and the pH-sensitive pHluorin (luminal side) (2) after the third helix of the vesicular marker protein synaptophysin, followed by a linker helix and the fourth synaptophysin helix. The resulting construct in the following referred to as pHoenix was expressed in murine hippocampal neurons using lentivirus.

In neurons, pHoenix colocalizes with the vesicular glutamate transporter 2 and resides on synaptic vesicles as shown by fluorescence measurements and electrophysiology. Next, we incubated autaptic cultured neurons with the V-type ATPase inhibitor bafilomycin for 2 hours and performed whole-cell patch clamp measurements. After action potential triggering no or very small excitatory postsynaptic currents (EPSCs) were observed indicating insufficient glutamate uptake in newly formed synaptic vesicles due to the lack of proton-motive force across the vesicular membrane. pHoenix activation by green light restored the proton gradient and recovered full EPSCs within 2 min. Acidification of synaptic vesicles was fast (5s) compared to glutamate uptake (60s) as was concluded from simultaneous pHluorin imaging and EPSC recordings. In the following pHoenix was applied to analyze vesicular release probability in dependence of the vesicular fill state. Therefore, we analyzed miniature EPSC amplitude and frequency, paired-pulse ratio and sucrose-induced vesicle release following complex illumination protocols. Our data confirms that release probability is higher for full vesicles compared to partially filled vesicles (3).

Finally, we created a pHoenix variant targeted to lysosomes by replacing the synaptophysin moieties by the transmembrane domains of the lysosomal marker protein CD63 (lyso-pHoenix). Lyso-pHoenix enabled reversible lysosomal acidification with light, both in bafilomycin-treated HEK and HELA cells. Chow BY et al. (2010). Nature 463(7277), 98-102. Miesenböck G et al. (1998). Nature 394(6689), 192-5. Herman MA et al. (2014). J Neurosci 34(35), 11781-91

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Neurovascular physiology and pathophysiology of brain pericytes

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Pericytes are contractile cells located on microvessels, and are found at highest density in the central nervous system. In the brain, pericytes dilate capillaries in response to neuronal activity, targeting blood flow to active neurons (Hall et al., 2014). However, following ischaemia, they irreversibly constrict capillaries and die in rigor. Pericytes also play many other critical roles in CNS development, physiology, and pathology, yet discord regarding their identification persists. We investigated the anatomy, physiology, and pathological dysfunction of pericytes. Methods: We used NG2-DsRed mice (n=13) and Sprague-Dawley rats for experiments. Data are presented as mean ± SEM. Acute brain slices were prepared from P12 rats (n=25) for physiological experiments and P21 (n=35) rats for ischaemia experiments. When examining the pericyte response to ischaemia in vivo, we used the intraluminal fill model of middle cerebral artery occlusion (MCAO) to simulate stroke. Rats (male, 250-300g, n=3) were anaesthetised with isoflurane (4% for induction) prior to maintenance with urethane (1.5g/kg IP) for surgery and MCAO. Ischaemia was induced for 90mins. Two hours after MCAO onset animals were sacrificed. Intravascular fluorescent gel perfusion was used to assess brain capillary diameter near pericytes in fixed tissue. MCAO animals were compared with naïve controls (n=3). Results: Pericytes are a distinct cell population and were readily distinguished from endothelial cells and juxtaglomerular microglia by their expression of the proteoglycan NG2, the receptor tyrosine kinase PDGFRβ, and their being surrounded by the basement membrane marker isolectin B4. Pericytes extend contractile processes along and around the endothelial cells forming capillaries. We found 33±3% of brain pericytes were contacted by microglial processes, potentially mediating surveillance of the blood-brain barrier. In cerebellar slices, NO (delivered as DETA-NONOate, 100μM) and PGE2 (1μM) dilated molecular layer capillaries by 13.5±2.3% and 17.7±3.8% of baseline diameter, respectively. Electrical stimulation of the cerebellar parallel fibres dilated capillaries by 14.6±3.4%. Dilation was mediated by PGE2 acting at EP4 receptors: dilation was blocked by the specific EP4 receptor blocker L161,982 (1μM, p=0.01, t-test). Capillary constriction at pericytes in brain slices occurred in ~15mins when ATP synthesis was inhibited with iodoacetate (2mM) and antimycin (25μM). After MCAO, capillaries were more constricted near pericytes compared to control (p=0.035, t-test). In ischaemic brain slices, capillary constriction was significantly delayed by blocking voltage-dependent calcium channels (nimodipine, 100μM, p=0.006, t-test) and 20-HETE synthesis (HET0016, 1μM, p=0.004, t-test), suggesting the use of such agents in ischaemic stroke should be re-evaluated.


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Brainstem astrocyte Kir4.1 channels contribute to central respiratory drive

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The brain regulates breathing in response to changes in tissue CO2/H+ via a process termed central chemoreception. The ventral medullary surface of the brainstem contains a region called the retrotrapezoid nucleus (RTN), which has been identified as a key locus for chemoreception and central control of respiration. Neurons in this region can sense CO2/H+ (i.e. are
capnea, we measured respiratory activity in awake mice by Kir4.1 cKO mice exhibit altered respiratory drive during hyper to changes in CO₂. Astrocytes may disrupt the ability of these cells to respond normally to pH changes (chemosensitive) in part by inhibition of TASK-2 channels. RTN astrocytes are also chemosensitive and CO₂-evoked release of the gliotransmitter ATP most likely occurs via connexin 26 hemichannels. This purinergic component appears to gain up RTN neuron chemosensitive responses by approximately 30%. However, RTN astrocytes also express a H⁺-sensitive current mediated by an inwardly rectifying potassium ion channel (Kir4.1-like). The contribution of this pH sensitive current in RTN astrocytes to the central respiratory drive has yet to be determined. Here we generate an inducible astrocyte specific Kir4.1 channel knockout (Kir4.1 cKO) using GFAP-CreERT2 and Kir4.1 floxed mouse lines. All animals were used in accordance with the National Institute of Health and University of Connecticut Animal Care and Use Guidelines. Immunohistochemistry was used to confirm reduced expression of Kir4.1 in GFAP positive cells in the hippocampus, cerebellum and RTN. Whole-cell voltage clamp recordings of RTN astrocytes in brainstem slices from Kir4.1 cKO mice showed the absence of a Kir4.1-like current. RTN neurons from brainstem slices were also recorded and preliminary data from Kir4.1 cKO mice appear to show a reduction in the purinergic component of their CO₂/H⁺ response, suggesting deletion of Kir4.1 from astrocytes may disrupt the ability of these cells to respond normally to changes in CO₂/H⁺. Therefore, to determine whether Kir4.1 cKO mice exhibit altered respiratory drive during hypercapnea, we measured respiratory activity in awake mice by whole-body plethysmography during graded increases in CO₂. We found that Kir4.1 cKO mice hypoventilate in 100% O₂ and show a reduced tidal volume response to CO₂ compared to controls. These results suggest that Kir4.1 channels in astrocytes contribute to the central drive to breathe.

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PKC-δ isozyme gene silencing normalizes Ca²⁺-signaling and decreases myocardial calcium sensitivity in aortic smooth muscle from streptozotocin-induced diabetic rats

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Diabetes is well known as a complex syndrome which leads to multiple malfunctions including vascular abnormalities. Changes in myocardial Ca²⁺-sensitivity plays a crucial role in complex interplay in contraction/dilation of vascular smooth muscle (SM) cells in diabetes which, in turn, alters protein kinase C (PKC) related mechanisms of Ca²⁺-signalling. In this study we have compared SM contractility and intracellular Ca²⁺ concentration ([Ca²⁺]) in three groups of rats: streptozotocin-induced diabetes rats (STZ), STZ PKC-δ isozyme gene silencing rats and control group of animals) during activation of SM with cumulative doses of phenylephrine (PE, 10⁻⁹-10⁻⁶ M). Animals were killed by cervical dislocation following ketamine (45 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) anesthesia on 3rd month of diabetes and on the 7th day after intravenous injection of siRNAs. Annealed siRNAs were injected (40 mgk / per rat) twice via tail vein with 24 hours interval.

All animal studies were approved by the Institutional Animal Care and Use Committee. Thoracic aortas obtained from of male Wistar rats (250-300 g) were cut into 1,5 mm-wide rings and loaded with 10 µM Fura 2-AM for 4 hours. The results of [Ca²⁺] measurements were presented as the ratio (R) of a 510-nm emission fluorescence intensity at 340- nm and 380-nm excitation signals. The sensitivity of contractile myofilaments to Ca²⁺ was measured as relationship between contractile force and R (FAR). Low concentration (10⁻⁶ M) of PE provoked asynchronous phasic contractions and increase in [Ca²⁺], which was followed by Ca²⁺-oscillations in STZ group likely due to over-activation in alpha-agonist stimulation while intact SM were without response to low PE concentration. ΔF/ΔR was significantly increased in STZ group up to 0.38±0.01 vs 0.21±0.02 in a control (n=12, P<0.05), suggesting that myofilaments sensitivity to calcium had increased under diabetes. sRNAs administration and following PKC-δ isozyme gene silencing restored normal SM sensitivity to alpha-agonist and normalized myofilament Ca²⁺ sensitivity in STZ rats (ΔF/ΔR = 0.23±0.03 in STZ PKC-δ group vs to a control (n=12, P>0.05). In conclusion, the data obtained clearly indicate an increased sensitivity of vascular SM to alpha-agonist stimulation as well an increment in sensitivity of contractile proteins to calcium in diabetic rats. The ability of siRNAs targeted to PKC-δ gene normalize Ca²⁺ signaling and vascular abnormalities supports PKC involvement in diabetic angiopathy development.

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Microvascular endothelial dysfunction is evident in patients with HER2 positive breast cancer who are newly-treated with Herceptin

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Breast cancer (BC) is the most common cancer in the UK and almost 25% of BC’s present with an aggressive form characterised by increased expression of human epidermal growth receptor 2 (HER2) proteins. In these patients, trastuzumab (TRZ) can be used to inhibit HER2 signalling pathways resulting in reduced tumour proliferation and improved survival. Unfortunately, TRZ can also cause cardiotoxicity which, via impairment of HER2 cardioprotective pathways, can result in congestive heart failure. At present, the precise mechanisms for this are unclear, although endothelial dysfunction has been hypothesised as a possible cause. Thus, the present study examined microvessel and large vessel endothelial function, arterial stiffness and carotid intima-media thickness (cIMT) in female breast cancer patients and healthy controls. Thirteen TRZ-naïve BC patients (age: 57 ± 15 years) with HER2 positive tumours who had been treated with TRZ for at least one year, as well as 15 healthy controls (age: 54 ± 9 years) underwent assessments of microvascular endothelial function (laser Doppler imaging with iontophoresis of endothelium-dependent agonist - acetylcholine (ACH) and endothelium-independent agonist - sodium nitroprusside), large vessel endothelial function (flow-mediated dilatation (FMD) and glyceryl-trinitrate mediated dilatation), arterial stiffness (pulse wave analysis), and cIMT. Cardiovascular disease risk was also calculated in both groups using the Framingham Risk Score (FRS) and QRISK2. Univariate Analysis of Variance revealed no significant differences in age, systolic or diastolic blood pressure, FRS or
QRI$\text{SK2}$ between groups (p’s > 0.3). However, TRZ patients had greater body mass index (BMI) and body fat percentage (Fat %) relative to healthy controls (BMI: 29 ± 6 and 26 ± 3kg/m$^2$ respectively, p = 0.04; Fat%: 37 ± 9 and 31 ± 5% respectively, p = 0.03). TRZ-treated patients also had significantly worse microvascular perfusion in response to ACh when compared with healthy controls (TRZ: 267 ± 130% vs. healthy controls: 545 ± 355%, p = 0.01), along with worse large vessel endothelial function (FMD: 9.2 ± 7.8 and 16 ± 7 % respectively, p = 0.04). Univariate Analysis of Covariance revealed that the difference in microvascular endothelium-dependent function between groups remained significant even after adjustment for BMI and fat % (p = 0.03), but large vessel endothelial function was no longer significantly different. No significant differences were found for arterial stiffness or cIMT. The present findings reveal that in BC patients treated with TRZ, there is a difference in microvascular endothelium-dependent function compared with healthy controls. Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

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The calcium-sensing receptor in vascular smooth muscle and endothelial cells elicits opposing effects on vascular tone

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Background: The extracellular calcium-sensing receptor (CaSR) is expressed in vascular smooth muscle cells (VSMC) and endothelial cells (EC) of blood vessels where its functions are not well defined. To elucidate the role of the CaSR in the VSMC, we generated mice with constitutive targeted CaSR gene ablation by crossing exon 7 LoxP-CaSR and Sm22α-Cre mice.

Methods and results: All results shown as mean±SEM. Ex vivo wire myography performed on aorta and mesenteric arteries of Cre-negative (wild-type, WT) and Sm22α-CaSR$^{lox/lox}$ (VSMC-knock-out, VSMC-KO) showed that and phenylephrine (PE)-induced contractility was significantly reduced in blood vessels from VSMC-KO animals compared to WT (e.g. at 1μM PE, aorta: N=18 KO and 19 WT, 78.9±6.3% vs 51.6±5.1% of max WT response, p<0.001) by extra sum-of-squares F-test for comparison of fitted curves (ESSqF) which was not abolished by endothelial denudation or inhibition of NO synthase by NG-nitro-L-arginine methyl ester (L-NAME). Furthermore, exposing the vessels increasing extracellular calcium concentrations [Ca$^{2+}$]o evoked contraction followed by relaxation in WT, but only relaxation in KO aortae (e.g. at 3mM Ca$^{2+}$, N=9, 19.7±5.8% contraction vs. 24.7±9.7% relaxation compared to baseline, p<0.001). These results suggest an endothe-lium-independent role for the VSMC-CaSR in contributing to blood vessel contractility.

In vitro, Ca$^{2+}$, or KCl elicited reduced intracellular Ca$^{2+}$ responses in isolated VSMC from KO animals compared to WT. As expected, CaSR deletion from VSMC had an effect on blood pressure in vivo, where we observed hypotension in VSMC-KO compared to WT using tail cuff and radiotelemetry which was greatest during the night when the animals were active (N=5, 112±2.6 vs. 127.3±4.9 mm Hg systolic p<0.05 and 81.3±2.2 vs 101.4±4.9 mm Hg diastolic p<0.01, Holm-Sidak post-test of two-way ANOVA, surgery under general anesthesia using s.c. flunisalone 10 mg/kg body-weight, fentanyl 0.3 mg/kg and midazolam 1 mg/kg).

To compare the effects of the VSMC-CaSR to those of the EC-CaSR, we assessed the vascular contractility of EC-CaSR knock out mice by crossing exon 7 Lox-P-CaSR mice and inducible platelet derived growth factor subunit B (iPDGFB)-Cre mice (5 days tamoxifen injection). In contrast to the VSMC-KO mice, aortic contractility in response to PE of these iPDGFB$^{+/+}$CaSR$^{flx/lox}$ mice was significantly enhanced compared to WT (e.g. at 1μM PE, N=9 KO and N=6 WT, 108.2±20.6 vs. 68.7±11.5, p<0.001, ESSqF) which was totally abolished by L-NAME, suggesting a NO-mediated vasodilatory effect of the EC-CaSR.

Conclusions: Our findings clearly demonstrate that the vascular CaSR contributes to modulation of vascular tone and blood pressure, with opposing effects depending on the vascular cell type: the VSMC-CaSR contributes to arterial contraction while the EC-CaSR mediates arterial dilation.

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Store-depletion activates Ga$q$-PLC activity through a STIM1/TRPC1-mediated but Oral1-independent mechanism in vascular smooth muscle cells

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Canonical transient receptor potential 1 (TRPC1) proteins form store-operated Ca$^{2+}$-permeable ion channels in vascular smooth muscle cells (VSMCs), which are involved in regulating contractility, and cell proliferation and migration (1). However, the mechanism involved in coupling depletion of internal Ca$^{2+}$ stores to activation of TRPC1 channels remains elusive. The present work investigates the idea that store-depletion triggers Ga$q$-mediated PLC and TRPC1 channel activities in freshly isolated and primary cultured rabbit and mice mesenteric artery VSMCs, measured using the PIP$^2$, fluorescent sensor PLC$\gamma$1-GH-GFP.

Application of BAPTA-AM, TPEN or CPA, which all deplete Ca$^{2+}$ stores, induced translocation of PLC$\gamma$1-GH-GFP-mediated signals from the plasma membrane to the cytosol, which were reduced by the PLC inhibitor U73122. BAPTA-AM-mediated translocation of PLC$\gamma$1-GH-GFP signals were greatly inhibited by transduction of cells with shRNA STIM1, and were absent in TRPC1$^{-/-}$ VSMCs. Co-immunoprecipitation, immunocytochemistry and proximity ligation assay showed that BAPTA-AM induced translocation of STIM1 from the cytosol to the plasma membrane, where it interacted with TRPC1. Moreover, store-operated STIM1/TRPC1 complexes associated
with Gaq and PLCβ1. In contrast, store-operated translocation of PLCβ1-GH-GFP signals, and interactions between STIM1, TRPC1, Gaq and PLCβ1 were not altered in Orai1−/− mice. These findings propose a novel mechanism for store-operated TRPC1 channels in VSMCs, through formation of STIM1/TRPC1 signalling complexes which drive activation of the classical Gaq/PLC pathway. Our results argue against a role for Orai1 in the activation of TRPC1 channels in VSMCs.


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A computational model of the ionic currents and action potentials underlying contraction of isolated urinary bladder smooth muscle

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Motivation: Urinary incontinence (UI) is the involuntary loss of urine that creates a social or hygiene problem. Among different types of UI, bladder over activity is one, which is an overactive detrusor smooth muscle (DSM). It is well known that electrophysiological phenomena generates spontaneous electrical activities that intern causes spontaneous contraction due to elevation of intracellular Ca2+ concentration [1,2]. In order to understand the ionic mechanisms which generate electrical activities such as action potentials (APs) and synaptic depolarizations, we aimed to establish a computational model of sufficient biophysical detail to simulate DSM APs which in turn can shed light on genesis of bladder over activity. Based on recent experimental evidence [3,4,5], we construct mathematical models for seven ionic currents of DSM, where the magnitudes and kinetics of each current is described by differential equations, in terms of maximal conductances, electro chemical gradients, and voltage-dependent activation/inactivation gating variables. The model is validated step by step by comparing simulated AP with experimental AP adapted from literature [4].

Methods: The model of active ion channels are based on classical Hodgkin-Huxley approach in parallel conductance model. Membrane capacitance (Cm) is taken as 1μF/cm2. The membrane resistance (Rm) is 138MΩ·cm2 and axial resistance is 1812Ω.cm. The time dependence of the membrane potential is governed by $\frac{dv_m}{dt} = -I_{ion}(t)/C_m$ where $v_m$ (in mV) represents the transmembrane potential, and $I_{ion}$ (in pA) represents the sum of the ionic currents crossing the cell membrane.

Results: Active conductances for rising phase are a large voltage gated L type Ca2+ current and small T type Ca2+ current current.Similarly voltage gated K+ current and calcium gated K+ currents are present in falling phase of the spike. Figure 1 shows both simulated AP and experimental AP in same scale, where Table 1 presents the validation in terms of resting membrane potential (RMP), Peak, after hyperpolarization (AHP) and AP duration. The AP generated from simulation resembles the AP from experiment up to a great extent. The simulation result doesn’t fully recreate the experimental AP. This may be absence of the medium conductance Ca2+ - activated K+ (IK) channel in our model.

Conclusion: The model reproduces successfully the generation of single spike in DSM that fit with the experimental data.Future perspectives of this work consist in adding more active channels, Na+–Ca2+ exchanger, plasma membrane Ca2+ ATPase pump and sarcoplasmic reticulum Ca2+ ATPase pump for a more comprehensive model.

Table I Comparison between simulated AP and Experimental AP [4]

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<thead>
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<th>RMP (mV)</th>
<th>Peak (μA)</th>
<th>AHP(mV)</th>
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Differential Kv7 subunit interaction in vascular smooth muscle cells of mesenteric and renal arteries in the normotensive and spontaneously hypertensive rat

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Background: KCNQ encoded Kv7 voltage-gated potassium channels (mainly Kv7.1, Kv7.4, Kv7.5), expressed in a range of vasculature, regulate vascular tone1 and are compromised in hypertension. Kv7 channel regulation is still being elucidated. Kv7 channels are tetramers of individual channel subunits; therefore these channels can be regulated by changes in subunit composition. We used Proximity Ligation Assay (PLA) technology2 to investigate the molecular architecture of Kv7 channels in renal (RA) and mesenteric artery (MA) vascular smooth muscle cells (VSMCs) in normotensive (NT) rats and compare any differences. We also investigated the molecular architecture of channels in the same arterial beds in spontaneously hypertensive rats (SHRs) for comparison to the NT rat.
Heart failure causes down regulation of the atrial L-type Ca^{2+} current (I_{CaL}) during heart failure. Downregulation of L-type Ca^{2+} current (I_{CaL}) in atrial myocytes has been suggested to involve reduction in both the expression and phosphorylation of the channel protein although the precise mechanisms remain unclear. The objective of this project was to examine the regulation of atrial I_{CaL} density in a surgical model of heart failure in rats. Procedures were approved by local ethics committees and performed in accordance with UK legislation. Adult male Wistar rats were subjected to surgical ligation of the left anterior descending coronary artery (CAL, N=10) or equivalent sham operation (Sham, N=12) under general anaesthesia (ketamine 75 mg/kg, medetomidine 0.5 mg/kg, ip) with appropriate analgesia (buprenorphine 0.05 mg/kg, sc). Left atrial myocytes were isolated 18 weeks after surgery. Single isolated cells were superfused with a Tyrode’s solution (37 °C) and whole-cell currents recorded from a holding potential of ~80 mV using a ruptured patch-clamp technique. I_{CaL} was activated by depolarisation to 0 mV (300 ms) following a pre-pulse to -40 mV to inactivate Na current, and measured as the difference between the peak inward current and the steady-state current at the end of the pulse. Data are presented as mean ± standard error of the mean and were compared by unpaired t-test. P<0.05 was used as the limit of statistical confidence. CAL rats had increased heart weight/tibia length (4.84±0.27 vs 3.02±0.07; P=0.0001) and lung weight/tibia length (4.19±0.30 vs 3.72±0.08; P=0.01) ratios, indicating early stage heart failure. CAL cells were hypertrophied compared to Sham (94.7±5.2 pF; n=63 vs 56.6±2.1 pF; n=47; P<0.0001). I_{CaL} density at 0 mV was reduced in CAL compared to Sham (3.3±0.4 pA/pF; n=31 vs 7.3±0.7 pA/pF; n=33; P<0.0001). The response of I_{CaL} at 0 mV to noradrenaline (NA; 1 μM) was increased from 3.2-fold in Sham to 6.8-fold in CAL so that there was no difference in I_{CaL} density between the two groups (CAL-21.9±3.0 pA/pF; n=9 and Sham-23.7±4.5 pA/pF, n=5; ns). The PKA inhibitor H-89 (10 μM) had no effect on basal currents but abolished the effect of NA in both CAL and Sham. The absence of difference in I_{CaL} density between CAL and sham myocytes in the presence of NA is consistent with a change in channel protein phosphorylation without a change in channel number. The regulation of L-type Ca^{2+} channel phosphorylation may be a potential therapeutic target for atrial arrhythmias in heart failure.

This work was supported by the British Heart Foundation

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**Down regulation of L-type Ca^{2+} current in rat atrial myocytes during heart failure**

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Heart failure causes down regulation of the atrial L-type Ca^{2+} current (I_{CaL}), which contributes to contractile and electrical dysfunction and increases susceptibility to atrial fibrillation. The reduction in atrial I_{CaL} density has been suggested to involve reduction in both the expression and phosphorylation of the channel protein although the precise mechanisms remain unclear. The objective of this project was to examine the regulation of atrial I_{CaL} in a surgical model of heart failure in rats. Procedures were approved by local ethics committees and performed in accordance with UK legislation. Adult male Wistar rats were subjected to surgical ligation of the left anterior descending coronary artery (CAL, N=10) or equivalent sham operation (Sham, N=12) under general anaesthesia (ketamine 75 mg/kg, medetomidine 0.5 mg/kg, ip) with appropriate analgesia (buprenorphine 0.05 mg/kg, sc). Left atrial myocytes were isolated 18 weeks after surgery. Single isolated cells were superfused with a Tyrode’s solution (37 °C) and whole-cell currents recorded from a holding potential of ~80 mV using a ruptured patch-clamp technique. I_{CaL} was activated by depolarisation to 0 mV (300 ms) following a pre-pulse to -40 mV to inactivate Na current, and measured as the difference between the peak inward current and the steady-state current at the end of the pulse. Data are presented as mean ± standard error of the mean and were compared by unpaired t-test. P<0.05 was used as the limit of statistical confidence. CAL rats had increased heart weight/tibia length (4.84±0.27 vs 3.02±0.07; P=0.0001) and lung weight/tibia length (4.19±0.30 vs 3.72±0.08; P=0.01) ratios, indicating early stage heart failure. CAL cells were hypertrophied compared to Sham (94.7±5.2 pF; n=63 vs 56.6±2.1 pF; n=47; P<0.0001). I_{CaL} density at 0 mV was reduced in CAL compared to Sham (3.3±0.4 pA/pF; n=31 vs 7.3±0.7 pA/pF; n=33; P<0.0001). The response of I_{CaL} at 0 mV to noradrenaline (NA; 1 μM) was increased from 3.2-fold in Sham to 6.8-fold in CAL so that there was no difference in I_{CaL} density between the two groups (CAL-21.9±3.0 pA/pF; n=9 and Sham-23.7±4.5 pA/pF, n=5; ns). The PKA inhibitor H-89 (10 μM) had no effect on basal currents but abolished the effect of NA in both CAL and Sham. The absence of difference in I_{CaL} density between CAL and sham myocytes in the presence of NA is consistent with a change in channel protein phosphorylation without a change in channel number. The regulation of L-type Ca^{2+} channel phosphorylation may be a potential therapeutic target for atrial arrhythmias in heart failure.

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The novel peptide ELABELA/toddler is expressed in the human cardiovascular system

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Introduction: The apelin receptor (a.k.a. AP), is a G protein-coupled receptor and mediates positive inotropic and vasodilatory effects when activated by the endogenous ligand apelin\(^1\,\,^2\). Apelin peptide knockout mice are viable and showed normal development. In marked contrast, apelin receptor knockout mice are not born in Mendelian ratio and show cardiovascular developmental defects\(^3\). The discrepancy between the phenotypes has suggested the existence of a further endogenous ligand. Two groups have independently identified a well conserved gene encoding a peptide named ELABELA (ELA)\(^4\) or Toddler\(^5\) (a.k.a. apela), required for cardiac development in zebrafish. The gene was identified in a region that had not previously been annotated as coding DNA and was predicted to express a 54 amino acid protein with a 32 amino acid mature peptide. The expression of the peptide has not been determined in the human cardiovascular system.

Hypothesis: Our working hypothesis is that ELA is expressed in adult human cardiovascular tissue.

Methods: RT-qPCR and immunohistochemistry were carried out with cDNA and sections (30µm) of adult human normal
Calcium-sensing receptor (CaSR) is an important regulator of fetal lung development through the cystic fibrosis transmembrane conductance regulator (CFTR)

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Immature lung development continues to be one of the major complications with preterm birth. Optimal postnatal respiratory function depends on the co-ordinated development of lung branching morphogenesis and fluid secretion in the fetal lung. These processes start during the pseudoglandular stage (weeks 9 – 17 in humans, and embryonic day (E)11.5 – 16.5 in the mouse), and their impairment results in significant and long-lasting morbidity which extends well into adulthood. Previously we have shown that lung development occurs in a relatively hypercalcemic environment (~1.7 mM for the fetus vs ~1.2 mM for a normocalcemic adult), and that this relative hypercalcemia is an important environmental signal for optimal lung development. Specifically, using organ explant cultures in chemically defined, serumless conditions, we have shown that fetal hypercalcemia suppresses lung branching morphogenesis via activation of the calcium-sensing receptor (CaSR) [1]. In contrast, CaSR activation by hypercalcemic conditions stimulates Cl- driven fluid secretion, resulting in a negative transluminal potential difference (PD), the magnitude of which reflects the rate of fluid secretion [2]. The aim of this work was to identify the mechanisms by which CaSR regulates fluid secretion ex vivo, using mouse and human fetal lung explant cultures and in vivo, by immunohistochemical methods. Our results show that a number of chloride transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotransporter (NKCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR) are expressed in Na-K-2Cl cotrans

Finney BA et al. (2008). J Physiol 586, 6007-6009


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These data suggest that bronchoconstriction exerts a more potent influence on NRD than changes in end expiratory lung volume during methacholine challenge.

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Can guided-inquiry lectures enhance student engagement and understanding of physiological concepts?

Y. Hodgson

Physiology, Monash University, Clayton, VIC, Australia

At Monash University, student enrolments in physiology are large (450-550) with lectures still the primary mode of teaching. Lectures develop students’ physiology knowledge, but not their communication, teamwork or critical thinking skills. The challenge is to modify lectures to encourage student engagement, active learning and the development of communication, teamwork, and critical thinking skills. In a previous study we found that real time digital inking on a Tablet PC in lectures stimulates student interest and understanding of physiology (1). In this study we have added guided-inquiry activities into these lectures. This is based on the constructivist theory of learning and an active learning approach termed POGIL (Process Oriented Guided Inquiry Learning; 3, 5) which has been shown to be effective in lectures, encouraging student participation and enhancing student learning (2, 3).

We developed guided-inquiry activities for three physiology lectures: (i) endocrine control of calcium homeostasis, (ii) the oxygen and haemoglobin dissociation curve and (iii) the chemical digestion of foods. During each guided-inquiry lecture, students were initially provided with background information relevant to the lecture concepts. This was followed by a guided-inquiry activity on the concept in which students interpreted graphs, answered questions and solved problems. An interactive discussion about the concepts followed the guided-inquiry activity. To evaluate the effectiveness of the guided-inquiry lecture approach, students were given a diagnostic multiple choice question quiz (pre-test) covering the core concepts at the beginning of semester. This quiz was repeated immediately after each lecture (post-test) to measure student learning. The results showed a significant improvement in student performance on the quiz questions between the pre and post tests (P<0.05). At the end of semester a survey was used to evaluate student perceptions of the guided-inquiry lectures (4; 55 responses). Students found that the guided inquiry lectures (i) encouraged them to take notes during the lecture (82%); (ii) stimulated class questions and/or discussions (91%); (iii) helped them to understand the lecture topics (89%) and (iv) encouraged them to attend lectures (75%). Written comments from students included:

“The guided inquiry lectures encouraged discussion with peers and lecturers. I felt that I could ask stupid questions.”

In conclusion, we have found that this guided-inquiry approach can be used in large lecture cohorts to motivate active student learning and enhance student understanding of core physiology concepts.


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Flipping respiratory physiology: supporting independent student learning in large cohorts

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Transition from school to University requires students to learn new factual content and, in many cases, new learning styles. The delivery of physiological content has traditionally been provided, and commonly remains, by a series of didactic lectures that may be complemented by small group tutorials (SGT). Whilst appearing to fulfil the requirements of curriculum delivery, such methodology fails to enable students to gauge accurately, at an individual level, their understanding of the subject and their focus of learning may naturally shift towards the security of rote learning the new content being presented. Such shifts in learning style are reflected by the increasing student demands for more didactic teaching, slide-based handouts and lecture capture. In many cases, the first independent test of understanding comes at a summative assessment and thus could be a major cause of the present demands for increased feedback from students following such assessments.

Respiratory physiology at the University of Birmingham is now taught to 350 year 1 MBChB students entirely using a ‘flipped classroom’ approach (Mazur, 2009). Students are provided with lecture podcasts (Panopto) via the VLE that they watch in their own time before attending non-didactic, question and answer sessions using interactive response technology (Turning Technologies). These sessions utilise peer teaching and lecturer-led explanations to test each student’s understanding of key concepts and to provide them with immediate, individualised feedback.

The flipped classroom is different from other lectures, requiring greater student input and effort before and during the lecture sessions and in order to stretch the most able students without undermining the confidence of other students, we found it necessary to provide a range of question difficulty. Our evaluation strongly suggests that this methodology is successful and that students appreciate the opportunities it provides and data collected over the past 2 years shows a trend towards greater appreciation of this methodology (see table 1).

The students received follow-up respiratory physiology SGT. Since the introduction of the flipped classroom these sessions have been able to focus more on the application of their knowledge in the context of clinical case scenarios, likely due to a higher level of core knowledge and understanding achieved prior to the SGT. We believe that this approach supports student learning by providing targeted and individualised feedback by enabling lecturers to tailor teaching towards the correction of misunderstandings.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Nesfatin-1 alleviates acetic acid-induced gastric ulcer healing via the activation of cyclooxygenase (COX) pathway

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We have previously shown that nesfatin-1 exerts neuroprotective and anti-apoptotic effects, and alleviates indomethacin-induced acute gastric ulcer¹,²,³. We aimed to investigate the anti-inflammatory effects of nesfatin-1 in a chronic gastric ulcer model and the involvement of cyclooxygenase (COX) pathway in nesfatin-1-affected gastroprotection. Experimental protocols were approved by the Marmara University Animal Ethics Committee. Under anesthesia (100 mg/kg ketamine+10 mg/kg chlorpromazine; intraperitoneally, ip) fasted male Sprague Dawley rats (250-300g) underwent a midline laparotomy, and half a milliliter of acetic acid (80%; ulcer group) was applied on the serosal surface of stomachs for 1 min. Control or ulcer groups were treated daily with either ip saline or nesfatin-1 (0.3 mg/kg) or non-selective COX inhibitor indomethacin (5 mg/kg) for 3 days. Rats were decapitated at the end of third day and their trunk blood was collected for the measurements of TNF-α, IL-1β and IL-10 using ELISA. Gastric lesions were scored macroscopically, and gastric samples were analyzed for levels of malondialdehyde (MDA; indicator of lipid peroxidation), nitric oxide, sensory nerves and vanilloid receptors. Peptides. 2013;49:9-20.


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

C48

Beta-catenin regulates KCNQ1 potassium channel expression in colon cancer cells

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The potassium channel KCNQ1 has been recently identified as a tumor suppressor in mouse and human colorectal cancer (CRC) tissues and as a β-catenin chaperone at the plasma membrane [1,2]. The transcriptional factor 4 TCF4 forms a complex with its transcriptional coactivator beta-catenin and plays important roles in carcinogenesis of colon epithelium [2,3]. We have investigated the role of KCNQ1 association with the β-catenin:TCF4 complex in colon epithelial cancer cell lines to get an insight into their contribution to the phenotype of tumour cells. We detected KCNQ1, E-Cadherin and N-Cadherin proteins using Western blotting. Beta-catenin and KCNQ1 immunostaining was used to determine their protein localisation. Beta-catenin:TCF4 complex and KCNQ1 associations were tested using pharmacological agents, and by KCNQ1 siRNA (SiQ1) and dominant negative TCF4 plasmid transfection (DNTCF4). Values are means ± S.E.M., compared by ANOVA.

In a human CRC patient database (n=290), the Kaplan relapse-free survival data showed that KCNQ1 expression was correlated with a better survival and that there was an inverse correlation between the expression of KCNQ1 and CTNNB1 (beta-catenin) genes. We observed that KCNQ1 was highly expressed in well-differentiated CRC cell lines and this was associated with a high expression of E-cadherin and a low expression of N-cadherin. Two inhibitors of Glycogen Synthase Kinase 3-Beta (GSK3- β), AR-A014 and GSK-3βiX, were used to activate beta-catenin in well-differentiated CRC cell lines. These inhibitors produced a reduction in KCNQ1 protein expression in HT29 cells (AR-A014: 52.91±7.3, n=5 p<0.05;
GSK-3iX: 55.8±10.58, n=4, p<0.05) and in C119A cells (AR-A014: 52.07±7.8, n=5 p<0.01; GSK-3iX: 41.8±9, n=4, p<0.01; also caused a reduction in the KCNQ1 staining intensity and in the beta-catenin nuclear localisation. The KCNQ1 expression was restored in the intermediate CRC differentiated DLD-1 cell line transfected with DNTCf4 plasmid (55±5.94, n=3, p<0.001). In C119A cells, the wound closure rate was decreased after using GSK-3iX treatment and the N-cadherin expression was increased after 24h and 48h of injury (n=3). In DLD-1 cells transfected with DNTCf4 plasmid, we observed an enhanced KCNQ1 expression (321±14 n=3, p<0.005) and an increased rate of wound closure 24h and 48h after injury. SiQ1 transfection and KCNQ1 pharmacological inhibition using HMR and C293B resulted in beta-catenin accumulation in the cytosol and a reduction in the rate of wound closure 24h after injury. In conclusion, the β-catenin:TCF4 complex represses KCNQ1 expression in colonic CRC cell lines. The expression as well as the function of KCNQ1 are directly associated with CRC patient survival, wound repair and cell differentiation processes. Our results indicate KCNQ1 as a promising new therapeutic target for the treatment of colorectal cancer.


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

Distinct mechanisms govern hormonal stimulation of the epithelial Na⁺

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Sodium absorption in the aldosterone-sensitive distal nephron is regulated by both steroid and peptide hormones. These stimulate sodium transport via the epithelial sodium channel (ENaC) by increasing both channel activity and number of channels at the apical surface [1]. A P38 kinase-mTORC2-SGK1 pathway has been implicated in both the rapid effects of the peptide hormone insulin, as well as the delayed responses elicited by steroid hormones [2]. The aim of this study was to compare the mechanisms by which these hormones stimulate ENaC-mediated Na⁺ absorption. Na⁺ transport via ENaC was measured by recording the aminolide-sensitive current (I₃₆₅₉) across mtkCCD₄₉ cortical collecting duct cells mounted in Ussing chambers. Individual components of the signalling pathway of interest were assessed by monitoring phosphorylation of downstream target proteins using Western blot analysis of whole cell lysates. qRT-PCR was used to monitor SGK1 mRNA abundance. Values are means ± S.E.M., compared by unpaired t-test. Dexamethasone (dex) stimulated I₃₆₅₉ from baseline (∆I₃₆₅₉) by 19.6±2.6 μAcm⁻² after 3h compared to control 1.5±1.9 μAcm⁻² (n=7, p<0.001). This was associated with a 42.7±8.8 fold increase in SGK1 mRNA abundance (n=6, p<0.001). Phosphorylation of downstream target of SGK1, NDRG1-Thr³₄₅, increased by 4.1±0.4 fold compared to control (n=10, p<0.01). However, phosphorylation of AktThr²₉₈ (n=9) and AktSer⁴₇₃ (n=5) remained unchanged following exposure to dex, indicative that PI3K/mTORC2 activity was unaltered. An inhibitor of protein translation cycloheximide (CHX) reduced the effect of dex on I₃₆₅₉ by 48.5±22.4 % (n=7), without altering the associated increase in SGK1 mRNA, 41.9±12.5 fold (n=6). After 1h exposure to insulin, ∆I₃₆₅₉ was stimulated by 6.8±1.7 μAcm⁻² compared to control 2.0±0.7 μAcm⁻² (n=6, p<0.01). SGK1 activity was increased indicated by a 1.7±0.1 fold increase in NDRG1-Thr³₄₅,3₆₆,3₃₆ expression (n=11, p<0.001). PI3K/mTORC2 activity was also increased with a 1.8±0.2 fold increase in Akt-Thr²₉₈ (n=17, p<0.01) and 1.7±0.1 fold increase in Akt-Ser⁴₇₃ (n=16, p<0.001) phosphorylation, respectively. CHX did not alter the stimulation of I₃₆₅₉ by insulin (n=6), however CHX reduced phosphorylation of all target proteins monitored, indicating reduced PI3K/mTORC2/SGK1 activity. Together these data indicate that dexamethasone stimulates ENaC-mediated Na⁺ transport by increasing transcription of SGK1 without increasing PI3K/mTORC2 activity. Insulin on the other hand increases PI3K/mTORC2/SGK1 activity, but does not evoke transcription of SGK1. Insulin can however still stimulate ENaC-mediated Na⁺ transport with significantly reduced activity of all components of this signalling pathway, suggesting either another signalling molecule is involved or that only a low level activity of this cascade is required to permit a response.


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C03

CO₂-dependent stimulation of Na⁺/[HCO₃⁻] cotransporter is a mechanism to facilitate recovery from CO₂-induced intracellular acidosis in human airway epithelia

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Efficient regulation of intracellular pH (pHᵢ) is of paramount importance for all human cells. Large changes in pHᵢ can elicit a wide range of changes in physiological processes, including protein synthesis, DNA replication and ion transport and, consequently, cells must possess mechanisms by which they can resist changes in pHᵢ. Diffusion of CO₂ from the blood to epithelia places an acid load on the cells, a process which is augmented in patients with lung disease who exhibit a high partial pressure of CO₂ in arterial blood (P CO₂, hypercapnia). We sought to assess the effect of hypercapnia on the pHᵢ regulatory mechanisms of a model of human airway epithelia. Calu-3 cells were loaded with the pH-sensitive dye, BCECF-AM, and pHᵢ was measured by fluorescent microscopy. Cells were initially perfused with solutions gassed with 5% CO₂ (normocapnia). Application of 10% CO₂ (hypercapnia) to both the apical and basolateral membrane caused a rapid, intracellular acidosis of 0.24 ± 0.02 units (n=6). Cells were able to recover pHᵢ from intracellular acidosis; a response which took ~20
mins. We observed that pH recovery in the absence of basolateral Na+ was -10.5 ± 21.4% of control (n=5; p<0.001) yet pH recovery in the presence of the Na+/H+ exchanger inhibitor EIPA (3μM) remained at a substantial 85.0 ± 8.4% of control (n=4; p>0.05) whilst the H+ channel inhibitor ZnCl2 (100μM) also did not significantly alter pH recovery (n=3; p>0.05). Conversely, the Na+/HCO3− cotransporter (NBC) inhibitor DIDS (100μM) caused a 56.7 ± 3.8% decrease in pH recovery (n=3; p<0.05). Together, these data implicated Na+-dependent HCO3− influx, as opposed to Na+-dependent H+ efflux, underlied pH recovery. Interestingly, BAPTA-AM (50μM), the phospholipase C inhibitor U73122 (10μM) and intracellular Ca2+ store depletion all reduced pH recovery by 63.1 ± 6.8% (n=5; p<0.001), 75.8 ± 9.0% (n=3; p<0.001) and 74.8 ± 8.1% (n=4; p<0.05) respectively, implying a Ca2+-signalling component was also involved in mediating pH recovery. End-point PCR revealed that both SLC4A4 (NBCe1) and SLC4A7 (NBCn1) were expressed in Calu-3 cells. Upon measuring NBC activity per se, we observed a 2.7 ± 0.3 fold increase (n=5; p<0.01) in activity in hypercapnic conditions. These findings suggest that CO2 stimulates an increase in NBC activity to facilitate increased HCO3− influx; a mechanism by which cells can maintain intracellular pH from CO2-induced acidosis. This work further reinforces the role of CO2 as a cell signalling molecule in human epithelia and demonstrates the adaptive mechanisms by which cells are able to maintain intracellular pH in response to acid loads.

Work supported by an MRC Studentship awarded to MT

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Airway epithelial cells secrete bicarbonate in response to acidic by-products of Pseudomonas aeruginosa metabolism

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The pH of the airway surface liquid (ASL) is tightly regulated to maintain optimal conditions for clearance of pathogens. In Cystic Fibrosis (CF), ASL pH is more acidic, thought to be due to reduced bicarbonate secretion1. This leads to impaired activity of antimicrobial peptides and defective bacterial killing in animal models of CF lung infections1. In addition ASL bicarbonate concentrations are raised in CF and further in CF-related diabetes, which is associated with increased colonisation with P. aeruginosa.2,3 The aim of this study was to investigate how the airway epithelium responds to acidic metabolic by-products produced by P. aeruginosa (PA01), a key pathogen in CF lung disease, and whether this is altered in CF cultures with and without hyperglycaemia. ASL pH and bacterial growth was monitored across primary human non-CF and CF bronchial epithelial cells (HBE) and Calu-3 cell line, cultured at air-liquid-interface, over 6 hours. Calu-3 and non-CF HBE cells were able to maintain ASL pH in the presence of P. aeruginosa (n=6). However ASL pH across CF HBE cells became more acidic after P. aeruginosa addition (decreased by 0.3 pH units after 6 hours, p<0.05, n=5). This acidification was enhanced by increasing the glucose content of the bathing solution (from 5 to 15mM), which correlated with increased P. aeruginosa growth (18±4% increase, p<0.05, n=5). ASL acidification in the presence of P. aeruginosa could be replicated in non-CF HBE and Calu-3 cultures by replacing bicarbonate with a HEPES-buffered bathing solution in order to limit epithelial bicarbonate secretion. Similar to CF cultures, increasing glucose led to a greater ASL acidification across infected non-CF HBE cells in the presence of HEPES (p<0.05, n=5). Replacing bicarbonate with HEPES increased P. aeruginosa growth across all cell types and growth was further enhanced by raising glucose concentration of the bathing solution (p<0.01, n=5). These results indicate that P. aeruginosa metabolic by-products stimulate airway epithelial bicarbonate secretion in order to maintain ASL pH optimal for bacterial killing. This process is impaired in CF epithelium leading to ASL acidification and bacterial growth both of which are enhanced by elevation of ASL glucose concentration. Overall the data are consistent with a critical role for CFTR-dependent bicarbonate secretion in airway innate immunity and the suppression of pathogen growth.


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Insulin requires A1 adenosine receptors expression to restore human fetal endothelial function in gestational diabetes

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Reduced adenosine uptake via human equilibrative nucleoside transporters 1 (hENT1) in human umbilical vein endothelial cells (HUVECs) from gestational diabetes (GD) is reversed by insulin by restoring hENT1 expression (Westmeier et al. 2015). In addition, GD-increased L-arginine transport via the human cationic amino acid transporter 1 (hCAT-1) is modulated by insulin in this cell type (Guzmán-Gutiérrez et al. 2012). Insulin modulation of L-arginine transport depends on expression and activation of A1 adenosine receptors (A1AR) (Guzmán-Gutiérrez et al. 2012). We studied whether the reversal of the associated effects of GD on transport by insulin depends on adenosine receptors expression and activation. hENT1 and hCAT-1 expression (mRNA number of copies, protein abundance) and activity (transport kinetics), and A1AR, A2AR and IR-A/IR-B expression in primary cultures of HUVECs from normal (n = 68) or GD (n = 68) pregnancies were assayed. Overall adenosine (0.15-500 μM adenosine) and L-arginine (0.75-1000 μM L-arginine) transport was measured in the absence or presence of 1 μM S-(4-nitrobenzyl)-6-thio-inosine (NBTI, ENT1 inhibitor), 2 mM hypoxanthine (ENT2 substrate), or both. Experiments were in A1AR, A2AR, IR-A, IR-B and IR-A/B knockdown cells in the absence or presence of insulin (0-10 nM, 8 h), CPA (A1AR agonist, 30 nm), DPCPX (A1AR antagonist, 30 nm), CGS-21680 (A2AR agonist, 30 nm), ZM-241385 (A2AR antagonist, 10 nM). Values are mean ± SEM (n = 27-31 different cell cultures). Results show that GD associates with higher mRNA and protein abundance of hCAT-1 (1.9 ± 0.3 and
Transcranial direct current stimulation improves isometric time to exhaustion performance of lower limbs

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Supraspinal fatigue is defined as the inability of the motor cortex (M1) to produce an adequate neural drive to excite and drive motoneurons adequately, and could contribute to the decrease in force production capacity (2). Recently, research studies have applied the use of transcranial direct current stimulation (tDCS) to manipulate corticospinal excitability in order to improve endurance performance (1). These interventions can be inhibitory (cathodal) or excitatory (anodal). Since there is no consensus on the standard placement of electrodes for improving endurance performance, we therefore tested the effect of two electrodes configurations.

Nine subjects underwent a control (CON), placebo (SHAM) and two different tDCS configurations sessions in a double blind, randomised and counterbalanced design. In one tDCS session, the anodal electrode was placed over the left M1 and the cathodal on contralateral forehead (HEAD) while for the other montage, the anodal electrode was placed over the left M1 and cathodal electrode above the contralateral shoulder (SHOULDER). tDCS was delivered for 10 min at 2.0 mA, after participants performed an isometric time to exhaustion (TTE) of the right knee extensors at 20% of the maximal voluntary contraction (MVC). Peripheral and central parameters were examined respectively by femoral nerve stimulation and M1 excitability via TMS at baseline, after tDCS application and immediately after TTE. Heart rate (HR), ratings of perceived exertion (RPE), and leg muscle PAIN were monitored during the TTE.

A one-way ANOVA with repeated measures was used to assess TTE duration, while two-way ANOVA with repeated measures was used to analyse central and peripheral parameters, HR, PAIN, and RPE.

None of the central and peripheral parameters showed any difference between conditions after tDCS stimulation (p>0.05). MVC significantly decreased after TTE (p<0.05) due to presence of central and peripheral fatigue, whilst motor evoked potential area (MEP) and cortical silent period increased after TTE (p<0.05) independently of the experimental condition. TTE was longer in the SHOULDER condition (p<0.05) although HR and PAIN did not present any difference between conditions (p>0.05). However, RPE was significantly lower in the SHOULDER condition (p<0.05). This is the first study showing an improvement of isometric TTE performance of the lower limbs after tDCS stimulation and further demonstrates that anodal tDCS over M1 improves isometric endurance performance of the knee extensors. Our findings suggest that SHOULDER montage is more effective than HEAD montage to improve endurance performance.


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gas exchange. Following confirmation of distribution of normality (Shapiro-Wilk Tests), data were analysed using independent sample t-tests.

Results: By design, the trained participants had a higher \( VO_{2\text{MAX}} \) (38 ± 6 vs. 22 ± 3 ml.kg\(^{-1}\).min\(^{-1}\); \( P < 0.05 \)) and subsequently a lower cPfPWV (Figure 1; \( P < 0.05 \)). Collectively, this translated into improved cerebral perfusion and better memory when assessed using the cued recall component of RAVLT (Table 1; \( P < 0.05 \)).

Conclusion: Our findings provide evidence of a link between reduced aortic stiffness, improved cerebral perfusion and ultimately the preservation of memory in older adults. This highlights the importance of maintaining good vascular health as a means of preserving memory with advancing age.

Table 1: Cerebral perfusion and memory performance

<table>
<thead>
<tr>
<th></th>
<th>Trained</th>
<th>Untrained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rey Auditory Verbal Learning Test (AVL; n)</td>
<td>42 ± 4</td>
<td>44 ± 10</td>
</tr>
<tr>
<td>Rey Auditory Verbal Learning Test (AVL; t)</td>
<td>1 ± 21</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>Repetition of Digits Forwards (s)</td>
<td>8 ± 2</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>Repetition of Digits Backwards (s)</td>
<td>6 ± 2</td>
<td>6 ± 1</td>
</tr>
</tbody>
</table>

Mean ± SD. n, number correct; † = \( P < 0.05 \) vs. untrained.

Figure 1: Aortic Stiffness.

Mean ± SD. † = \( P < 0.05 \) vs. untrained.

Hanon et al. (2005) Stroke; 36, 2193-2197.


Tarumi et al. (2013) J Hypertens; 31, 2400-2409.


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

Oral Communications

C56

Plasma nitrite and S-nitrosylhaemoglobin exchange across the human cerebral and femoral circulation; regulation by hypoxia and exercise

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Background and Aims: Hypoxic vasodilatation is a conserved physiological response that couples blood flow and oxygen delivery to tissue metabolic demand. Regulatory roles for the nitrite anion (NO\(_2^-\)) and/or S-nitrosylhaemoglobin (HbSNO) have been widely contested given their ability to conserve and transduce nitric oxide (NO) bioactivity (Haldar et al., 2013).

To provide further insight, we determined to what extent hypoxia and exercise influence local exchange measured across the cerebral and femoral circulation.

Methods: Ten participants completed a normoxic and hypoxic trial (10% F\(_O_2\)) with measurements performed at rest and following 30 min of cycling at a fixed power output equivalent to 35% of the maximum achieved in normoxia. Blood samples were obtained from the brachial artery (BA) and internal jugular (JV) and femoral (FV) veins. Plasma and red blood cells (RBC) were assayed for NO\(_2^-\) and HbSNO via ozone-based chemiluminescence (Bailey et al., 2010). Cerebral blood flow (CBF) was determined from transcranial doppler ultrasound and femoral venous blood flow (FBB) by constant infusion thermodilution. Net exchange was calculated as plasma or RBC flow × (JV or FV – RA). Data were analysed with three factor repeated measures ANOVA and Bonferroni-corrected paired samples t-tests.

Results: Both CBF and FBB were elevated during hypoxia with only the latter shown to respond to exercise (Table). Hypoxia reversed NO\(_2^-\) uptake resulting in net output with the greatest increase observed across the femoral circulation during exercise. Exercise elevated HbSNO output with the greatest increases confined to the femoral circulation. Both hypoxia and exercise-induced increases in FBF were associated with increased HbSNO output (\( r = 0.68-0.72, P < 0.05 \)) but not NO\(_2^-\) (\( P > 0.05 \)) output.

Conclusions: The present findings provide clear evidence for local NO metabolite formation and protected transport across the circulation proportional to the increase in tissue perfusion. The net output as opposed to loss of NO\(_2^-\), the latter traditionally taken to reflect consumption and corresponding liberation of NO, favours HbSNO as the more likely species responsible for transferring bioactivity.

<table>
<thead>
<tr>
<th>Site</th>
<th>Cerebral Flow</th>
<th>Femoral Flow</th>
<th>Plasma NO(_2^-)</th>
<th>RBC HbSNO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>Rest</td>
<td>Exercise</td>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>Normoxia</td>
<td>45 ± 6</td>
<td>43 ± 6</td>
<td>29 ± 14</td>
<td>211 ± 98</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>57 ± 17</td>
<td>57 ± 9</td>
<td>36 ± 16</td>
<td>254 ± 116</td>
</tr>
</tbody>
</table>

Sil: Site; Condition: Site x State (\( P < 0.05 \))

Values are mean ± SD; * different compared to rest for given site and condition; † different between sites for given condition and state; ‡ different between conditions for given site and state (all \( P < 0.05 \)).
Near-infrared spectroscopy versus transcranial doppler ultrasound for assessing dynamic cerebral autoregulation in clinical sepsis

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Near-infrared spectroscopy (NIRS) has been highlighted as a potentially useful non-invasive clinical tool for continuously monitoring dynamic cerebral autoregulation in sepsis, which is associated with a progressive impairment of cerebral haemodynamic function (Steiner et al, 2009). In the present study, we compared NIRS- to transcranial Doppler ultrasound (TCD)-based assessments of dynamic cerebral autoregulation in patients with severe sepsis.

We included ten mechanically ventilated patients (mean age 62, SD 10; 2 females) diagnosed with severe sepsis in the study. Dynamic cerebral autoregulation was assessed by transfer function analysis of spontaneous fluctuations in invasive mean arterial blood pressure and cerebral blood flow, measured by simultaneous ipsilateral and TCD. Bland Altman plots were constructed to compare TCD- and NIRS-based transfer gain and phase estimates in the low frequency range (0.07-0.20 Hz).

A total of fifteen simultaneous NIRS- and TCD-based assessments of dynamic cerebral autoregulation were obtained in the patients. Bland-Altman plot showed a relative bias of 1.20 cm mmHg⁻¹ sec⁻¹ with limits of agreement of -0.69 to 3.09 cm mmHg⁻¹ sec⁻¹ for transfer gain, and a relative bias of 0.29 radians with limits of agreement of -3.59 to 4.17 radians for phase (Figure). We found a poor agreement between NIRS- and TCD-derived estimates of dynamic cerebral autoregulation in sepsis. In particular, higher TCD-based gain values, which indicate impaired dynamic cerebral autoregulation, were not reflected by correspondingly high NIRS-derived values. This may be related to contamination of the NIRS signal by extracranial tissues, particularly blood flow through the scalp vessels (Toksvang et al, 2014). Our findings thus question the applicability of NIRS for monitoring dynamic cerebral autoregulation in sepsis.


Age, aerobic fitness and cerebral vascular reactivity to carbon dioxide

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Age is a major risk factor for cerebral vascular disease. Maintaining a high cardiorespiratory fitness has been proposed as a means of improving cerebral vascular health. Age and low cardiorespiratory fitness have been associated with reduced cerebral blood flow, however their influence on the cerebral vasodilatory response to carbon dioxide (CVR\textsubscript{CO2}) is debated. Using Doppler ultrasonic of the extracranial arteries, we sought to test the hypothesis that global cerebral blood flow and CVR\textsubscript{CO2} are higher in young and old individuals who are aerobically conditioned, compared to their unconditioned counterparts.

Eleven young aerobically conditioned (22±1 yr, VO\textsubscript{2max}: 65.3±0.9 mLkg⁻¹min⁻¹), ten young unconditioned (25±2 yr, VO\textsubscript{2max}: 51.1±1.3 mLkg⁻¹min⁻¹), eight older aerobically conditioned (65±1 yr, VO\textsubscript{2max}: 40.9±3.0 mLkg⁻¹min⁻¹) and nine older unconditioned (67±2 yr, VO\textsubscript{2max}: 30.4±1.2 mLkg⁻¹min⁻¹) individuals were recruited. Internal carotid (ICA) and vertebral (VA) artery blood flow were determined using Doppler ultrasound, along with middle cerebral artery mean flow velocity (MCA V\textsubscript{mean}; transcranial Doppler), mean arterial pressure (MAP; Finometer) and the partial pressure of end tidal carbon dioxide (P\textsubscript{ET}CO2; capnography) throughout. CVR\textsubscript{CO2} was determined using a hypercapnic test protocol whereby carbon dioxide was added to the inspired air to achieve a P\textsubscript{ET}CO2 of +1.5 and +6.5 mmHg above rest, while ICA blood flow and MCA V\textsubscript{mean} were recorded.

Resting bilateral ICA and global cerebral blood flow were higher in aerobically conditioned individuals (~33% and ~23% respectively, P<0.05 vs. unconditioned), with no significant effect of aerobic conditioning observed. In contrast, MCA V\textsubscript{mean} was reduced with age (60.7±2.2 cms⁻¹ young vs. 42.2±2.4 cms⁻¹ old, P<0.05) with no significant effect of aerobic conditioning observed. All groups exhibited similar increases in P\textsubscript{ET}CO2 (~4 mmHg) and MAP (~4 mmHg) during the hypercapnic test protocol. ICA and MCA CVR\textsubscript{CO2} were not different in the young and old, aerobically conditioned and unconditioned groups. These findings suggest that aerobic conditioning enhances global cerebral and bilateral ICA blood flow in both young and older individuals, but neither age nor aerobic conditioning impact upon cerebral vasodilatory capacity.

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

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The roles of exercise, heat and dehydration in exercise-induced hypotension and hypervolaemia

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Exercise and exogenous heat each stimulate multiple adaptations, but the role of the related stressor, dehydration, is unclear. While severe hypohydration potentially “silences” the long-term heat acclimated phenotype (in rats) [1], mild-to-moderate dehydration may stimulate cardiovascular adaptations [2, 3]. We tested the hypothesis that exogenous heat stress and dehydration can additively potentiate short-term haematological adaptations to exercise.

Five male and five female volunteers (mean ± SD: 173 ± 11 cm; 72.1 ± 11.5 kg; 24 ± 3 y) completed three trials in randomised order, involving 90-min orthostatically-stressful calisthenics, in: i) temperate (~22°C, 50% rh; Con); ii) exogenous heat (~40°C, 60% rh) whilst euhydrated (Hot+Euhy), and iii) exogenous heat with dehydration (12 h prior fluid restriction then permissive dehydration; Hot+Dehy). Time of day, menstrual phase, and activity levels were standardised. Measures recorded during exercise and intermittently until 24 h thereafter were rectal temperature, heart rate, mean arterial pressure (MAP) and Δ plasma volume. Plasma erythropoietin conc. (EPO) was measured before exercise and at 0 and 2 h after exercise.

Compared to Con, core temperature tended to rise more in Hot+Euhy (by 0.48°C; 95% Cl: 0.06 – 1.01°C; Cohen’s d=2.1; p=0.07), and more so with dehydration (by 0.47°C for Hot+Dehy vs. Heat+Euhy) 0.18 - 0.76°C; d=3.8 p=0.007; from ANOVA). Heart rate did likewise: (mean ± SD) +32 ± 7, +80 ± 30 and +95 ± 27 b/min for Con, Hot+Euhy, and Hot+Dehy respectively (all p<0.05). Post-exercise hypotension at 24 h was larger with the addition of heat (by 3 mm Hg for Hot+Euhy vs. Con; 1 – 5 mm Hg; d=0.9; p=0.03), and more so with dehydration (by 2 mm Hg for Hot+Dehy vs. Hot+Euhy; 1 – 3 mm Hg; d=2.1; p=0.008). Similarly, the hypervolaemic response at 24 h was larger with heat (by 4% for Hot+Euhy vs. Con; 1.9 – 6.9%; d=0.7; p=0.01), and more so with dehydration (by 8.8% for Hot+Dehy vs. Heat+Euhy; 3.2 – 14.3%; d=0.9; p=0.006). EPO was unchanged from baseline in any trial: 0.5 – 3.2; – 1.0 – 3.2; – 1.3 μU/mL change in Con. from baseline to post, and 2 h respectively; Δ-EPO =0.9 ± 2.1; – 2.8 – 0.9; and -1.6 ± 1.2; – 2.7 - 0.6 μM/L change in Heat+Euhy from baseline to post, and 2 h respectively; and 0.7 ± 2.0; – 0.6 – 2.1; and 0.2 ± 4.1; – 2.6 – 3.1 μM/L change in Heat+Dehy from baseline to post, and 2 h respectively. Trials were pooled for correlational analysis. The rise in core temperature was strongly and negatively related to the hypotension at 24 h (r = -0.7; p<0.0001), which was similarly related to the hypervolaemia at 24 h (r = -0.7) The rise core temperature was also strongly and positively related to this hypervolaemia (r = 0.7).

In conclusion, transient dehydration appeared to potentiate haematological (hypervolaemic) and cardiovascular (hypotensive) responses following exercise.


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Transient receptor potential canonical channels regulate body weight gain in hypercholesterolaemic mice

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Obesity is a major worldwide public health problem and there is a need to find new targets for the development of new treatments. Transient Receptor Potential Canonical (TRPC) channels are a family of cationic ion channels thought to have important roles in calcium signalling. We previously showed that TRPC channels are expressed by adipocytes and that inhibition of these channels increased the concentration of circulating adiponectin, an anti-inflammatory adipokine, without effect on leptin (1). The aim of this study was to investigate if there are implications of TRPC channels when there is metabolic stress. Mice with conditional global expression of dominant-negative ion pore mutant TRPC5 (DNTRPC5) were used to suppress ion permeation in TRPC channels in vivo in adults without deleting TRPC proteins. The study was carried out on ApoE-knockout mice which develop hypercholesterolaemia. Mice were fed with western-style high fat diet for 12 weeks and male litter-mates were compared with and without suppression of TRPC ion permeation. The results are expressed as mean±SEM and compared using Student’s t-test. The difference was considered statistically significant when the probability (P) was less than 0.05.

Analysis of adipose tissue showed that adiponectin mRNA was more abundant in the mice expressing DNTRPC5. mRNA of the pro-inflammatory cytokine TNFα was less abundant in adipose tissue of mice expressing DNTRPC5. These results suggest that TRPC channels are regulators of adiponectin and TNFα gene expression in adipocytes and that blocking TRPC channel could have an anti-inflammatory effect. Mice expressing DNTRPC5 showed significantly lower body weight as compared to controls after 6 weeks and 12 weeks of treatment (at 6 weeks: controls=35.8±1.46g, DNTRPC5=31.9±0.65g, P=0.036; at 12 weeks: controls=46.7±1.69g, DNTRPC5=40.1±0.99g, P=0.006; n=8-7). No obvious adverse effects on health were evident. Overall, this study suggests that ion flux through TRPC channels is a driver for weight gain and that ion pore blockers of TRPC channels could be an approach for protecting against obesity and related inflammation.

All procedures were carried out with ethical approval under UK Home Office license.


Support was received from the Wellcome Trust and the Medical Research Council.

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Downregulation of liver DNMT1 is associated with abnormally high adiponectin in a subset of obese patients: from clinical observation to human in vitro models

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Our group is working on the development of a human in vitro model to study epigenetic dysregulations in a personalized manner. Our goal is to identify secretory biosensors of the epigenome that could be used both in vivo and in vitro. Human in vitro models loaded with differentiated mesenchymal stem cells depend on reliable biosensors to study changes in the human epigenome (Shuler, 2012). We hypothesize that the liver is a major target organ of adiponectin the most abundant peptide hormone expressed in adipose tissue. And as a consequence the differential expression of liver target genes that regulate glucose and insulin homeostasis as well as fatty acid oxidation appear to be controlled by the adipose-liver signaling interactions. DNMT1 is a key regulator of normal liver function (Raggi, et al., 2014). CD14 is a peptide antigen associated with microvesicles/exosomes (Kranendonk, et al., 2014), which are important transportation vehicles for proteins and microRNAs in the human body. This in vivo and in vitro study is compliant with ethics review, as an IRB approved clinical trial (GHS # 1207-27). Subjects enrolled were patients scheduled to undergo gastric bypass surgery for weight loss; all donors (n=47) signed informed consent. Expression profiles of liver DNMT1, CD14 and GAPDH followed the MIQE guidelines. Fasting plasma was used for the assessment of plasma total adiponectin and CD14, as well as for the isolation of mature microRNAs. Differentiated mesenchymal stem cells (MSCs) isolated from the same donors are being used in an attempt to study cell signaling interactions in vitro (Stock, et al., 2010). Spearman’s correlation analysis reveal an inverse significant association between liver DNMT1 and liver CD14 (r=−0.5501, p=0.0180, n=18) as well as for plasma adiponectin (r=−0.7614, p=0.0002, n=18). To the best of our knowledge, this is the first report of the association between microRNA’s (hsa-miR148, hsa-miR-210, and hsa-miR22) have been identified for their potential regulatory loops with liver DNMT1 and their profiles are under evaluation. In summary fasting plasma total adiponectin, circulating CD14, mature microRNAs as well as liver expression profiles of DNMT1 and CD14 affect a multitude of regulatory signaling pathways that could potentially aid in the elucidation on the onset of obesity and the role of the adipose-liver signaling interactions.


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Ghrelin O-acyl transferase and the growth hormone secretagogue receptor mediate the adipogenic action of unacylated ghrelin in murine bone marrow revealing a novel endocrine mechanism

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Unacylated ghrelin (UAG) accounts for approximately 80% of circulating ghrelin, but does not bind to or activate the receptor for acylated ghrelin (AG), GHS-R1a (1). However, UAG exerts a distinct spectrum of activity, promoting adipogenesis in bone marrow (2), but not abdominal fat (WAT; 3) and inhibiting skeletal growth (4). In the absence of an alternative receptor, we investigated the potential role of GHS-R in mediating these skeletal effects of UAG. As previously shown in rats (2), intra-bone marrow (ibm) infusion of AG and UAG (from an osmotic minipump connected to a tibial ibm catheter implanted under isoflurane anaesthesia) induced adipogenesis in male wild-type (WT) mice, increasing the number of tibial marrow adipocytes from 22±3 cells/field (vehicle-infused; n=5) to 42±1 cells/field (AG; n=5; P<0.001) and 37±3 cells/field (UAG; n=4; P<0.05; all data mean±SEM, compared by 1-way ANOVA and Bonferroni post hoc test). Surprisingly, this effect was abolished in male loxTB-GHS-R (GHS-R-null) littermates, AG- and UAG-treated mice having 100 (n=5) and 88% (n=5) of the adipocytes in vehicle-treated GHS-R-null animals (n=5). Neither AG nor UAG had any effect on tibial epiphyseal plate width (EPW). Immunocytochemistry revealed that, unlike stomach (which expressed the activating enzyme, ghrelin O-acyl transferase (GOAT), but not the adipocyte-specific marker, PPARγ) and intra-abdominal WAT (which expressed PPARγ, but not GOAT), rat tibial marrow adipocytes co-expressed both PPARγ and GOAT. Furthermore, the adipogenic effect of a tibial ibm UAG infusion (as above) was abolished in male G0AT-null mice (41±2 cells/field in vehicle-treated (n=6) vs 48±3 cells/field in UAG-treated (n=6)), compared to a 120% increase in UAG-treated male WT littermates (16±3 in vehicle-treated vs 35±6 in UAG-treated; n=5;6; P<0.05). Again, UAG had no effect on tibial EPW. However, both GHS-R-null and GOAT-null mice displayed elevated marrow adiposity compared to their WT littermates (P<0.01), suggesting that elevated circulating UAG may produce maximal adipogenesis in these mice. ELISA of terminal plasma samples revealed that circulating ghrelin (total) was not elevated in either GHS-R-null or GOAT-null mice.

Thus, our data demonstrate that the adipogenic effect of UAG in bone marrow is dependent upon acylation by GOAT and activation of GHS-R. The ability of cells expressing these proteins to activate UAG prior to signalling through GHS-R represents a novel endocrine mechanism. Indeed, the cell-specific distribution of these proteins in conjunction with the differential uptake mechanisms for AG and UAG may thereby determine the unique activity spectra of these two hormones.


Davies JS et al. (2009) Molecular Endocrinology 23:914-924.


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Metformin treatment in obese pregnant mice reduces hepatic steatosis in the adult offspring

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Introduction: Prevalence of maternal obesity is rising, contributing to increased risk of metabolic disease, such as non-alcoholic fatty liver disease (NAFLD), in adult offspring. Maternal lifestyle changes have little impact on reducing the offspring’s risk, highlighting the need for alternative treatments. Metformin (MET) is used to treat gestational diabetes; it reduces maternal hyperglycaemia and inflammation. However, the impact of MET treatment during obese pregnancy on offspring NAFLD risk is unknown.

Aims: To investigate the effects of MET treatment in obese pregnant mice on expression of genes involved in lipid homeostasis and on the degree of hepatic steatosis in adult offspring.

Methods: Female C57BL6J mice (n=18) were fed a control (C, 7% kcal fat) or a high-fat diet (HF, 45% kcal fat) 6 weeks prior to mating through, pregnancy and lactation. Half of C and HF dams were given MET in drinking water (250mg/kg bodyweight/day) during pregnancy and lactation. Female offspring were weaned onto C or HF diet, creating 8 groups (n=5/8/group): from untreated dams C/C, C/HF, HF/C & HF and from MET-treated dams Cm/C, Cm/HF, HFm/C & HFm/HF. Offspring were killed at 30 weeks of age. A portion of their left livers was fixed and processed for histological examination. The rest was snap-frozen for gene expression analysis. Blinded point counting and Kleiner scoring was used to assess steatosis, inflammation and hepatocyte ballooning in stained liver sections. Expression of genes involved in hepatic de novo lipogenesis (FAS, ACC, ACPG) and fatty acid b-oxidation (CPT1) was measured by quantitative real-time RT-PCR. Data was analysed using a mixed effects model.

Results: Maternal and/or postnatal HF diet increased steatosis in offspring livers. Treating obese pregnant dams with MET reduced steatosis in HFm/C vs C/HF. Offspring from MET-treated lean dams (Cm/HF) had increased hepatic steatosis (2.07 fold, p<0.001) vs C/HF. Maternal HF diet reduced steatosis, inflammation and hepatocyte ballooning in stained liver sections. Expression of these genes involved in hepatic de novo lipogenesis (FAS, ACC, ACPG) and fatty acid b-oxidation (CPT1) was measured by quantitative real-time RT-PCR. Data was analysed using a mixed effects model.

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MET-treated lean dams (Cm/C & Cm/HF) vs those of untreated dams (2.28 fold, p<0.05).

Conclusions: MET treatment in obese pregnant dams offers some protection against offspring developing hepatic steatosis. Thus MET may reduce the burden of maternal obesity on the offspring’s future health. However, MET treatment in lean pregnant mothers predisposes offspring to increased hepatic steatosis. Caution should therefore be taken when prescribing MET to these women.

Funded by Diabetes UK

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Supplementation of the maternal diet with canola oil during lactation leads to increased UCP1 levels in adipose tissue of the offspring

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Background and Aims: Brown adipose tissue (BAT) has been identified as a potential target to combat obesity. This is due to its propensity to expend large amounts of energy via non-shivering thermogenesis, a process that is mediated by Uncoupling Protein (UCP) 1 (1) (1). In sheep, during postnatal development, BAT is rapidly replaced by white adipose tissue, an adaptation determined in part by the maternal metabolic and endocrine environment (2). One dietary factor that may influence this process is the milk content of conjugated linoleic acid (CLA), specifically the cis-9, trans-11 isomer, which has been shown to promote the abundance of UCP1 in vitro (3). The aim of this study was to determine whether maternal dietary supplementation of canola oil, to increase the amount of the cis-9, trans-11 isomer in the milk, would promote the retention of BAT during postnatal development.

Methods: With UK Home Office approval, from the first day of lactation, sheep were fed daily either a standard diet (con) or the same diet plus 3% canola oil (canola; n=8). Each mother raised 2 lambs. Milk samples were collected from each mother at 7 and 28 days of lactation, immediately before one lamb was euthanased with an intravenous injection (Pentobarbital Na, 200mg/kg body weight) and tissue sampled. Samples of perirenal adipose tissue were weighed and fixed in formalin for immunohistochemical (IHC) analysis. Samples were sectioned and stained with UCP1 antibodies. The total area of tissue and the area stained were measured, and the percentage of UCP1 staining calculated. Values are means ± S.E.M compared by Mann-Whitney test (IHC) or unpaired t test (milk analysis).

Results: Supplementation of the maternal diet with canola oil resulted in an increase of cis-9, trans-11 CLA in the milk at 28 days (control, n=8, 6.3±0.5 mg/g fat; canola, n=8, 10.9±0.5 mg/g fat; p<0.0001) but not 7 days of age (control, n=6, 37.8±3.2; canola, n=6, 37.0±3.5).

Conclusions: Supplementation of canola oil during lactation delays the rate of loss UCP1 from perirenal adipose. The mechanisms mediating this response are currently being examined.

C66

The effect of 2 and 14Hz sacral neuromodulation on cortical evoked potentials and polysialated neural cell adhesion molecule expression in a rodent model of pudendal nerve injury

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Severe faecal incontinence, a common and debilitating disorder affecting mostly multiparous women, is routinely treated with sacral neuromodulation (SNM). It is hypothesized that SNM acts upon somatosensory afferent fibres, but its mechanism of action is not yet fully understood. This study aims to elucidate its effects on afferent transmission to the somatosensory cortex by utilizing a model of pudendal nerve injury, designed to mimic the trauma of childbirth(1), and cortical evoked potential (EP) recording. Two stimulation frequencies, the clinically used one (14Hz) and an experimentally derived optimum (2Hz(2)), were tested.

Using 24 urethane (1.5g/kg, i.p.) anaesthetized female Wistar rats anal EPs were recorded by a 32-channel multi-electrode array over the primary somatosensory cortex before, during and after a 60min inflation of 2 retro-uterine balloons(1.5mLs each). Two or 14Hz SNM was applied after balloon deflation (left S1, 10min, 1ms pulse duration, at motor threshold: 4.8V±0.5V). Four groups (N=8 each) were studied: Sham, balloon, 2 and 14Hz SNM. Immunohistochemistry for polysialated neural cell adhesion molecule (PSA-NCAM) was performed on harvested brains (N=4/group) as described by Griffin et al.(3). Data are means±S.E.M. The criterion for statistical significance was P<0.05.

EP amplitude diminished during balloon inflation to -69±6% in balloon, 2 and 14Hz groups. Recovery in the balloon group was partial (-35±2%), while 14Hz SNM increased EPs to (-3%±3%) and 2Hz SNM above the initial level (33%±4%). In the recovery period, EPs in SNM groups were larger than in the balloon group and in the 2Hz group larger than in the 14Hz group (two-way repeated-measures ANOVA, group factor: P<0.0001).

The frequency of PSA-NCAM positive cells per field of view (250μmx250μm) was Poisson distributed. Their number was increased in the right (stimulated) hemisphere of both SNM groups (P<0.0001, Poisson test), but not in the Sham or bal-
loin group (P=0.43, P=0.74 respectively). Their numerical density incl. P values (Student’s t-test) are shown in Table 1. Their location changed after SNM: Unstimulated cortex showed a small cluster in layer IV and stimulated cortices in layer I/II, IV and V (Fig. 1). SNM causes a long term potentiation like phenomenon in the somatosensory cortex. Increased PSA-NCAM expression was seen in layer I/II, IV and V. In electrophysiological experiments 2Hz is more effective in ameliorating diminished EPs than 14Hz SNM, but on a cellular level changes in PSA-NCAM expression were similar for both frequencies.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham group</th>
<th>Bulbar group</th>
<th>14Hz SNM group</th>
<th>2Hz SNM group</th>
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<td>7900/mm² ±1100/mm²</td>
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<tr>
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<td>810/mm² ±90/mm²</td>
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<td>P=0.01</td>
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</tr>
</tbody>
</table>

Figure 1:

A. Anti PSA-NCAM Immunocytochemistry of the anorectal representation in the primary somatosensory cortex. Cell nuclei are stained red (propidium iodine) and PSA-NCAM green.

B. Close up of PSA-NCAM positive cells. Cells where greater than ½ of the nucleus is surrounded by PSA-NCAM are counted as positive.

C. Nissl stain of the somatosensory cortex and location of PSA-NCAM positive cells. Data from the 14Hz SNM group is shown. Note the increase in PSA-NCAM positive

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Plasticity of visual cortex function in an adult mouse model of retinal ganglion cell loss

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Injury to optic nerve (ON) axons plays a major role in glaucoma progression. ON crush is an established model of an acute unilateral axonal injury. The crush results in retrograde degeneration and death of retinal ganglion cells (RGCs). However it is unknown how signal transmission to higher visual structures such as primary visual cortex (V1) is affected. Separate cohorts of C57Bl/6 mice of >8 month old were used for this study. Unilateral ON crush was performed on left eyes under general anaesthesia (2 % isoflurane). V1 function of the contralateral (right) hemisphere was assessed by optical imaging (OI) under anaesthesia (0.8-1.0% isoflurane) of normal adult mice (n=6) and 30min (n=2), 7d (n=3), 14d (n=1) and 30 days (n=3) after ON crush. RGC numbers were quantified by counting Hoechst labelled cells in flat-mounted retinas. An additional group of mice (n=5) were imaged longitudinally by optical imaging (OI) before and at multiple time points up to 60 days after ON crush. We found a significant (p<0.001) cell loss in the RGC layer compared to normal adults after 30 days ON crush. RGC layer cell loss occurred progressively over time with 15% of cells lost after 7 days and 43% of cells lost 14 days after ON crush. OI experiments demonstrate a significant shift in ocular dominance index (p<0.001) from 0.17±0.04 to -0.67±0.12 towards the ipsilateral eye and significant reduction (p<0.001) of signal magnitude in V1 in response to contralateral eye stimulation from 0.79±0.08 in normal adult control to 0.15±0.05 in all ON crush animals. Surprisingly response magnitude to ipsilateral eye stimulation was significantly increased (p<0.05) from 0.55±0.08 to 0.72±0.15 after long term ON crush (n=7). This study shows an acute and permanent loss of signal transmission from the retina to V1 via a crushed ON and much slower RGC loss. Additionally we demonstrate a significant increase of responsiveness in V1 to non-crushed eye stimulation, which indicates that acute and severe injury of the ON in adulthood may evoke plasticity that is normally seen during the critical period of visual cortex development.

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Distinct 5-HT receptors mediate serotonin-induced \([\text{Ca}^{2+}]_i\) responses in astrocytes

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Astrocytes provide neurones with metabolic and structural support. There is also evidence that these numerous glial cells may also play an active role in controlling neuronal excitability, synaptic transmission, plasticity and, therefore, information processing. Astrocytes residing in different parts of the central nervous system are tuned to sense local microenvironment, including neurochemical changes associated with enhanced activities of local and projecting neurones. Previous studies demonstrated that astrocytes show elevations in intracellular \([\text{Ca}^{2+}]_i\) in response to 5-HT (1-3). In this study we aimed (i) to determine whether hippocampal, cerebellar and brainstem astrocytes are equally sensitive to 5-HT; (ii) to identify the receptor(s) which mediate the effects of 5-HT on astrocytes residing in different parts of the CNS. Primary astroglial cultures were prepared from the hippocampus, cerebellum and the brainstem tissue of neonatal rats (P2-3). After 6 days in culture, cells were loaded with a conventional Ca\(^{2+}\) indicator Fura-2 and changes in intracellular \([\text{Ca}^{2+}]_i\) evoked by 5-HT (10 \(\mu\)M) were recorded using fluorescent microscopy. 5-HT receptor agonist and antagonists were used to characterize the identity of 5-HT receptors which mediate the recorded effects of serotonin. Astrocytes cultured from all three brain regions responded to 5-HT with vigorous elevations in intracellular \([\text{Ca}^{2+}]_i\) (EC\(_{50}\) 7-10 \(\mu\)M). Phenylbiguanide (PBG, 5-HT\(_3\) agonist) in concentrations 0.1-0.3 \(\mu\)M mimicked the effects of 5-HT (induced increases in \([\text{Ca}^{2+}]_i\)) in hippocampal astrocytes. PBG had no effect on \([\text{Ca}^{2+}]_i\) in both cerebellar and brainstem astrocytes. 5-HT-evoked \([\text{Ca}^{2+}]_i\) responses in hippocampal astrocytes were completely blocked by 5-HT receptor antagonist granisetron (20 \(\mu\)M), suggesting that only 5-HT\(_3\) receptors are expressed by these cells. \([\text{Ca}^{2+}]_i\) responses elicited by 5-HT in cerebellar and brainstem astrocytes were blocked by phospholipase-C (PLC) inhibitor U73122 (5 \(\mu\)M), suggesting that 5-HT\(_2\) receptors might be involved. The 5-HT\(_3\), antagonist ketanserin (0.01-1 \(\mu\)M) blocked 5-HT-induced \([\text{Ca}^{2+}]_i\) responses in both cerebellar and brainstem astrocytes (but not in hippocampal astrocytes). Neither 5-HT\(_{2C}\) agonist WAY161503 (0.01-5 \(\mu\)M) nor 5-HT\(_{2A}\) agonist BW723C86 (0.001-1 \(\mu\)M) had an effect on \([\text{Ca}^{2+}]_i\), in astrocytes of any of three brain regions studied. These data indicate that astrocytes residing in different parts of the central nervous system express different functional 5-HT receptors. These differences are retained in a highly reduced culture conditions. Hippocampal astrocytes express ionotropic 5-HT\(_3\) receptors, while cerebellar and brainstem astrocytes express G-protein-coupled 5-HT\(_{2A}\) receptors. These data imply that 5-HT actions in the CNS may (at least in part) be mediated via the actions of this transmitter on 5-HT\(_3\) and 5-HT\(_{2A}\) receptors expressed by astroglia.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Recovery of human motoneurones after prolonged voluntary contractions

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Motoneurones often fire repetitively and for long periods. In sustained voluntary contractions the excitability of motoneurones declines (e.g. McNeil et al. 2009; Manning et al. 2010). To understand this better, we mapped the time course of recovery of motoneurones in human volunteers who gave written informed consent to the study. We recorded the discharge of single motor units (n=30) with intramuscular wire electrodes inserted into triceps brachii during weak isometric contractions. Recordings lasted 40-60 min. Subjects (n=15) discharged single motor units at a constant frequency (~10 Hz) with visual feedback for prolonged durations (3-7 minutes) until the rectified surface electromyogram (EMG) of triceps brachii increased by ~100%. After rest intervals which varied from ~2-240 s, subjects resumed the constant-frequency contraction (with 3-4 intervals being tested per motor unit). Compared to baseline, the level of EMG required to re-recruit the target motoneurone at the test frequency increased after the sustained contraction (for rest intervals from 2-60 s, p = 0.001-0.038). This increased EMG indicates that greater excitatory drive was needed to discharge the motoneurone at the test rate. The increased EMG returned to baseline levels with an exponential time course (time-constant 28 sec) and was fully recovered after a rest period of ~240 s. We suggest that the decline in motoneurone excitability in a sustained weak contraction depends on repetitive activation of the motoneurones. The lack of apparent muscle fatigue with weak contractions indicates that the CNS compensates for this decreased excitability.


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BACE1 overexpression induces impaired oxidative metabolism in SH-SYSY cells

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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 research focuses on analysing the brain once hallmark amyloid plaque and tau tangle pathologies are present, their appearance is extremely end stage. In addition, to date, any attempts at alleviating them have failed to halt symptom progression. It may therefore be beneficial to look at earlier events; with reduced glucose metabolism and a regional switch to aerobic glycolysis observed prior to symptom presentation (1, 2). These changes may subsequently precipitate cognitive decline as glucose is a required substrate for induction of long-term potentiation (3) and conversion of short- to long-term memories. Evidence implicates the aspartyl protease β-site amyloid precursor protein cleaving enzyme 1 (BACE1) as a key enzyme in the aetiology of AD. Previous work from our laboratory has also suggested a role in metabolism, with BACE1 knock-out mice displaying enhanced whole body glucose metabolism and insulin sensitivity (4). We have also previously presented that overexpression of BACE1 impairs glucose uptake and use (5). The present study aimed to elucidate the mechanisms underlying this altered glucose handling in SH-SYSY human, neuroblastoma cells stably overexpressing BACE1. 14C-radiolabelled glucose oxidation
and extracellular flux analyses were utilized to assess cellular respiration in real-time. Enzyme assay kits were used to monitor the activity of protein complexes regulating glucose metabolism (pyruvate (PDH), isocitrate (IDH) and α-ketoglutarate dehydrogenases (α-KGDH)). All data are presented as mean ± standard error of the mean and statistical significance determined by Student’s t-test or ANOVA. Chronic elevation of BACE1 resulted in impaired activity of key fuel partitioning enzymes: PDH (reduced to 69 ± 8 per cent, p < 0.01, n = 5), total IDH (reduced to 49 ± 3 per cent, p < 0.001, n = 4) and α-KGDH (reduced to 61 ± 6 per cent, p < 0.01, n = 6). This attenuated enzyme functioning lead to a preferential use of aerobic glycolysis over oxidative phosphorylation for ATP generation (oxygen consumption rate (OCR) reduced to 65 ± 9 per cent, p < 0.01, n = 7 and extracellular acidification rate (ECAR) increased to 165 ± 16 per cent, p < 0.01, n = 7). These deficits in glucose oxidation could however be effectually attenuated through supplementation of the cells with α-lipoic acid or ketone bodies. Taken together these data show that overexpression of BACE1 effectively phenocopies some of the earliest pathophysiological changes seen in the brain during progression towards AD. It also highlights novel targets and supports a potential role for neutraceutical supplementation to alleviate these deficits at the cellular level.

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Identification and anatomy of cardiac pericytes in the NG2-DsRed mouse

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Pericytes are contractile microvascular mural cells, known to have important functions in regulation of the vasculature in health and disease. In the CNS they regulate capillary diameter and initiate an increase in blood flow in response to neuronal activity (Hall et al., 2014). In ischaemia pericytes irreversibly constrict capillaries, contributing to the “no-reflow” phenomenon following stroke. This phenomenon is also observed after myocardial infarction, raising the possibility that myocardial pericytes contribute to ischaemia-evoked constriction at the capillary level (O’Farrell & Attwell, 2014). We investigated the anatomical characteristics of murine ventricular myocardial pericytes to determine the feasibility of a potential role in regulating cardiac capillary diameter and blood flow. Methods: Adult NG2-DsRed mice (n = 5; 3 males, 2 females) were sacrificed using Schedule 1 methods. The hearts were removed and immersion fixed in PFA. Immunohistochemistry was performed on 150 μm transverse sections. Ventricular pericytes were labelled for PDGFRβ and capillaries were labelled with FITC-conjugated isoclin B4. Z-stacks for pericyte quantification were acquired using laser-scanning confocal microscopy and data were analysed using ImageJ software (NIH, Bethesda, MD, USA). Data are presented as mean ± SEM. Results: Pericytes were present in the lateral, septal, and anterior/posterior (not distinguishable in transverse slices) left ventricle (LV) of adult mice at a density of 2.6x10^4/cm^3, 2.5x10^4/cm^3, and 2.5x10^4/cm^3 respectively. The overall LV pericyte density was 2.6x10^4/cm^3. Mouse myocardial pericytes extend NG2- and PDGFRβ-positive processes along and around the surface of coronary capillaries, providing a possible anatomical substrate for control of capillary diameter. In the LV 99.2±0.6% of NG2-DsRed⁺ pericytes were also labelled by anti-PDGFRβ (2874 pericytes analysed in 36 z-stacks). Moreover, 93.2±0.6% of PDGFRβ⁺ pericytes were also NG2-DsRed⁺. Pericytes are as abundant as cardiac myocytes in ventricular tissue. Their high density and anatomical configuration is consistent with them having a role in regulating myocardial blood flow. Use of the NG2-DsRed fluorescent reporter line will enable cardiac physiologists to investigate pericyte physiology and pathology in vivo.


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Protein phosphatase 1 beta regulates focal adhesion turnover and cytoskeletal reorganization in endothelial cell angiogenesis

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Background/Aim: Protein phosphatase 1 (PP1) belongs to a major family of serine/threonine specific phosphatases and is universally expressed in all eukaryotic cells. PP1cβ is a specific isoform of the catalytic subunit of PP1 enzyme and its physiological activity contributes to the normal maintenance of some cellular functions such as endothelial cell barrier protection and DNA repair. We have previously showed that PP1cβ also plays a key role in endothelial cell angiogenesis. In particular, PP1cβ regulates the early stage of cell migration during the angiogenic process, rather than the subsequent phase of tube formation. Since previous studies have reported PP1cβ localization to focal adhesions, we hypothesized that PP1cβ regulation of endothelial cell migration would involve cytoskeletal reorganization through impairment of focal adhesion kinases (FAK) activation. Hence, the mechanism by which PP1cβ regulates endothelial cell angiogenesis was investigated in this study.

Methods. The effect of PP1cβ pharmacological inhibition, in addition to knocking down and overexpressing PP1cβ, on endothelial cell migration and morphogenesis was examined using in vitro wound healing scratch assay. The PP1cβ knockdown effects on F-actin reorganization (phalloidin staining) and focal adhesion formation (vinculin) were evaluated by immunocytochemical staining with specific antibodies.

Results: PP1cβ knockdown significantly reduced endothelial cell migration, with this effect being restored to the control level upon consecutive transfection with PP1cβ cDNA. The mechanism, by which PP1cβ regulates endothelial cell migra-
tion, involves the interplay of actin cytoskeleton proteins and focal adhesion molecules signalling. In particular, PPT1 regulates focal adhesion turnover and actin polymerization pathways.

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C74

Characterisation of the anti-angiogenic effects of two calmodulin-dependent protein kinase II (CaMKII) inhibitors in the retina in vitro and in vivo

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Abnormal angiogenesis underlies vision loss in a number of ocular diseases. Growth factors (GFs) such as vascular endothelial (VEGF), fibroblast (FGF), hepatocyte (HGF) and insulin (IGF) drive ocular angiogenesis (1). Current anti-VEGF therapy or laser photocoagulation are either not effective in all patients or destructive to the retina. We have previously shown that targeting endothelial CaMKII may represent a more effective therapeutic strategy (2), and in the present study we examine the effects of two CaMKII inhibitors on retinal angiogenic signalling in vitro and invivo. GF-induced changes in total and phospho-CaMKII levels in human retinal microvascular endothelial cells (hRMECs) were assessed by Western blotting. The effects of the CaMKII inhibitors, KN93 and CK59 (10μM), on GF-induced EC proliferation, migration, tube formation and sprouting angiogenesis were also evaluated (2). Downstream signalling pathways stimulated by GF activation of CaMKII in ECs were determined using phospho-kinase arrays. In vivo effects of KN93/CK59 were tested using the mouse model of oxygen-induced retinopathy (OIR). OIR was induced in C57BL6 mice as previously described (3). Mice were anaesthetised at P15 with ketamine (60mg/kg, IP) and xylazine (6mg/kg, IP) and given 1ul intravitreal injection of CaMKII inhibitor/control agents. At P17 mice were killed by schedule 1 methods, eyes were enucleated and retinas stained with isolectin for quantification of avascular (AVA) and neovascular areas. Data were analysed by ANOVA. Values are mean±SEM. GFs (VEGF, HGF, FGF and IGF1; 50 ng/ml for 24 hr) increased total and phosphorylated CaMKII protein levels in ECs (n=3). Treatment of ECs with KN93/CK59 reduced the number of angiogenic sprouts formed in vitro assays from 48.0±6.9 to 14.6±2.7 and 29.1±0.7 respectively (p<0.001 in both cases). The inactive analogue of KN93, KN92 (10μM), and vehicle controls (0.01% DMSO) were without effect. A number of kinases were phosphorylated following GF stimulation in a CaMKII-dependent manner including Erk 1/2 and MSK 1/2 and the Src family kinases, Yes and Fgr. In the OIR model, revascularisation of the retina was inhibited by 10μM KN93 (AVA 47.8±2.0%) and CK59 (AVA 50.7±4.7%) treated mice compared to untreated P17 controls (AVA 31.1±1.8%, p<0.001 in both cases). These inhibitors also reduced retinal neovascular tufts (KN93, 11.8±1.3%; CK59, 11.5±1.8%, compared to untreated P17 controls(19.6±2.6%, p<0.05 in both cases). These data strongly suggest that targeting CaMKII may provide a novel therapeutic approach for treating neovascular diseases of the eye.


British Heart Foundation

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C75

VEGF-A165b and inhibitors of SRPK1 prevent monocyte adherence to human synoviocytes in vitro

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Tumour necrosis factor-a (TNFa) is upregulated in inflamed synovium and drives inflammation and pain. Vascular endothelial growth factor-A (VEGF-A) is a family of proteins derived from the vegf-a gene by alternative pre-mRNA splicing. Endogenous control of splicing by activation of serine-arginine-rich protein kinase-1 (SRPK1) increases the expression of pro-angiogenic VEGF-A165. VEGF-A165 increases adhesion molecule (ICAM-1) expression in normal synoviocytes leading to ICAM-1-dependent inflammatory cell recruitment. We tested the hypotheses that anti-angiogenic, anti-nociceptive VEGF-A165b, or control of VEGF alternative splicing in favour of VEGF-A165a by SRPK1 inhibition would reduce monocyte adherence to human synoviocytes in vitro.

Rheumatoid arthritis (RA) and normal human fibroblast-like synoviocytes (HFS, passage 6-12; Sigma) and human THP1 monocytes (passage <30; Sigma) were cultured to confluence. HFS were split into 96 well plates at 12,000 cells/well and incubated for 24h with TNFa 20ng/ml alone or with one of the following: anti-ICAM-1 (100ng/ml); VEGFR inhibitor PTK787 (200nM); VEGF-A165b (0.01-400ng/ml); SRPK1 inhibitors SRPIN340 (5μM, N-[2-(1-piperidinyl)-5-(trifluoromethyl)phenyl]isonicotinamide, Ascent Scientific, Bristol UK) and SRPIN4 (N-(2-(4-(2-dimethylamino)ethyl)piperazin-1-yl)-5-(trifluoromethyl) phenyl) isonicotinamide), (5μM, synthesised by JDR and JM, IC50=261nM). After 24h of treatment fluorescein-labelled THP1 cells were added to HFS at 30,000 cells/well, and incubated for 2h, rinsed, and fixed in 4% paraformaldehyde. Relative fluorescence, indicating monocyte adherence to HFS, was quantified on a multi-plate reader. Background fluorescence was subtracted and results normalised to untreated and TNFa treated conditions.
Treatment of either RA or normal HFS with TNFa increased fluorescence, which was blocked by cotreatment with anti-ICAM-1. TNFa-induced fluorescence was also significantly reduced by treatment with PTK787 or VEGF-A_{165b}. Inhibitors of SRPK1, SRPIN4 or SRPIN340 significantly reduced fluorescence compared to TNFa treatment (10.0±19%, n=5 c.f. SRPIN4 49±5%, n=7 p<0.01; SRPIN340, 56±8% n=7; 1-way ANOVA+ Dunnett’s multiple comparison test).

Monocyte adherence to TNFa-stimulated normal and RA HFS was dependent on both endogenous VEGF-A/VEGFR and ICAM-1. Inhibiting VEGF receptors with PTK787 or VEGF-A_{165b} significantly prevented monocyte attachment to HFS cells. Results indicate monocyte attachment is mediated by VEGF-A_{165b} and VEGF-A_{165b} may act as a competitive antagonist for VEGF-A_{165b} at VEGFR2. Reduction of monocyte adherence by inhibition of SRPK1 using SRPINs suggests that control of alternative RNA splicing of VEGF may represent an anti-inflammatory strategy.


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The role of sympathetic innervation in cerebrovascular tone and autoregulation, and changes associated with ageing

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It is known that the cerebral vasculature is innervated with sympathetic fibres originating primarily from the superior cervical ganglion (SCG). However, the role that these sympathetic fibres play in control of the cerebral circulation remains controversial. Some evidence suggests a tonic sympathetic constrictor influence on cerebral blood flow (CBF) and a role in autoregulatory vasoconstriction in response to increases in arterial blood pressure (ABP) towards the upper limit (UL), giving protection of the blood brain barrier. Ageing is generally associated with impaired sympathetic functioning; involving changes in both innervation density and transmitter biosynthesis. But how this affects the cerebral circulation and autoregulation of CBF is unknown.

We hypothesised that sympathectomY (sympX) would lower the UL in young (Y) rats, due to a reduced capacity for autoregulatory vasoconstriction. In old (O) rats, as this vasoconstriction may already be impaired, we hypothesised no effect of sympX on the UL, and also that the UL would be lower in O than intact Y rats. In Alfaxan-anaesthetised (12-30mg.kg.hr\(^{-1}\) i.v.), Y (6-8 weeks, n=7) or O (52-58 weeks, n=10), male Wistar rats, we isolated the SCG and performed bilateral sympX. Gross CBF was recorded from the common carotid arteries after external carotid ligation. Data is expressed as mean±SEM, and statistics used were paired (*) or unpaired (†) t-tests. P<0.05 was considered significant.

In Y rats, bi sympX significantly increased basal CBF from 1.9±0.1ml.min\(^{-1}\) by 0.6±0.1ml.min\(^{-1}\). In O rats, basal CBF was significantly higher than Y (2.9±0.3ml.min\(^{-1}\)) and bi sympX similarly and significantly increased CBF by 0.7±0.1ml.min\(^{-1}\). Phenytoxine was then infused (0.1–200µg.kg.min\(^{-1}\) i.v.) to raise ABP and the autoregulatory UL was determined by dual-linear regression analysis of CBF vs ABP. The gross UL after sympX in Y rats was higher than that in intact Y rats (178±3* vs 168±2mmHg). In O rats, the gross UL after sympX was significantly higher, or even abolished, compared to intact O rats (190±3* vs 180±3mmHg), and also when compared to Y rats; both intact and post-sympX.

We propose that sympathetic fibres have a role in maintaining basal cerebrovascular tone, and that this is similar for Y and O rats. Further, sympathetic innervation appears to limit the autoregulatory UL in both Y and O rats, such that removal of sympathetic input raises the UL. The mechanism responsible for further vasoconstriction in the absence of sympathetic activity may be the myogenic response. Structural differences between the cerebral vessels of Y and O may therefore account for the higher UL in O.

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C77

Non-invasive measurement of cerebral arterial compliance during post exercise ischemia

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Introduction It remains unclear whether the main source for vascular resistance in the brain is located in the major cerebral arteries or the smaller arterioles. Here we use our recently introduced non-invasive measurement of cerebral arterial compliance (AC) to measure the cerebrovascular response to post exercise ischemia (PEI) over the cerebrovascular tree. AC is inversely related to cerebrovascular resistance; if AC decreases vascular resistance increases. The aim of this study is to investigate where in the cerebral arterial tree AC decreases during PEI, when blood pressure and sympathetic nerve activity are elevated.

Data acquisition 8 healthy young men underwent an MRI scan session in which two AC measurements (one rest, one PEI) were done. PEI was induced following isometric forearm contraction (IFC) at 40% of each participant’s maximum grip (3 mins). After 2 mins of IFC, a blood pressure cuff was inflated to 100 mmHg above systolic BP, on the gripping arm, to induce PEI. During PEI (10 mins), pulsed arterial spin labelling (ASL) with 32 tag-control pairs for each of seven inversion times (250-850 ms, 100 ms spacing) was performed. Cardiac traces were monitored with a probe at the left index finger. Muscle sympathetic nervous activity (MSNA) in rest and during IFC was measured in a separate session with microneurography of the peroneal nerve in the lower leg. In both sessions (MRI...
and microneurography) blood pressure was monitored at the left thumb. Data analysis ASL tag and control images were retrospectively grouped according to their acquisition time relative to the cardiac cycle. Maps of diastolic and systolic arterial blood volume ($aBV_{Dia}$ and $aBV_{Sys}$) were calculated by voxelwise fitting of an arterial input function$^4$ to the average difference signal over time. Average pulse pressure was used to obtain maps of $AC$ ($\%$/mmHg) according to: $AC = 100 \times \frac{(aBV_{systole} - aBV_{diastole})}{(aBV_{diastole} \times PP)}$. Regional median $AC$ values were drawn for the flow territories encompassing the major brain feeding arteries, which are the internal carotid and basilar arteries (ICA, BA), and the bilateral middle cerebral arteries (MCA).

Results Mean arterial blood pressure and MSNA were significantly increased (paired t-test, $p < 0.05$) during PEI compared to rest ($+9.2 \pm 10.6$ mmHg and $+10.5 \pm 7.8$ burst/100 heartbeats, respectively). Figure 1 illustrates that during PEI the arteries inferior to and at the level of the Circle of Willis show a decrease in $AC$, while the MCA flow territories distal from the Circle of Willis show no change in $AC$.

Implications The increase in cerebrovascular resistance (decrease in $AC$) in the ICA and BA is caused by an increase in vascular smooth muscle cell tone in the arterial walls. This result suggests that the major cerebral arteries proximal to the circle of Willis play an important role in determining cerebral vascular resistance in humans.

![Compliance](image.png)

Figure 1. Group average compliance plotted against slice location. Note that the data is averaged over the major brain feeding arteries below the Circle of Willis (CoW) and the bilateral MCA at the level of and above the CoW. Error bars indicate standard error of the mean.


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PC001

Possible mechanisms of cardiac contractile dysfunction and electrical changes in ammonium chloride induced chronic metabolic acidosis in Wistar rats

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Background and Aim: Metabolic acidosis could occur due to either accumulation of endogenous acids or bicarbonate loss from the gastrointestinal tract or more commonly from the kidney. This study aimed to investigate the possible underlying mechanism(s) of chronic acidosis-induced cardiac contractile and electrical changes in rats.

Materials and Methods: 24 adult Wistar rats, of both sexes, were randomly divided into control group and chronic metabolic acidosis group, which received orally 0.28 M NH₄Cl in the drinking water for 2 weeks. At the end of experimental period, systolic and diastolic blood pressures were measured. On the day of sacrifice, rats were anesthetized by i.p. pentobarbitone (40 mg/kg B.W.), transthoracic echocardiography and ECG were performed. Blood samples were obtained from abdominal aorta for complete blood count and determination of plasma pH, bicarbonate, chloride, sodium, potassium, troponin I, creatinine kinase, interleukin 6 and aldosterone levels.

Results: Compared to control group, chronic metabolic acidosis group showed anemia, significant systolic and diastolic hypotension accompanied by significant reduction of ejection fraction and fraction of shortening, significant bradycardia, prolonged QTc interval and higher widened T wave, as well as, significantly elevated plasma levels of aldosterone, troponin I, creatinine kinase and interleukin 6. The left ventricular wall of acidosis group showed focal areas of degeneration and areas of hypertrophied cardiomyocytes with vacuolated pale acidophilic sarcoplasm and deeply stained pyknotic nuclei.

Conclusion: Chronic metabolic acidosis induced negative inotropic and chronotropic effects and cardiomyopathy, possibly by elevated plasma aldosterone and interleukin 6 levels.

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PC002

Chronic type 1 diabetes mellitus induces structural changes, fibrosis and hypertrophy in the rat kidneys

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Chronic kidney failure is the leading cause of morbidity and mortality in individuals with diabetes mellitus (DM). To date, the underlying cause for this remains poorly defined and this has prevented the development of drugs to prevent/slow its development. This study investigates longitudinal changes in morphology, fibrosis and expression of extracellular matrix (ECM) proteins in kidneys of rats with type 1 DM. Type 1 DM was induced in male Wistar rats (n = 16) using a single dose of streptozotocin (STZ; 60 mg/kg body weight, ip) dissolved in citrate buffer. Age-matched control rats (n = 16) received an equivalent volume of citrate buffer alone. Eight and sixteen weeks after STZ injection animals were euthanized and perfused with phosphate buffered saline. Kidneys were then harvested and processed histologically to examine morphological changes using conventional staining and quantitative assessment of fibrosis, respectively. Immunohistochemical studies were also conducted to assess apoptotic markers governed by Caspase-3. Molecular biology techniques were also employed to examine gene expressions of proteins encoding ECM components, TGFβ-1 and hypertrophy biomarkers ANP and BNP along with ELIZA to quantify the level of total and active TGFβ-1. The project had the relevant Home Office Ethical Clearance.

After 8 and 16 weeks of DM, body weights were reduced (ANOVA, Bonferroni corrected t-tests, p<0.05. Mean±SEM, 270±5.78 vs. 343±9.99 and 221±13.02 vs. 378±10.63 compared to controls. Blood glucose, kidney weights, kidney weight to body weight ratio, were higher in DM rats compared to controls. Typically, blood glucose levels (mg/dl) and kidney weight/body weight ratio (g/100 g body weight) after 2 and 4 months DM were 443±12.2 and 0.15±0.02 and 446±18.8 and 0.20±0.03 compared to 98±3.79 and 0.94±0.01 and 97±3.04 and 0.82±0.01 (n=8), in controls. DM induced structural changes in the kidneys including dilated tubules and severe disorganization of the glomeruli. Electron microscopy study revealed significant increase (p<0.05) in glomerular basement membrane, meanwhile morphometric analysis indicated (p<0.05) increments in fibrous tissue proliferation and apoptosis compared to controls. This was accompanied by (p<0.05) increases in expressions of genes encoding a variety of extracellular matrix proteins including collagen 1α, collagen 3α, fibronectin, elastin, MMP9, TIMP4, CTGF, integrin α5, vimentin TGFβ-1, and hypertrophy biomarkers ANP and BNP compared to controls. Additionally, TGFβ-1 total and active levels increased significantly (p<0.05) in DM compared to age-matched controls. These alterations were more pronounced (p<0.05) after 4 months compared to 2 months DM rats. The results reveal that type 1 DM is detrimental to kidney structure and function leading to Diabetic Nephropathy (DN), symptomatic of TGFβ-1 activity.

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PC003

Increased methylglyoxal is an underlying cause for loss of endothelial progenitor cells in type 1 diabetic rats

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Diabetes mellitus (DM) is a major metabolic disorder currently affecting over 380 million people worldwide. DM can induce oxidative stress in the body leading to the generation of elevated levels of the endogenous reactive carbonyl species (RCSs), including methylglyoxal (MGO) which can elicit vascular dysfunction. Elevated MGO is believed to reduce the pool of endothelial progenitor cells (EPCs) responsible for maintaining the vasculature. This study isolated EPCs from the blood and characterizing them, measured endogenous level of MGO in blood and tissues during DM and investigated the mechanism(s) whereby MGO can elicit a reduction of EPCs or cell death. Young adult male Sprague Dawley rats weighing 180-200g were rendered diabetic using a single dose of intraperitoneal injection (ip) containing 45 mg/kg body weight streptozotocin (STZ) dissolved in citrate buffer. Age-matched animals were given the same volume (0.3 ml of the buffer alone. Fasting blood glucose was assessed four days prior to STZ injection and on the day (8 weeks later) when the animals were humanely killed for experimentation using an ACCU-CHEK Aviva glucose meter (Roche, Mannheim, Germany) to confirm DM. EPCs were incubated with MGO for 24 hours using varying concentrations (0-500 μM) to determine the effect of MGO on EPC viability using MTT assay. Western blot analysis was used to determine the relative levels of the MGO scavenging enzyme glyoxalase following its overexpression. Confocal imaging was used to assess the effect of MGO on cytopenic and mitochondrial calcium and ROS production. STZ-treated rats had significantly (Mean ± SEM; 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 45±7.8 mg/dl and 116.7±4.38 mg/dl and heart to body mass ratios (g/100g body weight) were 0.28±0.08 and 0.3±0.07 for diabetic (n=5) compared to control (n=5) rats. Serum insulin (ng/ml) was 1.07±0.16 and 0.29±0.05 in diabetic and control rats, respectively. The results show that MGO level is significantly (p<0.05) elevated in serum, whole blood, heart and brain tissues, but not in the kidneys, during DM compared to healthy age-matched controls. In contrast, the number of EPCs decreased significantly (p<0.05) in the blood of diabetic rats compared to control. The MTT assay showed that increasing concentrations of MGO had the ability to kill EPCs with EC₅₀ of 100 μM. Over-expression of glyoxalase enzyme blunted the effect of MGO on cell viability. Confocal microscopic results have indicated significant (p<0.05) time-dependent increases in both cytosolic Ca²⁺ and mitochondrial Ca²⁺ release from either fluo-3 or rhodamine-loaded EPCs treated with 100 μM MGO, respectively. MGO can also elevate mitochondrial ROS production from EPCs. It is concluded that the RCS-generated MGO has the ability to induce death of EPCs through a mechanism involving superoxide production and mitochondrial calcium overloading.

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

PC004

Evidence that ghrelin may be associated with the development of human breast cancer

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Ghrelin, a peptide hormone made of 28 amino acids, is distributed and expressed in many tissues. Two major functions of ghrelin involved the stimulation of growth hormone release and food intake. Ghrelin exerts a physiological effect via its functional receptor called growth hormone secretagogue receptors (GHSR1a). Ghrelin also plays an autocrine/paracrine role in a number of processes related to cancer progression, including cell proliferation, cell migration and invasion, and in apoptosis. This study investigated the expression of ghrelin hormone and its functional receptor in a total of 56 human mammary tissues (16 healthy, 16 benign and 24 cancerous) using immunohistochemistry method. The correlation between tissue expression to the hormone and its receptor and the histopathological characteristics of the cancerous tissues were examined for comparison. The work had ethical approval from both institutions. The healthy tissues were obtained from women who underwent mammoplasty for cosmetic reasons.

The results show that breast tissues invaded with cancer were structurally different from normal healthy tissues having prominent, lobules and ducts. Cancerous tissues appeared characteristic of ductal carcinoma containing dense fibrous stroma. There was a significant (Student’s t-test; p<0.05) and an exclusive and differential immune-reactivity to ghrelin in the cancerous mammary tissues compared to normal and benign tissues which showed little or no immune-reactivity to ghrelin. All tissue morphological types showed immune-reactivity to the ghrelin hormone functional receptor, (the GHS-R1a). There was a significant (p<0.05) down regulation of the receptors in malignant tissues showing either no or weak immune-reactivity to the GHS-R1a receptor. Immuno-reactivity to ghrelin and its receptor was independent of the histopathological characteristic of breast cancer, except for the lymph node status which is the extent of axillary metastasis to the lymph nodes for the breast cancer. There was also a significant (p<0.05) correlation between strong receptor immune-reactivity at the cancerous tissues and low metastases to the lymph nodes. The majority of the victims had invasive ductal cancer (90%) compared to ductal carcinoma in situ and invasive lobular carcinoma (8.3%). These correlated strongly with the stages (8.3 %, 12.5%, 50.5%, 8.3% and 37.5 % for stages 0, I, II, III and -IV, respectively), grades (12.5%, 37.5% and 50% for grades I, II and III, respectively). Similarly, over 75% of the victims had positive expressions for oestrogen, progesterone and HER-2. These results demonstrated a close relationship between breast cancer and ghrelin in human subjects from the UAE.
Sodium channel haploinsufficiency and structural change in age and gender associated ventricular arrhythmogenesis

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Normal cardiac excitation involves an orderly sequence of electrical activation and recovery dependent on the voltage-gated, sodium channels (Na\textsubscript{1.5}). Mutations in Na\textsubscript{1.5} are associated with arrhythmic conditions such as Brugada Syndrome and progressive cardiac conduction defect which are associated with sudden, often fatal, ventricular tachycardia or fibrillation. The arrhythmic phenotype is associated not only with the primary biophysical change but also additional, anatomical, abnormalities, in turn dependent upon age and sex that themselves exert arrhythmic effects. In this scientific review, we aim to summarize a collection of experimental studies of physiological importance that now provide evidence for a unified binary scheme for the development of arrhythmia. Biophysical studies using mouse Scn5a\textsuperscript{+/-} hearts suggested that Na\textsubscript{1.5} deficiency produces a background electrophysiological defect compromising conduction producing arrhythmic substrate typically unmasked by flecainide or ajmaline challenge. More recent reports further suggest a progressive decline in conduction velocity and increase in its dispersion in Na\textsubscript{1.5} haploinsufficient compared to WT hearts, particularly in ageing male animals. This appears to involve an increased expression of slowly conducting pathways and a demonstrable change in their frequency distributions. These changes were accompanied by increased cardiac fibrosis. It is the combination of the structural and biophysical changes that accentuate arrhythmic substrate to a degree sufficient to cause spontaneous arrhythmic events. This resulting binary scheme explains the requirement for a combination of separate, biophysical and structural, changes, occurring in ageing males associated with Na\textsubscript{1.5} haploinsufficiency, to produce clinical arrhythmia.


We are grateful to the Ministry of Education, Malaysia, Fundamental Research Grant Scheme (FRGS/2/2014/SKK01/Perdana/02/1), the MacVeigh Beneaction, the Medical Research Council UK (MR/M001288/1) and the Wellcome Trust (105727/Z/14/Z) and the Merdeka Award Grant for Internationa Attachment for support.

Diabetes deteriorates the epithelium mediated mechanisms through frailty of COX pathways in diabetic-antigen sensitized airways of guinea pigs

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Studies on the incidence of diabetes and asthma together indicate that they seldom occur in the same patient. If asthma develops in diabetic patients it is less severe than that in the residual population (1). Our aim was to investigate whether diabetes, modulates/alters the bronchial responses in hyper-reactive airway smooth muscle (ASM) and whether the two of the epithelium mediated pathways: COX, and EpDHF were affected.

Experimental models of guinea pigs (n=30) with airway hyper-reactivity (ovalbumin (20mg/kg, ip for two alternate days), and diabetic guinea pigs along with airway hyperreactivity (streptozotocin (180mg/kg (2)), four weeks after ovalbumin, ip for two alternate days) were developed.

Numerical data is expressed as mean ± SEM of the number of animals used in each experiment. The results were analyzed by one way analysis of variance (ANOVA) followed by post-hoc Tukey’s test. Differences were considered statistically significant at p<0.05. Denudation of tracheal rings from healthy guinea pigs significantly reduced the IP induced relaxation, suggesting that the relaxant response to IP is mediated in part through the epithelium. When the same experiment was repeated on the epithelium intact trachea of guinea pigs with hyperreactivity, a smaller response to IP was produced, indicating partial loss of epithelium mediated relaxation. A significantly smaller relaxation response was observed in guinea pigs with diabetes along with airway hyperreactive condition to IP in comparison to healthy and hyperreactive guinea pigs (Fig 1) suggesting that diabetes worsens the loss of epithelium mediated relaxation. Epithelium denudation did not alter the IP induced relaxant response in guinea pig with diabetes and airway hyperreactivity validating complete loss of epithelium mediated relaxation (Fig 2). The relaxant response to IP in presence of glibenclamide was significantly reduced in healthy trachea (14.7±1.2%, *p<0.05), but not in the hyperreactive trachea (4.6±0.6%) and in diabetes along with airway hyperreactive trachea (3.5±0.9%). The results suggest that in both hyperreactivity and diabetes along with hyperreactivity EpDHF pathway was damaged. The relaxation response of healthy trachea (14.5±1.2%, *p<0.05) and hyperreactive trachea (9.6±2.2%, p<0.05) to IP after addition of indomethacin, significantly reduced. In contrast, there was
no alteration in the relaxant response to IP in trachea where diabetes occurred along with with airway hyperreactivity (3.2 ±1.4%), after incubation with indomethacin. This implies that diabetes aggravates epithelial dysfunction by disrupting the COX pathway in diabetes along with hyperactivity. Therefore we can conclude that diabetes further deteriorates the epithelium mediated bronchiorelaxation, specifically the COX mediated pathway in the guinea pigs having both diabetes and airway hyperreactive condition.


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

PC007

Computational modelling of the mouse sino-atrial node: Exit block due to apoptosis

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Background and Aim: The sinoatrial node (SAN) is electrically and structurally heterogeneous [1, 2]. SAN cell apoptosis may accentuate the heterogeneity causing SAN exit block.

Methods: Our previous model [3] was enhanced by including a histology based 2D mouse SAN geometry [2]. A semi-insulating SAN-atrial boundary was randomly assigned a number of SAN-atrial exit pathways. A simple electrophysiological cell model simulated a range of SAN pacemaking firing rates, as well as atrial excitable action potentials (APs) (see Figure). The spatial distribution of SAN cells was assigned randomly. Gap junction coupling (GJC) in the atrium was adjusted to produce a 0.6 m/s conduction velocity. SAN GJC distribution was made heterogeneous. Maximum SAN GJC was 10 times smaller than in the atrium. Inactive apoptotic cells and the insulating tissue were assigned a small electrical conductivity. Each simulation produced 5 s of electrical activity data. To detect the SAN exit block, the number of oscillations at each location were recorded. Bradycardia was defined as when number of SAN oscillations exceeded those in the atrium. Standard deviation of each SAN cell’s oscillations identified the leading pacemaker locations [4, 5]. The estimated leading pacemaker locations was verified by studying time animations of the 2D voltage distribution. Three simulation experiments were performed. In the first experiment, the number and size of exit pathways was gradually increased till a robust pacemaking-propagation pattern was achieved. This estimate of the exit pathway density along the insulating border was used in the next two experiments. In the second experiment, the occurrence of SAN exit block due to uniformly distributed apoptotic cells (0% and 10%) was tested. The third experiment was similar to the second experiment, except that the apoptotic cells were confined to be within 0.2 mm of the insulating border.

Results: The outcome of the first experiment indicates that the amount of exit pathways should be 17.5% to achieve a robust SAN-atrial pacing. The leading pacemaker location was found to coincide with the location of minimum GJC in the SAN. The results of simulations 2 and 3 show that with progressive apoptosis, SAN-atrial pacing was increasingly intermittent. Multiple distinct leading pacemakers separated by 0.1 to 0.3 mm were observed. Whereas a total arrest of atrial pacing was observed at 8% in simulation 2 (uniformly distributed apoptosis) [3], total arrest was observed at a lower 4% apoptosis in simulation 3 (apoptosis close to SAN-atrial boundary).

Conclusions: The model behaviour indicates that only a small part of the SAN has to be in contact with the atrium to achieve physioloical pacing. In contrast to our previous study, the new model is capable of reproducing the experimental data of missed beats and sinus block at modest levels of apoptosis.
Action potentials recorded from a 2D simulation

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SAN and atrial APs in the 2D model of mouse SAN.


British Heart Foundation.

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PC008

Octanoate eliminates the cardioprotection elevated extracellular glucose impacts on isolated cardiomyocytes

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Under normal physiological conditions, fatty acids are the preferred metabolic substrate for the myocardium. However during ischaemia, fatty acid oxidation ceases and anaerobic glycolysis becomes the main source of ATP production (1). Upon reperfusion, due to the build up of fatty acids during ischaemia, inhibition of glucose oxidation can occur, this can be detrimental (2). Improved re-coupling of glycolysis and glucose oxidation via stimulation of pyruvate dehydrogenase can relieve this detrimental effect (2, 3 and 4). This study investigates the effects of elevated extracellular glucose in the absence or presence of the medium chain fatty acid octanoate, and whether pyruvate has any effect on the actions of octanoate. Ventricular cardiomyocytes were enzymatically isolated from adult male Wistar rats which had been culled humanely in accordance with Home Office guidelines. Contractile function of the isolated cardiomyocytes was assessed using a simulated ischaemia/reperfusion protocol, where cardiomyocytes were stimulated to contract at 1Hz by electrical field stimulation and continuously perfused with Tyrode solution at 32±2°C. Ischaemia was simulated by perfusing a metabolic inhibition (MI) solution of Substrate-Free Tyrode solution containing cyanide (2mM) and iodoacetic acid (1mM) for 7 minutes, followed by 10 minutes of ‘reperfusion’ with Tyrode solution. These data show that perfusing isolated cardiomyocytes with 0.5mM pyruvate and an elevated extracellular glucose concentration (20mM) significantly increased the percentage of cardiomyocytes regaining contractile recovery from 27.6 ± 3.6% (5mM glucose control) to 51.5 ± 2.2%*** (Results presented as mean ± S.E.M; N= 6 experiments, >169 cardiomyocytes for each experiment, ***P<0.001 Unpaired T-test). The replacement of pyruvate (0.5mM) with octanoate (0.5mM), under conditions of elevated extracellular glucose, significantly decreased the percentage of cardiomyocytes able to regain contractile function from 51.5 ± 2.2% to 12.9 ± 2.1%**** (Results presented as mean ± S.E.M; N= 6 experiments, >156 cardiomyocytes for each experiment, ****P<0.0001 Unpaired T-test). The addition of 5mM pyruvate to octanoate, under conditions of elevated extracellular glucose, increased the percentage of cardiomyocytes regaining contractile recovery from 12.9 ± 2.1% to 25.5 ± 6.1% (Results presented as mean ± S.E.M; N= 6 experiments, >156 cardiomyocytes for each experiment, P=0.08 Unpaired T-test). These data show that the cardioprotection imparted by perfusion with an elevated extracellular glucose concentration, is eliminated by octanoate and that the effect of octanoate could be attenuated by pyruvate. We hypothesis these effects are due to the coupling status of glycolysis and glucose oxidation upon reperfusion.

Lopaschuk G.D et al (1993). An imbalance between glycolysis and glucose oxidation is a possible explanation for the detrimental effects of high levels of fatty acids during aerobic reperfusion of ischemic hearts. The Journal of Pharmacology and Experimental Therapeutics 264, 135-144.

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PC009

Orchidectomy enhances myocardial Ca²⁺ transient, SERCA2 expression and prevents ventricular dysfunction after myocardial infarction in rats

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Introduction: The incidence of cardiovascular disease increases significantly in women after the fall in the production of sex hormones (Corona et al, 2011). Studies have shown that the absence of testosterone in males, after myocardial infarction (MI), could mitigate the effects of cardiac remodeling (Cavasin et al., 2006). Objectives: This study aims to evaluate the cardiac contractility of the right and left chambers 60 days after myocardial infarction in rats with suppression of male hormones. Methods: Anaesthetized 2 month old male Wistar rats were subject to bilateral orchidectomy with a mixture of
ketamine (90 mg/kg)/xylazine (10 mg/kg) by intraperitoneal injection. One week after orchidectomy (OQT) the animals underwent surgery to induce MI through the anterior descending branch of the left coronary artery ligation. The groups were divided in to: OQT, MI, OQT + MI (OQT-MI), and control (Sham). Eight weeks after MI surgery, the animals were anesthetized for hemodynamic measurements and then were sacrificed to obtain the functional assessment of the data by using the contraction force of the papillary apparatus. To evaluate myocardial contractility, strips of the right and left ventricle papillary were collected. Calcium curves were performed (0.62, 1.25, 2.5 and 3.75 mM) and isoproterenol (10^(-8) to 2^(-5) M) Ventricular myocytes were isolated for intracellular calcium (Ca^{2+}) measurements, and assessment of Ca^{2+} handling proteins. Data are expressed as mean ± S.E.M. Statistical analysis was performed using ANOVA-2 then post hoc Bonferroni. Results: The contractility was preserved in the OQT group and OTQ-MI group compared to the MI group in response to calcium (Force in g / g in Ca^{2+} 3.75 mM Sham: 608 ± 69.76 (n = 11); OQT: 590 ± 37.37 (n = 16); OQT-MI: + 594 ± 75.6 (n = 7); MI: 311 ± 33.1 (n = 9); p <0.05) and Isoproterenol (Force g / g 10^{-2} Isoproterenol: Sham: 516 ± 60.2 (n = 11) OQT 525 ± 50.4 (n = 17) MI-OQT: 463 ± 74 (n = 8) MI: 210 ± 29.3 (n = 10); p <0.05). At the cellular level, MI cardiomyocytes did not show significant changes in peak Ca^{2+} transient amplitude, or SERCA protein expression levels. Although PLN phosphorylation levels at Thr^{17} were significantly reduced in these cells when compared to cells from Sham rats. On the contrary, OQT-MI cells presented enhanced Ca^{2+} transient, and increased SERCA2 expression levels. Both changes were observed in OQT ventricular myocytes. Conclusion: Left and right myocardial contractility was preserved in the Orchidectomy rats after myocardial infarction. The results suggest that testosterone contributes to the impairment of cardiac function and contractility after MI involving myocardial Ca^{2+} transient. Financial support: FAPES, CNpq, CAPES.


Maria A. Cavasin, Zhen-Yin Tao, Ai-Li Yu, Xiao-Ping Yang. Testosterone enhances early cardiac remodeling after myocardial infarction, causing rupture and degrading cardiac function. American Journal of Physiology - Heart and Circulatory Physiology Published 1 May 2006 Vol. 290 no. 5, H2043-H2050

Financial support: FAPES, CNpq, CAPES.

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PC011

QT interval among apparently healthy adults in Enugu, South – East, Nigeria

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Increased prevalence of heart diseases is being reported in World (Araoye, 1982). Ventricular arrhythmias seen in people with long QT interval is a known predisposition to sudden cardiac death (SCD) (Chou, 1991). Electrocardiogram (ECG) is the readily available, cheap, and non-invasive cardiac investigation in use in most hospital and researches. There is paucity of local data on values of the respective waves, intervals and segments of ECG in Nigeria. Establishing the Qr interval range of our people will help identify people who have long QT, thus at risk of ventricular arrhythmias and sudden cardiac death. One hundred age-matched consenting male and female subjects who are staff of the University of Nigeria Teaching Hospital (UNTH), Enugu and volunteers, who had no clinical evidence of cardiovascular diseases had a resting 12 – lead surface ECG recorded. There were no controls since this was a baseline study. Structured questionnaires were used to obtain their bio-data. The result showed that there was no significant difference between QT intervals of our subjects and the Caucasians. The mean QT_{c} interval was 0.38 +/- 0.025 (males) and 0.41 +/- 0.029 (females) respectively. Abnormal ECG was seen in 14% of subjects studied. QT_{c} was prolonged in 5% males.
while all the females had normal values (Oluranti and Taiwo, 2007; Oluwadare and Adams, 2012). The QTc in the subjects were of wider range compared with Caucasian values. The apparently healthy adults had abnormal ECG, and prolonged QT intervals, thus we suggest that the populace should be advised to go for regular medical check – up. The result provides a baseline data for establishing a reference value for QT interval in our locality.


Oluwadare, O.J., Adams, O. Gender differences in electrocardiogram of young adults in South - Western Nigeria. EJSR 81: 1 - 5.

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PC012

Blocker-induced changes in ion permeation through the cardiac ryanodine receptor also affect channel gating

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Precisely controlled Ca\(^{2+}\) release from the sarcoplasmic reticulum in the cardiomyocyte, mediated by the cardiac ryanodine receptor Ca\(^{2+}\) release channel (RyR2), is essential for rhythmic contraction of the heart. In spite of the paucity of detailed structural information for the RyR2 pore and the gating entities it contains, functional studies have indicated the possibility of two gates. The Ca\(^{2+}\) (ligand) controlled gate at the inner helix bundle crossover (IHBx) region of the RyR2 pore was found to be mechanistically distinct from that of the gating at the selectivity filter (SF) region that is Ca\(^{2+}\) independent. As ion channel blockers have previously been shown to be effective antiarrhythmic agents, it is envisaged that a similar mechanism of drug action could be employed in the case of RyR2 dependent arrhythmia. This study looks at the effect of RyR2 channel block by large tetraalkylammonium cations and Shaker B inactivation peptides. The blockers' ability to reduce the availability of permeant ions in the pore, previously shown to stabilise the SF, preventing its collapse in other ion channels. This study demonstrates that RyR2 blockers could inhibit channel activity due to their effects on gating in addition to reducing single channel current by physical block of the pore. Values are means ± SEM, data compared by t-test.


Funded by BHF

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PC013

Altered luminal accessory protein regulation of mutant cardiac ryanodine receptors as a putative mechanism of sudden cardiac death

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Human cardiac ryanodine receptors (hRyR2) play an indisputable role in excitation-contraction coupling, allowing Ca\(^{2+}\) to pass from the sarcoplasmic reticulum (SR), into the cytoplasm of cardiomyocytes. These Ca\(^{2+}\) release channels form quaternary complexes with luminal accessory proteins calsequestrin (CSQ2), junctin (JUN) and triadin (TRD1), which are thought to regulate the response of hRyR2 to changes in luminal Ca\(^{2+}\) within the SR. Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is an arrhythmogenic disorder, caused by mutations in hRyR2 and CSQ2 genes and defective channel sensing of cytosolic and luminal Ca\(^{2+}\) plays an important role in disease pathogenesis. Given the proposed role of CSQ2 and JUN in modulating the response of hRyR2 channels to activating Ca\(^{2+}\), altered association or dysfunctional regulation by these accessory proteins could be a contributing factor in the aberrant Ca\(^{2+}\) release identified in CPVT-linked mutant hRyR2. In this investigation we examine the interaction and response of wild-type (WT) and CPVT-linked mutant (A4556T and N4104K) eGFP-hRyR2 channels to CSQ2 and JUN co-expression in HEK293 cells. Luminal Ca\(^{2+}\) events were monitored by examination of spontaneous Ca\(^{2+}\) release (SCR) events in fluo-3 loaded cells using confocal microscopy. Interaction with hRyR2 was assessed using pixel co-localisation of the co-expressed eGFP-hRyR2 with Alexa-594 labelled CSQ2/JUN in fixed

107P
Demonstrating functional heterogeneity between hRyR2 mutations, A4556T-hRyR2 displayed a similar pattern of SCR to WT, whilst N4104K-hRyR2 displayed a remarkably altered profile characterized by an increased frequency of events (WT 2.1±0.15 vs. NK 8.1±1.5 events/min, p<0.001 n=14) and decreased amplitude (ΔF/F₀ WT 1.59±0.16 vs NK 0.79±0.08, p<0.05 n=12). Co-expression with CSQ2 (either in the presence or absence of JUN) had inhibitory effects on WT and N4104K-hRyR2, increasing inter-event duration (WT 36±3.69 vs NK 7.31±0.96 secs, p<0.001 n=13), thereby decreasing SCR event frequency (WT 1.34±0.13 vs NK 3.10±0.29 events/min, p<0.001 n=14), yet this inhibitory effect was not observed with A4556T-hRyR2 (AT+CSQ2 2.43±0.28 vs AT only 2.37±0.29 events/min n=14), suggesting that this CPVT mutant is unable to be regulated by this accessory protein. In agreement with this, immunofluorescent co-incidental pixel counting suggested reduced CSQ2 and JUN association with A4556T-hRyR2 compared to WT (for example, WT 93±1.46 vs AT 80±3.0, p>0.05 n=12 (CSQ2 association in cells expressing hRyR2+CSQ2+JUN), whereas association with N4104K-hRyR2 was unchanged (85±6.75). This suggests that A4556T affects hRyR2 regulation by CSQ2, possibly as a result of defective protein-protein interaction.


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can be modulated by IP<sub>3</sub>-R activation. Whilst the results with 2-APB are consistent with a role for constitutively active IP<sub>3</sub>-R in AVN electrophysiology, they also raise the possibility of other direct ion channel effects of 2-APB.


Hancox JC, Yuill KH, Mitcheson JS, Convery MK (2003). Progress and gaps in understanding the electrophysiological properties of morphologically normal cells from the cardiac atrioventricular node. Int J Bifurcation and Chaos 13, 3675-3691.


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**PC016**

Remodelling of ventricular pH regulation in cardiac hypertrophy and heart failure includes enhanced Na<sup>+</sup>-HCO<sub>3</sub><sup>-</sup> co-transporter activity and reduced intracellular H<sup>+</sup> mobility

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<sup>1</sup>Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK, <sup>2</sup>Institute of Cardiovascular Sciences, University of Manchester, Manchester, UK and <sup>3</sup>Department of Pharmacology, University of Oxford, Oxford, UK

Intracellular pH (pH<sub>i</sub>) in ventricular myocytes (VMs) is normally controlled by acid extrusion on sarcosomal Na<sup>+</sup>/H<sup>+</sup> exchange (NHE1) and Na<sup>+</sup>-HCO<sub>3</sub><sup>-</sup> co-transporters (NBCs). Upregulated NHE1 activity has been suggested to induce maladaptive hypertrophy and heart failure (HF), while pharmacological inhibitors of NHE1 may attenuate disease progression.<sup>1</sup> We have now investigated NHE1 and NBC activity in a large-animal model of HF, the tachypaced sheep.

HF was induced in adult female sheep by transvenous right ventricular tachypacing (pacemaker implanted under general anaesthesia using isoflurane inhalation, 1.5-2.5% v/v). In iso-

To explore if prominent NBC upregulation was unique to ovine models, a compensated cardiac hypertrophy (which can pre-figure HF), was induced in adult C57BL/6 mice by subcuta-

sneous delivery of isoprenaline (10 mg/kg/day) for 1-2 weeks, via subcutaneously implanted osmotic mini-pumps (Alzet).<sup>3</sup> Mini-pump implantation was performed under general anaesthesia using isoflurane inhalation, 1.5-2.5% v/v. In iso-

lated VMs, NHE1 and NBC flux appeared to be enhanced, but only the latter reached statistical significance (NHE1 increase of 3.1±1.7 mM/min at pH 6.9, n = 6-10, P = 0.0001, t test). This effect was accompanied by increased NHE1 and NBC<sub>e1</sub> protein expression (Western blot, n = 2-4).

In conclusion, murine and ovine models of maladaptive hyper-

trophy and HF display prominent upregulation of ventricular NBC activity, while H<sup>+</sup> mobility is significantly reduced in HF. These results suggest a profound remodelling of the pH reg-

ulatory system, beyond that previously reported for NHE1. Results also suggest that, in response to stress, the remodel-

ling may occur early in the pathological process and be sus-

tained as the heart decompensates. Whether NBC upregula-

tion plays a causal role in the development of hypertrophy/ HF remains to be evaluated.


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

**PC017**

Inhibition of catalase in Langendorff rat heart augments injury following ischemia and reperfusion

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Generation of reactive oxygen species (ROS) during ischaemia and reperfusion (I/R) is a key determinant of cardiomyocytes death. Therefore, anti-oxidants can play a critical role in cardio-

protection. The endogenous anti-oxidant enzyme catalase has been implicated in protection but there is no direct evidence showing its involvement. We have recently shown that direct inhibition of endogenous catalase, using 3-Amino-1,2,4-tri-

azole (3-AT), significantly increases the rate of ROS produc-

tion in freshly isolated ventricular cardiomyocytes (Boles et al., 2014). The aim of the present study was to investigate the effect of catalase inhibition using 3-AT on I/R injury in whole hearts.

Experiments were carried out on Langendorff-perfused rat heart. Rats were dispatched by cervical dislocation and hearts immediately extracted. Hearts were subjected to 25 mins stabilization followed by 25 mins global normothermic isch-

emia (37°C) and 120 mins reperfusion. Hearts were assigned to two groups; control (n=4) or treatment with 3-AT (n=4). After 10 mins stabilization, the treatment group was perfused with 15mM 3-AT in Krebs-Henseleit buffer for 15 mins. Per-
Impaired P2X1 receptor-mediated adhesion in eosinophils from asthmatic patients

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Background: Eosinophils contribute to the pathogenesis of asthma. They can be activated by extracellular nucleotides released following cell damage and inflammation. This study aimed at identifying the ATP-gated P2X receptor(s) present on eosinophils and determining their contribution to eosinophil biology.

Methods: Human eosinophils were isolated from the peripheral blood of healthy and asthmatic volunteers. qPCR and Western blots were used to determine respectively eosinophil P2X receptor mRNA and protein expression level. Conventional whole cell patch-clamp experiments were performed to identify eosinophil functional P2X receptor subtypes. Eosinophil CD11b cell surface expression was measured by flow cytometry and cell adhesion was assessed as residual eosinophil peroxidase activity of adherent eosinophils as previously described by Zhu et al. (2000). Data are expressed as mean±SEM. For electrophysiology experiments, currents from at least 3 cells were averaged per donor. Student t-test was performed when Gaussian distribution was observed, otherwise the Wilcoxon matched-pairs and Mann-Whitney tests were used for paired and unpaired values respectively. CD11b flow cytometry experiment significance was assessed by the ratio paired t-test. Friedman test and Dunn’s multiple comparison test were used for the adhesion assays.

Results: Transcripts for P2X1, P2X4 and P2X5 receptors were expressed in healthy and asthmatic eosinophils (n=6 for each). The P2X1 receptor agonist α,β-melATP (10 μM) evoked rapidly activating and desensitizing inward currents (peak 18±3 pA/pF at -60 mV, n=3) in healthy eosinophils, typical of P2X1 homomeric receptors, which were abolished by the selective P2X1 antagonist NF449 (1 μM) (3±2 pA/pF, n=8, p=0.008). These currents were smaller in eosinophils from asthmatic patients (8±2 versus 27±5 pA/pF for healthy, n=12 and 13 respectively, p<0.001), but were rescued following treatment with a high concentration of the nucleotide apyrase (17±5 pA/pF for 10 IU/ml and 11±3 pA/pF for 0.32 IU/ml, n=6 for both, p=0.031), indicating that the channels are partially desensitised by extracellular nucleotides. α,β-melATP (10 μM) increased active CD11b from the integrin complex expression in eosinophils from healthy, but not asthmatic donors (143±21% and 108±11% of control response, n=13 and 10 respectively, p=0.041). Because α4β1 integrin mediates eosinophil adhesion to ICAM-1 and BSA (Zhu et al., 2000), we investigated P2X1 receptor contribution to eosinophil adhesion on BSA-coated plates. α,β-melATP increased healthy (18±2% compared to control 10±1%, n=6, p=0.014) but not asthmatic eosinophil adhesion (13±1 compared to control 10±0%, n=6). NF449 (1μM) inhibited α,β-melATP-mediated healthy eosinophil adhesion (12±1%, n=6, p=0.0447), but had no effect on asthmatic eosinophil adherence properties (9±1%, n=6).

Conclusion: Healthy human eosinophils express functional P2X1 receptors whose activation leads to eosinophil α4β1 integrin-dependent adhesion. P2X1 responses are constitutively reduced in asthmatic compared to healthy eosinophils, probably due to higher basal release of nucleotide(s). This could be a mechanism for retention of eosinophils in a tissue compartment.


We thank all our healthy and asthmatic volunteers.

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

Reverse-remodelling of cardiac β-adrenergic responsiveness in metoprolol-treated pulmonary hypertensive rats

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Heart failure (HF) is an inability to reach a cardiac output sufficient for the body’s metabolic demands. Following an initial insult (e.g. pressure overload), maladaptive remodelling of the myocardium takes place. One of the main elements of this is a desensitisation of the β-adrenergic response (secondary to an increase in sympathetic drive) which is characterised by decreased β1-adrenoceptor (β1,AR) expression and receptor uncoupling. β-blockers are routinely used in the treatment of left ventricular (LV) HF and promote re-sensitisation of the β1,AR as well as inhibiting pathways that lead to adverse remodelling. In patients with pulmonary artery hypertension (PAH), leading to right ventricular (RV) hypertrophy and
failure, β-blockers are not usually prescribed. We examined whether a selective β1AR blocker metoprolol could show similar re-sensitisation of β1AR when treating RV HF induced by PAH. Male Wistar rats (180-220g) received an i.p. injection of saline (CON group) or 60 mg/kg of monocrotaline (MCT). MCT animals were given a selective β1AR blocker (10 mg/kg metoprolol; MCT+BB group) or placebo (MCT group) daily by oral administration from 15 days post injection. MCT induces RV hypertrophy which progresses to RV HF by 21-28 days post-injection when clinical symptoms are apparent (e.g. weight loss). MCT+BB and CON animals were time-matched to MCT animals; all rats were humanely killed. Langendorff-perfused hearts were used for ventricular myocyte isolation (for functional experiments) or preparation of myocardial homogenates (for protein expression via Western blotting).

RV myocytes from MCT animals showed a blunted increase in fractional shortening to selective β1AR stimulation compared with CON (P<0.05; n=5-20; one-Way ANOVA). This difference was absent in MCT+BB cells (P>0.05; n=9). MCT myocytes also showed a slower time to 50% relaxation with selective β1AR stimulation compared with CON (P<0.05). Again this difference was absent in cells from MCT+BB. A similar trend was seen in Ca1+ transient amplitude and decay. Together this suggests a stepped improvement in β-AR responsiveness with metoprolol treatment. Myocardial β1AR expression was reduced in MCT vs. CON (P<0.05; n=5), but this effect was absent in MCT+BB (P>0.05; n=3). This graded improvement in β1-AR responsiveness with metoprolol treatment may come in part from the observed increase in myocardial β1AR expression. These results showing an improvement in function, along with other studies showing cardioprotective effects1, provide support for the beneficial outcomes of treatment with selective β1AR blockers in RV failure. Small observational clinical trials2 have seen no increase in mortality risk with PAH patients already taking β-blockers. The exact molecular mechanism by which this reverse remodelling is achieved still requires further investigation.


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PC021

Human Ether-a-go-go-Related Gene (hERG) channel activators and vulnerability to re-entrant arrhythmias: A computational study of the roles of potassium and sodium currents

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hERG activators increase the rapid delayed rectifier K+ current IKr and have been proposed as therapeutic interventions for reducing arrhythmias associated with prolongation of the ventricular action potential, as seen in heart failure (HF) and long QT syndrome 2 (LQT2) for example. However, we have shown that one hERG activator, NS1643, can have pro-arrhythmic side effects as it increases transmural dispersion of repolarisation (TDR) and, therefore, increases vulnerability to re-entrant arrhythmias (Peitersen et al., 2008). Furthermore, NS1643 can block IKr at high concentrations (Bilet & Bauer, 2012). The

For macroscopic IKr recordings, the extracellular superfusate was acidified whereas for cell-attached recordings unpaired comparisons were made between recordings made with pipette solutions of pH 7.4 and 6.3. Data are presented as mean ± SEM. Macroscopic IKr responded to a reduction in pH4 by a modest positive shift in activation, accelerated deactivation and reduced whole cell IKr conductance (from 0.69 ± 0.12 nS/pF at pH 7.4 to 0.46 ± 0.09 nS/pF at pH 6.3; n=8 cells; P < 0.001, two-tailed paired t-test). Single channel recordings were made with symmetrical K+ concentrations of 140mM. Current measurements were made at a series of repolarization voltages following a depolarizing command to +40 mV. A reduction in pH4 caused a reduction in single channel amplitude with a mean amplitude, at -100mV, of 1.11 ± 0.03 pA at pH 7.4 (n=8 cells) compared to 0.93 ± 0.08 pA at pH 6.3 (n=7 cells). Slope conductance values derived from current-voltage relations between -120 and -40 mV were 11.31 ± 0.17 pS for pH 7.4 (n=8 cells) and 9.39 ± 0.44 pS for pH 6.3 (n=7 cells; P < 0.01, two-tailed unpaired t-test with Welch’s correction) respectively. Thus, a reduction in single channel conductance contributes to the attenuation of macroscopic IKr by extracellular acidosis.


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PC020

Effects of extracellular acidosis on single channel conductance of hERG potassium channels

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Potassium channels encoded by the human Ether-à-go-go-Related Gene (hERG) are believed to underpin the rapid delayed rectifier K+ current (IKr), which plays a key role in ventricular repolarisation in the heart. Acidosis occurs in a number of pathological situations such as cardiac ischemia and is known to modulate macroscopic hERG channel current, IKr (e.g. Bérubé et al. 1999; Terai et al. 2000; Bett & Rasmusson 2003; Du et al. 2010; Van Slyke et al. 2012). We have studied the effect of extracellular acidosis on single wild-type hERG channels, to identify actions that may underpin reported effects of acidosis on macroscopic IKr. Whole-cell and cell-attached patch clamp measurements of IKr from HEK-293 cells stably expressing hERG channels were made at room temperature.
novel hERG activator MC-11-157c does not block \( I_{Na} \) at higher concentrations, but it can block the \( Na^+ \) current \( I_{Na} \) (Guo et al., 2014). We used computational models to simulate MC-II-157c effects on TDR and quantify the interactions of \( I_{Kr} \) activation and \( I_{Na} \) block on vulnerability to re-entry. A model of human ventricular cell electrophysiology (O’Hara et al., 2011) was used, with modifications to simulate HF or LQT2. MC-II-157c effects were modelled by modifying the \( I_{Kr} \) formulation (conductance decreased by 12%, activation shifted by -14 mV and inactivation by 14 mV, and deactivation slowed 3.3-fold) and blocking \( I_{Na} \) by up to 90%. These cell models were incorporated into a one-dimensional model of the heterogeneous left ventricular wall, which was used to map out the vulnerable window (VW), the spatiotemporal region where an extra stimulus applied during the repolarisation phase results in unidirectional propagation block (Benson et al., 2011).

In single HF cells paced to 1 Hz periodic steady-state, TDR was 190 ms. The \( I_{Kr} \) modification alone decreased TDR to 148 ms, mainly by decreasing midmyocardial action potential duration (APD). 50% \( I_{Na} \) block alone decreased TDR to 179 ms, due to an increased epicardial APD. MC-II-157c (i.e. the \( I_{Kr} \) modification with 50% \( I_{Na} \) block) decreased TDR to 148 ms through a combination of reduced midmyocardial APD and increased epicardial APD. In the HF tissue model (TDR = 85 ms) the same pattern of TDR reduction as in single cells was observed: TDR for \( I_{Kr} \) modification = 85 ms, for 50% \( I_{Na} \) block = 79 ms and for MC-II-157c = 60 ms. \( I_{Na} \) block exerted an additional decrease in TDR through slowed transmural activation: epicardial tissue was excited, and thus repolarised, later. Accordingly, the maximum temporal width of the VW decreased with \( I_{Kr} \) modification and with 50% \( I_{Na} \) block (from 35.7 ms to 19.0 and 25.8 ms respectively), and these reductions were enhanced when both effects were combined as in MC-II-157c, when \( VW = 14.3 \) ms. Qualitatively similar results were found for the LQT2 model. Thus, we found that the hERG activator MC-II-157c reduces vulnerability to re-entrant arrhythmias due to the cumulative effects of \( I_{Kr} \) activation and \( I_{Na} \) block.


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**PC022**

**Effect of flecainide derivatives on sarcoplasmic reticulum \( Ca^{2+} \) release confirms a lack of direct action on the cardiac ryanodine receptor**


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The class 1c anti-arrhythmic flecainide is a well-characterised use-dependent blocker of the cardiac \( Na^+ \) channel. Elucidation of the mechanisms involved in this block has been facilitated by the use of fully charged (QX-FL) and neutral (NU-FL) derivatives of flecainide. In these studies it was established that Na’ channel block requires entry of the cationic form of the molecule into the cytosolic vestibule of the open channel. Flecainide has recently been shown to be effective in the treatment of catecholaminergic polymorphic ventricular tachycardia (CPVT), however the mechanism of flecainide action is contentious.

In this study, we have sought to determine how the flecainide derivatives influence RyR2-mediated Ca\(^{2+}\)-release from the sarcoplastic reticulum and whether this correlates with their effectiveness as \( Na^+ \) channel blockers and/or blockers of RyR2. To investigate this, we compared the ability of flecainide, QX-FL and NU-FL to modulate the properties of Ca\(^{2+}\) sparks in intact adult rat cardiac myocytes with their ability to block cation flux in individual recombinant human RyR2 channels reconstituted into planar lipid bilayers. NU-FL was synthesized by a novel route. Cardiomyocytes were dialyzed via patch pipette with either vehicle control solution, or with flecainide, QX-FL or NU-FL (5\u201dM) and were stimulated via depolarization from -80 to +30 mV (2Hz, 30s). Spontaneous Ca\(^{2+}\) sparks were recorded during a subsequent 20 second quiescent period. Data are given as mean±SEM. Intracellular application of flecainide or QX-FL resulted in equivalent suppression of Ca\(^{2+}\) spark frequency (34.96±6.23% and 28.49±9.86% reduction, respectively, p<0.05 (unpaired t-test) compared to control cells) whereas NU-FL did not reduce Ca\(^{2+}\) spark frequency. Flecainide, QX-FL and NU-FL had no measurable effect on Ca\(^{2+}\) spark amplitude or mass. For single channel studies, we reconstituted recombinant human RyR2 channels into planar lipid bilayers under voltage clamp conditions in symmetrical 610mM KCl. We found that both QX-FL and NU-FL were dose- and voltage-dependent open channel blockers of the non-physiological cytosolic to luminal flux of ions through the channel but were significantly less effective than flecainide (n=6). The fractional conductances of the blocked state were different for each compound. However, neither cytosolic QX-FL nor NU-FL was able to influence the luminal to cytosol flux of cations through the RyR2 channel. Given its inability to block this physiologically-relevant cation flux through RyR2, the effect of QX-FL on Ca\(^{2+}\) sparks is likely, by analogy with flecainide, to result from block of the \( Na^+ \) channel. Our data reveal important information about differences in the nature of the interaction of flecainide with sites in the cytosolic vestibules of Na’ and RyR2 channels.


Supported by the BHF.

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**PC023**

**Zinc modulates RyR2 function and may lead to “leaky” channels in heart failure**


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In heart failure, cardiac ryanodine receptors (RyR2) become abnormally active or “leaky” and are unable to remain closed...
during diastole (1). This results in unwanted irregular contractility. Inhibition of channel gating by Mg\(^{2+}\) helps prevent inappropriate activation of RyR2. Ablation Zn\(^{2+}\)-homeostasis is associated with chronic heart failure (2) raising the question to the role of Zn\(^{2+}\) in regulating RyR2 function. Here we have studied how Zn\(^{2+}\) influences the gating of single native RyR2 channels. Sheep RyR2 channels were incorporated into planar phosphatidylethanolamine lipid bilayers under voltage-clamp conditions as previously described (3). Unless stated, single-channel recordings were obtained with 250mM HEPES, 80mM Tris, 10mM HEPES, pH to 7.2 with Ca(OH)\(_2\) (free [Ca\(^{2+}\)] approximately 50mM) on the trans (luminal) side of the bilayer. The luminal chamber was voltage-clamped at ground. Open probability (Po) was determined over 3 min of continuous recording. Student’s t-test was used to assess the difference between mean values. In cardiomyocytes the resting intracellular Zn\(^{2+}\) concentration is reported to be at 100pM (4) Interestingly the addition of 100pM Zn\(^{2+}\) to the cytosolic face of the channel significantly increased RyR2 activity from 0.10 ± 0.03 to 0.45 ± 0.04 (S.E.M; n=4; P<0.05). Under these conditions, channel activation was still dependent on the presence of activating levels of cytosolic Ca\(^{2+}\). At concentrations of free Zn\(^{2+}\) reported to occur under pathophysiological conditions (>1nM) Zn\(^{2+}\) became the main activating ligand and the dependency on Ca\(^{2+}\) was removed. In line with previous studies (5), we show that exposure of RyR2 to 1mM cytosolic Mg\(^{2+}\) reduced channel Po from 0.34 ± 0.05 to 0.05 ± 0.01 (S.E.M; n=2). In the continued presence of 1mM Mg\(^{2+}\) the subsequent addition of 100pM Zn\(^{2+}\) to the cis chamber caused a marked increase in channel Po (0.36 ± 0.04; S.E.M, n=2). This suggests that Zn\(^{2+}\) may play a key role in regulating channel function enabling RyR2 to operate under conditions of systole. When the cytosolic [Ca\(^{2+}\)] was lowered to 100nM, our preliminary data (n=1) reveal that in the continued presence of 1mM Mg\(^{2+}\) the subsequent addition of 1nM cytosolic Zn\(^{2+}\) caused channel Po to increase from 0.06 to 0.19. Under these conditions RyR2 channels are expected to remain closed. This suggests that pathophysiological levels of Zn\(^{2+}\) may lead to irregular opening of RyR2 channels during diastole. We propose that Zn\(^{2+}\) plays a key role in shaping intracellular Ca\(^{2+}\)-dynamics through modulation of RyR2 and that pathological perturbations in Zn\(^{2+}\)-homeostasis may lead to inappropriate release of Ca\(^{2+}\) leading to the progression of heart failure and fatal arrhythmias.

Meissner G et al. (1986). Biochemistry 25, 236-244.

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PC024

Actions of a multi-component medication, SKT, on skeletal, smooth and cardiac muscle
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To investigate the actions of Shakuyaku-kanzo-to (SKT), a standardized 1:1 combination of two root powder extracts, paeony and licorice, we have studied its actions and those of its components on skeletal, smooth and cardiac muscles. The skeletal muscle experiments were performed on guinea-pig and rat phrenic nerve-diaphragm preparation using phrenic nerve stimulation and direct muscle stimulation. Smooth muscle experiments were done on guinea-pig ileum using the Magnus method to record both resting tension and responses to field stimulation. Whole heart experiments were performed on guinea-pig using Langendorff perfusion. The guinea-pigs were Duncan Hartley, 300-500g. Rats were male Long Evans or Wistar, 200-500g. Both were stunned by cervical dislocation.

We found (1) A large difference in time course of inhibition of contraction in smooth and skeletal muscles. In skeletal muscles the full effect develops over 20-30 minutes. By contrast in smooth muscle both resting and stimulated tension fall completely within 1-2 minutes. (2) The action on skeletal muscle is directly on the muscle, not necessarily or primarily on neuromuscular transmission. (3) The dose-response curve in skeletal muscle was found to be very steep, with a threshold around 1 mg/ml, and full inhibition around10 mg/ml. (4) Conditions for actions at lower concentrations as shown in a second abstract. (5) Experiments at 0.2mg/ml on Langendorff perfused whole heart show a small increase in force before a small slow fall. There is a fall in pressure, with only small effects on rhythm.

These results could be consistent with synergistic actions between multiple components but also with the idea that there is a cascade of events in a regulatory network of inter-actions. The time course of action in skeletal muscle sometimes appears to be step-wise, which might indicate that the cascades develop with different time course in different motor units. Further experimental work is required to investigate these and other possibilities.

The animals were killed according to UK legislation.

Left: rapid action of SKT in relaxing ileum muscle subjected to electrical field stimulation at a frequency of 1/min. SKT at a dose of 10 mg/ml was added to the bath at the vertical dotted line. Both resting and active tension are abolished within 1-2 minutes. Right; slow action of 10 mg/ml in skeletal muscle.
Dose-response curves for the action of SKT on skeletal muscle twitch for concentrations of TJ-68 between 1 and 10 mg/ml in the presence of normal potassium concentration. The half maximal concentration (IC50) is about 5 mg/ml. Vertical bars show standard error, n = 8.

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Extracellular potassium may potentiate the action of a multi-component medication, SKT, in skeletal muscle

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An accompanying poster (Sam et al 2015) on Shakuyaku-kanzo-to (SKT) showed that in normal physiological conditions the threshold for its action in inhibiting contraction in skeletal muscle is relatively high (1 mg/ml) compared to likely therapeutic levels. What could explain how much lower levels can achieve inhibition and why does it not inhibit general skeletal musculature? We determined whether interstitial potassium could be involved since experiments on humans show that interstitial potassium rises substantially during exercise (Green et al, 2000). Using the same methods as in Sam et al (2015), increasing extracellular potassium from 5.4 mM to 10.8 mM itself achieves inhibition of muscle contraction which develops with a time course remarkably similar to that produced by SKT. When SKT is then also applied further inhibition of contraction occurs (Fig 1), but with a much lower threshold concentration, around 0.1 to 0.2 mg/ml. Both interventions might act on a common component of the relevant cell networks to potentiate each other. Further experiments are required to investigate these and other possible explanations.

We used the Shorten et al (2007) model to determine whether interstitial [K] changes similar to those recorded experimentally can be predicted. The model was coded in CellML format to enable it to run in OpenCOR (http://www.opencor.ws/). Figure 2 shows that even modest frequencies of stimulation of contraction can achieve a significant rise in interstitial potassium. This model is now being developed to enable hypotheses to be explored that might help to explain the synergy between potassium and SKT.

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Changes in some electrocardiographic parameters amongst children with sickle cell anemia in Port Harcourt, Nigeria

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Sickle cell anemia is a genetic blood disorder affecting mostly Africans, Hispanics, Indians and people of Middle Eastern descent; involving major organs of which the heart is the most fatal and commonest cause of morbidity and mortality. The present study is aims to determine some electrocardiographic parameters of sickle cell anemia children attending the sickle cell clinic at the University of Port Harcourt Teaching Hospital, Nigeria.
A total of 118 subjects comprising of 55 sickle cell anemia (HbSS genotype) patients (Group A) and 63 normal controls consisting of 40 subjects (HbAA genotype) Group B and 23 subjects (HbAS genotype) Group C were recruited into the study. Control subjects were matched for weight and sex with sickle cell anemia (HbSS genotype) (Group A) patients. All subjects were aged between 2 and 15 years. Height, weight, body mass index, hemoglobin concentration and heart rates were determined and a thorough physical examination conducted to exclude the presence of co-morbidities. Electrocardiographic parameters were subsequently determined using a standard resting 12-lead electrocardiogram. Results were analyzed using analysis of variance; a p value < 0.05 was considered significant.

Results obtained showed no significant differences in age, body mass index and heart rate between patients with sickle cell anemia (HbSS genotype) and the control groups. However, control subjects had significantly higher haemoglobin concentration compared to sickle cell anemia (HbSS genotype) patients (p<0.05). Differences were also observed in some electrocardiographic parameters of sickle cell anemia (HbSS genotype) patients as compared to control subjects. For instance, higher percentages of left and right ventricular hypertrophy, ST segment depression, ischemic changes and axis deviation were observed amongst sickle cell anemia (HbSS genotype) patients compared to control subjects. Further, the QTC interval of Group A sickle cell anemia (HbSS genotype) patients was significantly higher than values for Group C (HbAS genotype) control subjects (p<0.05); however, the p axis of Group A sickle cell anemia (HbSS genotype) patients was significantly lower than values for Group C (HbAS genotype) control subjects (p<0.05).

Our study is of value and confirms suggestions that routine electrocardiography amongst children with sickle cell anemia can help detect those prone to arrhythmia, ischaemic changes and sudden cardiac death and therefore enhance mortality and morbidity.


The authors acknowledge the assistance of Professor OJ Odia in the course of this study.

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PC028

Changes in the expression of connexins and Ca\(^{2+}\)-handling proteins at the Purkinje-ventricular junction in a rabbit model of heart failure

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The cardiac His-Purkinje system conducts electrical activity throughout the ventricles and, in heart failure, electrical conduction within the His-Purkinje network is impaired and about 36% of patients show left bundle branch conduction block. Intercellular gap junctions conduct the electrical signals from one cardiomyocyte to another and these junctions are composed of proteins known as connexins (Cx). We have developed a rabbit model of congestive heart failure (CHF)
The effects of a 2.5% weight loss on systolic blood pressure, baroreceptor sensitivity and autonomic modulation in young women

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The prevalence of obesity and hypertension among women in Saudi Arabia is increasing at an alarming rate (Al-Nozha et al., 2005). The relationship between obesity and hypertension is well established with obese subjects tending to have higher BP than the non-obese, even in the normotensive range (Kotsis et al., 2010). Most clinical weight loss interventions recommend a five to ten percent weight loss – a level which has been shown to result in a significant drop in blood pressure especially among young women (Andrade et al., 2012). This level of weight loss is however difficult to achieve and maintain (Sarlio-Lahteenkorva et al., 2000). We therefore evaluated the effects of a minor (~2.5%) loss of body weight on systolic blood pressure (SBP), baroreceptor sensitivity (BRS) and sympathetic modulation in young women. Twenty-five women volunteers were randomly assigned to a 4-week dietary weight-loss or weight-maintenance program. Spectral analysis of beat-to-beat blood pressure variability was used to derive sympathetic modulations prior to and after 4 weeks. Baroreceptor sensitivity was determined using regression coefficients between the rise in systolic pressure and the widening of the RR interval following Valsalva’s maneuver. Statistical analyses used were a two- (control vs experimental group) by two (pre and post treatment) analysis of variance (ANOVA) and posthoc analyses. The weight loss group lost ~2.5% of their weight; this resulted in a significant drop of 7.5% in SBP (p < 0.05), a significant increase in BRS (p < 0.05), and a significant decrease in sympathetic modulation at rest (p < 0.05), compared with no significant changes in the weight maintenance group. Our study suggests that minor weight loss may decrease cardiovascular disease risk factors in young women. We propose conducting longitudinal studies involving minor but repeated additive weight losses as a non-pharmacological intervention for obesity.

Within and between group results for the variables of weight, systolic blood pressure (SBP), baroreceptor sensitivity (BRS) and sympathetic modulation (LF\textsubscript{SBP})

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Weight loss group</th>
<th>Weight maintenance group</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>20±2.7</td>
<td>21±3.7</td>
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<tr>
<td>24</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Weight (kg)</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>70±2</td>
<td>68.5±11.8</td>
<td>69±2</td>
<td>67.9±16.5</td>
</tr>
<tr>
<td>67.5±10.4</td>
<td>64±2.2</td>
<td>66±2</td>
<td>71.5±16.3</td>
</tr>
</tbody>
</table>

SBP (mmHg)

| 127±10.5 | 118±14.9 | 124±18.8 | 125±6.5 |

BRS (ms/mmgHg)

| 6.4±2.1 | 4.8±2.4 | 6.4±2.7 | 7.7±3.3 |

LF\textsubscript{SBP} (m/sac)

| 61.7±10.4 | 64±12.7 | 51.8±15.5 | 54.6±10.4 |

Values are given as mean ± S.D. * Denotes significant differences within groups; † denotes significant differences between groups.

Al-Nozha et al. (2005). 26, 824-829
Kotsis et al., (2010). Hypertension Res 33, 386-393

This work was funded by an internal grant from Alfaisal University College of Medicine
Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC029

The statin paradox: Cardiovascular protection versus skeletal myopathy, comparison of spark characteristics between heart and skeletal muscle

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Statins, inhibitors of HMG CoA reductase, are the most widely prescribed drugs for prevention of cardiovascular disease. The primary reason for cessation of statin therapy is skeletal myopathy but the underlying mechanism and apparent absence of detrimental effects on cardiac muscle function is not understood. Here, we compare the effect of chronic statin treatment in vitro on Ca\textsuperscript{2+} sparks in heart and intact skeletal muscle. Male Wistar rats were given simvastatin (40 mg/kg) daily by oral gavage over a 4 week period; control animals received saline. Ventricular myocytes and flexor digitorum brevis (FDB) fibres were isolated by collagenase digestion and
Effects of flecainide on Ca\(^{2+}\) sparks and waves in saponin permeabilised rat ventricular myocytes

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Flecainide is effective at preventing delayed after depolarisations and associated arrhythmias in catecholaminergic polymorphic ventricular tachycardia (CPVT), however its primary mode of action remains contentious (1-4). While initial studies using CPVT transgenic mice concluded that flecainide had a direct action on the ryanodine receptor (RyR2), which reduced Ca\(^{2+}\) wave frequency (1), subsequent reports suggested that its effects are entirely due to Na\(^{+}\) channel (Nav1.5) block (2-4). In this study, the effects of flecainide on sarcoplasmic reticulum Ca\(^{2+}\) regulation were investigated in saponin permeabilised ventricular myocytes isolated from adult male Wistar rats. Rats were killed according to Schedule 1 methods. Following isolation and permeabilisation, cells were perfused with a mock intracellular solution containing fluo-3 pentapotassium salt (15 μM) and Ca\(^{2+}\) release events were visualised using line scan confocal microscopy. Ca\(^{2+}\) sparks were automatically detected and analysed using the Sparkmaster plugin for ImageJ. Statistical significance (p<0.05) was determined using a paired Student’s t-Test or one way repeated measures ANOVA. Values are mean ± S.E.M. n=cell number. Ca\(^{2+}\) wave frequency was investigated after 3 min perfusion with or without 25 μM flecainide and results were grouped according to initial wave frequency. At low wave frequencies (<5 waves/min or 6–9 waves/min), 25 μM flecainide significantly reduced wave frequency by 25.8 ± 7.8 % (p<0.01, n=15) and 14.6 ± 4.6 % (p<0.01, n=17) respectively. At high initial wave frequencies (>10 waves/min) there was no significant change (p>0.05, n=14). There was no significant change in wave frequency in time-dependent controls within any group (p>0.05, n=7). In separate experiments, the effects of flecainide on Ca\(^{2+}\) sparks were studied in the absence of Ca\(^{2+}\) waves. The amplitude of Ca\(^{2+}\) sparks decreased by 15.5 ± 2.97 % (p<0.001, n=30); width significantly decreased by 15.9 ± 1.66 % (p<0.001, n=30); frequency significantly increased by 36.0 ± 10.4 % (p<0.01, n=30); and there was no significant change in spark duration (p>0.05, n=30). There was no significant change in spark properties in time-dependent controls (n=10, p>0.05). These experiments provide evidence that in wild-type rat myocytes, flecainide can produce qualitatively similar changes in Ca\(^{2+}\) sparks and waves to those reported in permeabilised myocytes from CPVT mice, albeit at a higher drug concentration (25 vs 6 μM) (1). The data are consistent with an effect on RyR2 gating under conditions where the channel remains in situ and the influence of Nav1.5 is excluded. The apparent ineffectiveness of flecainide at high initial Ca\(^{2+}\) wave frequencies may contribute to recent discrepancies in the literature. Hilliard FA, Steele DS, Laver D, Yang Z, Le Marchand SJ, Chopra N, Piston DW, Huke S, Knollmann BC. Flecainide inhibits arrhythmogenic Ca\(^{2+}\) waves by open state block of ryanodine receptor Ca\(^{2+}\) release channels and reduction of Ca\(^{2+}\) spark mass. Journal of molecular and cellular cardiology. 2010 Feb;48(2):293-301.


Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
widely used in the clinical treatment of atrial fibrillation; it blocks hERG/I\textsubscript{sc} at clinically relevant concentrations (Breindahl 2000; Paul et al. 2002) and, rarely, can induce life-threatening ventricular tachycardia (Torsade de Points), associated with rate corrected QT interval prolongation that is not fully accounted for by widening of the QRS complex (Thevenin et al. 2003; Oguayo et al. 2014). Although hERG block by flecainide has previously been reported (Paul et al. 2002), the flecainide binding site on hERG has not yet been identified. The aim of this study was to determine which residue(s) in the hERG channel pore is/are responsible for the interaction. Flecainide was tested on alanine-mutants of hERG transiently expressed in HEK293 cells. hERG current (I\textsubscript{hERG}) was recorded using whole-cell patch-clamp at 37°C. Concentration response relations from which half maximal inhibitory concentrations (IC\textsubscript{50}) were derived, were produced from application of multiple drug concentrations (Na\textsubscript{5} at each concentration). The selected residues included V625, G648, Y652 and F656, all known to be determinants for hERG block, located within the pore region of the channel (Mitcheson et al. 2000). I\textsubscript{hERG} tails were elicited at -40mV or -120mV after a depolarizing step from -80mV to +20mV. GOLD docking software was then employed to simulate the interaction between flecainide and a MthK-based hERG homology model. Docking outputs were ranked according to their energy scores and the best poses (lowest energy scores) were selected for further analysis. Flecainide was ~142-fold less potent on F656A (compared to its WT under similar conditions). Docking simulations supported these results: the inner mouth of the pore cavity, with the benzamide and in the best low energy score poses, flecainide docked low into the aromatic ring of F656.

Oguayo KN et al. (2014). Pharmacotherapy 34, e30-e33.

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

Comparison of cardiac ion channel remodelling in acute heart failure and chronic hypertrophy in the rabbit

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Cardiac hypertrophy and failure give rise to disability and death on account of arrhythmias and pump failure. However, the cardiac phenotype can vary widely. Here we used a rabbit (NZW) model of volume and pressure overload of the left ventricle (LV) - two degrees of volume overload were induced. Aortic regurgitation was induced by catheter in anaesthetised rabbits (ketamine 25 mg/kg i.m. with 2.5% isoflurane in O\textsubscript{2}) to give increases in aortic pulse pressure of ~100% in the acute heart failure group (AHF, n=8) and ~50% in the chronic hypertrophy group (CHT, n=8). After 3 weeks, pressure overload was induced by abdominal aortic banding. The rabbits were kept for 8 (AHF) or 78 (CHT) weeks. Study was conducted in accordance with the Animals (Scientific Procedures) Act 1986. After sacrifice, ion channel transcript expression in different parts of the heart was measured using quantitative PCR (differences tested using 2-way ANOVA).

The AHF but not the CHT group showed evidence of heart failure - serious cavity effusions and pulmonary congestion. Echocardiography showed increased LV internal dimensions in both groups. At termination, fractional shortening was reduced to ~25% in the AHF group and preserved at ~35% in the CHT group. Intrinsic heart rate was slower and the PR interval and QRS duration were prolonged in the AHF group while unchanged in the CHT group. The CHT group, however, showed 75% incidence of ventricular arrhythmias after automatic blockade.

In the AHF group, marked remodelling was seen mainly in the sinoatrial node (SAN) and left Purkinje fibres with 12 and 19 out of 25 transcripts studied downregulated (and 1 upregulated in SAN). LV displayed 5 upregulated (Nav1.5, Kir2.1, SUR2a, NCX1, RYR2) and 1 downregulated (KChIP2) transcripts. Surprisingly, there were no changes in the right ventricle (RV) in the AHF group. In contrast, changes in the CHT group occurred more evenly throughout the heart. The RV showed the most striking remodelling with upregulation of Nav1.5, Cav1.2 and Cav3.1 and most Kv channels; only Kv1.4 was downregulated. The right Purkinje fibres displayed upregulation of 2 Ca\textsuperscript{2+} channels, 4 K\textsuperscript{+} channels, RYR3 and SERCA2a. The SAN had 3 channel subunits downregulated (Cav1.3, KChIP2, KvQTL1). Right atrium showed reduction of 2 K\textsuperscript{+} channels and 2 gap junction channels, and upregulation of HCN4. LV showed upregulation of Cav1.3, Cav3.1 and RYR3. In conclusion, difference in the severity of volume overload has produced 2 distinct models with contrasting responses at whole animal and mRNA levels. More severe overload causes acute HF with major downregulation of ion channels in the SAN and left Purkinje fibres, possibly induced by stretch. Less severe volume overload leads to compensated hypertrophy with a more uniform response, and in particular upregulation of gene expression in the RV and right Purkinje fibres.


BHFRC/06/005
Novel mitochondrial potassium channel openers uncouple respiration and decrease reactive oxygen species production in isolated rat heart mitochondria

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Background. A large body of evidence unequivocally demonstrated that pharmacological modulation of the mitochondrial ATP-sensitive potassium channels (mitoKATP) is protective against myocardial ischemia/reperfusion injury. The present work was aimed at assessing the dose-dependent effect of four novel mitoKATP openers - benzopyranyl analogues (L-1487, KL-1492, KL-1495, and KL-1507) on mitochondrial respiration and reactive oxygen species production in isolated rat heart mitochondria.

Materials and methods. Experiments were performed on Sprague Dawley (SD) adult female rats (4-6 months, n = 5-6/group) anesthetized by the intraperitoneal administration of a mixture of ketamine (30 mg/kg) and xylazine (5 mg/kg). Rat heart mitochondria were isolated by differential centrifugations at 4°C. Mitochondrial respiratory function was assessed by high-resolution respirometry at 37°C (Oxygraph-2k, Orophoros Ltd.). Hydrogen peroxide (H2O2) release was determined with the Amplex Red fluorescence assay. Four different concentrations: 50, 75, 100 and 150 μM of the compounds were tested.

Results. When applied in higher concentrations (100 and 150 μM) a significant increase of state 2 and state 4 respiratory rates for mitochondria respiring in the presence of both complex I (CI) and complex II (CII) substrates was found. However, the maximal concentration (150 μM) elicited an important decrease of the oxidative phosphorylation. When applied in the higher concentrations (100 and 150 μM) all investigated compounds determined a significant decrease of mitochondrial H2O2 production in mitochondria respiring on complex I substrates (glutamate/malate).

Conclusion. The novel mitochondrial KATP openers are able to elicit mild uncoupling and decreased oxidative stress in isolated rat heart mitochondria, properties that are likely to be effective in cardioprotection.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Caveolin-3 (Cav-3) plays an important role in localizing L-type calcium current (I_{Ca}) to the t-tubules of cardiac ventricular myocytes, via localized protein kinase A (PKA)-dependent phosphorylation (1). Ageing is associated with a decline in cardiac function, and a decrease in I_{Ca} that appears to occur predominantly at the t-tubules (2). We have, therefore, investigated the role of Cav-3 and PKA in this decrease, using ventricular myocytes isolated from 3 month (3 mo) and 24 month (24 mo) old wild type (WT) and cardiac-specific Cav-3 overexpressing (Cav-3 OE) mice.

Animal procedures were approved by local ethics committee and conducted in accordance with UK legislation. Ventricular myocytes were isolated by enzymatic digestion of hearts from male Cav-3 OE or WT littermate mice. I_{Ca} was recorded using the whole-cell patch-clamp technique in intact cells and following acute detubulation (DT, using formamide-induced osmotic shock (3)), at 22-25°C. I_{Ca} was elicited by a 500 ms step depolarisation from -40 to 0 mV following a 200 ms pre-pulse from -80 to -40 mV, at 0.2Hz and measured as the difference between peak current and that at the end of the pulse. I_{Ca} recorded from DT myocytes represents I_{Ca} at the surface membrane and the difference in I_{Ca} between intact and DT myocytes gives I_{Ca} at the t-tubule membrane. Data are expressed as mean±SEM (n cells). Student’s t-test or ANOVA were used for statistical analysis, with the Bonferroni post hoc test; the limit of statistical confidence was p<0.05.

In WT mice, I_{Ca} density was ~ 28% (p<0.001) smaller in myocytes isolated from 24 mo old compared to 3 mo old mice. In contrast, I_{Ca} density was not significantly different in myocytes isolated from 24 mo and 3 mo old Cav-3 OE mice (3 mo, -4.8±0.3 (n=25); 24 mo, -5.1±0.3 (n=28), pA/pF, p<0.01) but decreased to the same level following DT. However, in intact cells from Cav-3 OE mice in the presence of H-89, I_{Ca} density was smaller in myocytes from 24 mo compared to 3 mo WT mice (3 mo, -2.5±0.2 (n=12); 24 mo, -1.6±0.1 (n=11), pA/pF, p<0.01) but decreased to the same level following DT. In Cav-3 OE myocytes isolated from 3 mo and 24 mo old Cav-3 OE mice; thus, calculated I_{Ca} density in the t-tubules was not significantly different at the two ages in Cav-3 OE mice (3 mo, -9.1±0.4; 24 mo, -9.3±0.3, pA/pF). In the presence of the PKA inhibitor H-89, I_{Ca} density was smaller in myocytes from 24 mo compared to 3 mo WT mice (3 mo, -1.7±0.2 (n=10); 24 mo, -1.6±0.1 (n=17), pA/pF, ns) and decreased to the same level following DT. These data suggest that Cav-3 OE may be protective against the decrease in t-tubular I_{Ca} density observed in aged WT mice. The effect of age, and the protective effect of Cav-3 OE, persist in the presence of H-89 and may therefore reflect, in part, t-tubular calcium channel density.


This work was supported by the British Heart Foundation.

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PC037

Ca transients in aged murine ventricular myocytes are not altered by caveolin-3 overexpression

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Ageing is associated with a decline in cardiac output and a decrease in t-tubular L-type Ca current (I_{Ca}), which comprise the trigger for sarcoplasmic reticulum (SR) Ca release, and...
Thus contraction, in ventricular myocytes (1). This decrease in t-tubular IC₃ is absent in mice that overexpress cardiac caveolin-3 (Cav-3), which appears to localize protein kinase A activity at the t-tubules. We therefore investigated whether cardiac overexpression (OE) of Cav-3 (2) also protects against changes in the systolic Ca transient and its regulation with age.

Animal procedures were approved by the local ethics committee and conducted in accordance with UK legislation. Cells isolated from the ventricles of Cav-3 OE and wild type (WT) littermate control mice at 3 or 24 mo of age were field-stimulated at 0.1, 0.2 and 1.0 Hz at room temperature, and intracellular Ca transients monitored using Fluo-4/AM in conjunction with confocal microscopy. β-adrenergic stimulation was provided by 100 nM isoprenaline (ISO). Data were analysed using custom routines written in MATLAB (R2014b) and 2-way repeated measures ANOVA was used for statistical analysis.

Fig. 1A shows that WT myocytes had a relatively flat Ca transient amplitude-frequency relationship over the range of stimulation frequencies studied, and that Ca transient amplitude was slightly, but not significantly, smaller at each frequency in the cells from 24 mo compared to 3 mo WT mice. These changes were associated with little change in time to peak (TP) or the time to decay to half amplitude (T½). Fig. 1A also shows that OE had no significant effect on the force-frequency relationship in either age group, nor did it significantly affect TP or T½. In 3 mo WT myocytes, ISO increased Ca transient amplitude by ~74% and TP by ~33%, and decreased T½ by ~30%, but had no significant effect in 3 mo OE myocytes. In contrast, 24 mo WT and OE cells showed a similar response to ISO (Fig. 1B).

These data show that the decrease in t-tubular IC₃ with age is accompanied by only a modest decrease in Ca transient amplitude, and that the effects of Cav-3 on t-tubular IC₃ density are not reflected in the Ca transient, suggesting that other factors are determining Ca transient amplitude. However, Cav-3 OE may cause age-related changes in the response to β-adrenergic stimulation.

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PC038

Altered distribution of Na-Ca exchange in ventricular myocytes from failing rat hearts

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The balance between Ca influx and efflux regulates intracellular Ca (Ca_{i}) in cardiac myocytes (1). The main Ca influx pathway, I_{Ca}, has recently been shown to be redistributed from t-tubules (TT) to surface sarcolemma (SS) in ventricular myocytes from failing hearts (2). However, whether the main Ca efflux pathway, Na-Ca exchange (NCX), is redistributed in heart failure is unknown.

Animal procedures were performed in accordance with UK legislation and approved by the local Ethics Committee. Coronary artery ligation (CAL) and sham operations (Sham) were performed in adult male Wistar rats under surgical anaesthesia (ketamine 75 mg/kg, medetomidine 0.5 mg/kg ip) with appropriate analgesia (buprenorphine 0.05 mg/kg sc). 18 weeks after surgery hearts were excised under pentobarbitone anaesthesia (140 mg/kg ip) and left ventricular myocytes isolated by enzymatic digestion. NCX current (I_{NCX}) was measured at -80 mV during application of 10 mM caffeine (to release sarcoplasmic reticulum Ca), using whole-cell patch-clamp. Ca_{i} was measured simultaneously using fluo-4. Recordings were made in intact (Sham n=12, CAL n=8) and detubulated (DT; Sham n=11, CAL n=7) myocytes at room temperature. Data are presented as mean±SEM and analysed by 2-way ANOVA with Bonferroni post-test.

The rate of decline of the caffeine-induced Ca transient (k_{Caff}, a measure of sarcolemmal Ca efflux) in Sham cells was significantly slowed by DT (0.80±0.07 vs. 0.35±0.04 s⁻¹, P<0.001). k_{Caff} was slower in CAL than in Sham cells (0.41±0.05 s⁻¹ P<0.05) but was unaffected by DT (0.48±0.03 s⁻¹). Exposure to caffeine was repeated in the presence of 10 mM Ni (to inhibit NCX) and the rate of Ca removal via NCX calculated as the difference between the rate of removal in the absence and presence of Ni. In Sham cells, DT decreased the rate of Ca removal via NCX (k_{NCX}, 0.65±0.06 vs. 0.26±0.04 s⁻¹; p<0.001). k_{NCX} in CAL cells was not statistically different from Sham (0.35±0.05 s⁻¹), and was not altered by DT (0.35±0.03 s⁻¹). I_{NCX} density at 400 nM Ca_{i}, during the descending phase of the caffeine-induced Ca transient, was not significantly different between intact Sham and CAL cells (0.27±0.08 vs. 0.38±0.08 pA/pF). However, calculating TT-I_{NCX} from the difference in I_{NCX} between intact and DT myocytes confirmed that I_{NCX} was located predominantly at the TT in Sham myocytes, as reported previously (3). However, I_{NCX} in CAL cells was ~53% lower at the TT and ~270% higher at the SS, compared with Sham cells. These data demonstrate that I_{NCX} is redistributed from TT to SS in the rat CAL model of heart failure. Changes in the localization of NCX function are likely to contribute to abnormalities in Ca homeostasis and susceptibility to triggered pro-arrhythmic events in heart failure.


Feridooni HA et al. (2014). J Mol Cell Cardiol (In press).


This work was supported by the British Heart Foundation.


This work was supported by the British Heart Foundation. Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC039

Paracrine crosstalk between hypoxic cardiomyocytes and cardiac fibroblasts via extracellular vesicles

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Introduction: Altered expression of non-coding RNAs (ncRNAs) has been connected to many cardiac disease conditions (1). A hallmark of these conditions is insufficient oxygen supply (hypoxia). Some studies already showed that ncRNAs are transported by extracellular vesicles (EVs) and transferred to their target cells (2, 3). However, if ncRNA-enriched vesicles might be involved in myocyte-fibroblast crosstalk during hypoxia is not known so far. In this study, the paracrine communication mechanisms between hypoxic cardiomyocytes and cardiac fibroblasts via extracellular vesicles and the impact of hypoxic cardiomyocyte-derived vesicles mediating cardiac fibrosis is addressed.

Methods and Results: Murine cardiomyocytes (HL-1 cells) were exposed to normoxic (21% O2) and hypoxic (0.2% O2) conditions for 24h following 4h reoxygenation. EVs were purified from the conditioned medium of hypoxic and normoxic cardiomyocytes by differential centrifugation and ultracentrifugation steps. To investigate the morphology of isolated vesicle subsets, including apoptotic bodies, microvesicles and exosomes, we used transmission electron microscopy, showing a typical shape and structure of all vesicle subtypes. EVs were further analyzed by Western Blot and nanoparticle tracking, which revealed that cardiomyocyte-derived vesicles differ in protein composition and concentration. Moreover, we studied the secretion and uptake mechanisms of cardiomyocyte-derived vesicles by confocal laser microscopy and found that the vesicles were taken up by fibroblasts in a temperature- and time-dependent manner. To examine the effect on fibroblasts, we co-cultured them with conditioned medium of normoxic and hypoxic cardiomyocytes and measured the gene expression of fibrotic markers by qPCR. We observed that conditioned medium of hypoxic cardiomyocytes led to an increase in fibrotic markers such as collagen I, collagen III, connective tissue growth factor (p<0.05, n=4) and matrix metalloproteinase 2 (p=0.064, n=4) in fibroblasts (P-values are determined by Student’s t-tests). Co-culture of fibroblasts with individual fractions of isolated vesicles revealed that mainly the microvesicle fraction contributes to a pro-fibrotic response. The results further indicate a paracrine cardiomyocyte-fibroblast crosstalk mediated by microvesicles. Moreover, analysis of the RNA content of cardiomyocyte-derived vesicles identified a large amount of small RNAs, but also larger RNAs which might play a role in this paracrine communication.

Conclusion: In response to hypoxia, cardiomyocytes produce and secrete different subtypes of EVs which are taken up by fibroblasts, triggering a fibrotic response in cardiac fibroblasts. Moreover, cardiomyocyte-derived vesicles contain small non-coding RNAs which might be involved in the paracrine crosstalk between cardiomyocytes and cardiac fibroblasts during hypoxia.


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PC040

T-tubule organisation is not altered in aged murine cardiac ventricular myocytes

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Ageing is associated with smaller, slower Ca transients in cardiac ventricular myocytes, which have been attributed to reduced Ca current and ryanodine receptor density and slowed Ca uptake by sarcoplasmic reticulum (1). However, the possible role of changes in the organisation of transverse (t-) tubules, invaginations of the ventricular cell membrane that are crucial to excitation-contraction coupling, is unknown. The role of caveolin-3 (Cav-3), a structural protein that has been implicated in the formation of t-tubules (2), in determining t-tubule structure with age, is also unknown.

We therefore investigated t-tubule structure in ventricular myocytes isolated from wild type (WT) and cardiac-specific Cav-3 overexpressing (OE) mice at 3 weeks (w), 3 and 24 months (mo) of age. Animal procedures were approved by local ethics committee and conducted in accordance with UK legislation. Myocytes stained with di-8-ANEPPS were imaged on a confocal microscope. Image stacks were deconvolved and then, using a novel algorithm that does not rely on threshold (e.g. 3, 4), t-tubules were skeletonised (Fig. 1) and t-tubule density and orientation quantified. T-tubule regularity was determined using a discrete two-dimensional Fast Fourier Transform (FFT). Data are expressed as mean±SEM of n cells and were analysed using the Kruskal-Wallis or Mann-Whitney test as appropriate, with significance taken as p<0.05.

T-tubule structure was not significantly different at the three ages examined. In 3w (n=11), 3mo (n=22) and 24mo (n=15) WT myocytes, FFT analysis showed no significant difference in the spatial frequency of the first harmonic (~0.56 μm) or associated power (0.08±0.02, 0.05±0.01 and 0.10±0.03). Nor was there any change in t-tubule density (0.76±0.09, 0.69±0.03 and 0.60±0.04 μm/μm2) or proportion of longitudinal tubules (44±3, 44±1 and 43±3%) with age. Similar data were obtained in OE cells: in 3w (n=28), 3mo (n=30) and 24mo (n=34), spatial frequency was ~0.56 μm and FFT power was 0.07±0.01, 0.06±0.01 and 0.10±0.03, respectively. T-tubule density was also similar (0.70±0.03, 0.71±0.03 and 0.61±0.03 μm/μm2), as was the proportion of longitudinal tubules (48±2, 43±3 and 41±%}.
AMPK activity may thus compromise the capacity to accommodate modulation of the respiratory network. Deficits in the ability to breathe during hypoxia and resist apnoea via right-side left asymmetry (Vallortigara et al., 2005) with respect to ventilation. Surprisingly AMPK deletion revealed pronounced deficits in those that comprise the hypoxia-responsive respiratory network, from carotid body to brainstem (Guyenet, 2000). Moreover, mice lacking AMPK exhibit marked reduced breathing associated with metabolic syndrome-related disorders (Chau et al., 2012; Ruderman et al., 2013).


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PC041

AMPK couples oxygen to energy supply at the whole-body level by delivering increased drive to breathe during hypoxia and thus protects against apnoea

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The mechanisms by which hypoxia increases drive to breathe remain controversial. We assessed the role in this process of the AMP-activated protein kinase (AMPK), which is central to cell autonomous adaptations during metabolic stress (Hardie, 2007). Hypoxia-evoked increases in minute ventilation were abolished by deletion of AMPKα1 and α2 catalytic subunits in TH(tyrosine hydroxylase)-expressing cells, which comprise a subset of those that comprise the hypoxia-responsive respiratory network, from carotid body to brainstem (Guyenet, 2000). Moreover, mice lacking AMPK exhibited marked hypoventilation and apnoea during hypoxia, rather than hyper- ventilation. Surprisingly AMPK deletion revealed pronounced right-left asymmetry (Vallortigara et al., 2005) with respect to the activation by hypoxia of dorsal and ventral nuclei of the caudal brainstem; assessed by functional magnetic resonance imaging. We conclude, therefore, that AMPK increases drive to breathe during hypoxia and resists apnoea via right-side dominant modulation of the respiratory network. Deficits in AMPK activity may thus compromise the capacity to accommodate respiratory depression during hypoxia, and identifies this signalling pathway as a therapeutic target for sleep disordered breathing associated with metabolic syndrome-related disorders (Chau et al., 2012; Ruderman et al., 2013).

Modern education should aim to assist the mind to think rather than just packing it up. Individualism in learning methods remains a major pedagogic concern for the academicians yet today. Additionally, the trainees’ own forte and flaw also adjunct in learning process, significantly. We therefore assessed students’ preference following sensory modalities of learning approach using visual, aural, read/write and kinesthetic (VARK) questionnaire. Moreover, to figure out any significant variations in such preference affected by variables like, genders or study years. The qualitative study was conducted among the undergraduates of Holy Family Red Crescent Medical College, Dhaka, Bangladesh and Km. University, Dhaka, Bangladesh.

Learning style preference of undergraduate medical students: A VARK Analysis

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Learning style preference of undergraduate medical students was considered as significant while analyzing the data using Pearson’s Chi square and ANOVA. Value of p<0.05 was considered as significant while analyzing the data using SPSS v. 16. Out of 180 respondent 141 female and 123 were from year 1. The responses were tallied and assessed for gender and level of study according to learning style preference. The qualitative study was conducted among the undergraduates of Holy Family Red Crescent Medical College (HFRCMC), Bangladesh in December 2014. This was a descriptive, cross-sectional, questionnaire based survey where the year 1 and year 2 medical students were included. Ethical approval was obtained from institution ethics committee. Consent was taken from the participants. The stepping stone method (1) was employed to collect the data from respondents including certain socio-demographic information. Copyright permission was granted from the VARK developers. The stepping stone method (2) was applied to comment on the respondent’s particular preference. While mean was calculated for quantitative variables, frequencies were determined for qualitative data, which were then analyzed with Pearson’s Chi square and ANOVA. Value of p<0.05 was considered as significant while analyzing the data using SPSS v. 16. Out of 180 respondent 141 female and 123 were from year 1. The responses were tallied and assessed for gender and level of study according to learning style preference. More than half (55.6%) of the participants were found to have multimodal learning preferences, with the most common being tri-modal (17.5%), followed by bimodal (13.1%) and unimodal (15.9%). The hierarchy of single mode preferences (44.4%) were as follows: 46% ofread, 26% of aural, 25% of visual, and 25% of kinesthetic.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
5 visual followed by 3 read/write. No significant association was existed in sensory preferences with gender, academic background or year of study. It became evident from this study, that majority of HFRMCM undergraduates preferred multimodal learning strategies. This is remains the pioneer study of its kind and rarely reported from this region indicating medical teachers to assimilate their presentation style to help out the medicos towards productive education. Hence, re-designing instructional methods in accordance with students’ inclination demand to be tailored further to potentiate learning.


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PC044

**Engaging with Dementia: From Bench to Bedside**

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There is significant public interest in science. Further, sixty-five percent of the UK adult population would like scientists to spend more time discussing their science and ethical implications of this science with them (Ipsos-Mori, 2014). The Faculties of Biological Sciences and Medicine and Health at the University of Leeds undertake significant research into the causes of Alzheimer’s disease and therefore the aim of this project was to engage the public with this research Engaging with Dementia: From Bench to Bedside comprised of three dissemination activities. For Key Stage 5 students, a workshop comprising of a “hands-on” laboratory session in which students recorded intracellularly from snail brain neurones and investigated the effects of ion channel modulators on neuronal firing, followed by a discussion of the ethical issues arising from research into Alzheimer’s disease, was developed. For the general public, a series of 24 open educational resources which took the them from the basic science underpinning neuronal function and Alzheimer’s disease, through more advanced concepts to current research being undertaken at Leeds was created and uploaded onto the project website. These resources included computer interactive, text articles and extended video podcasts of researchers talking about their research. Finally, a two day interactive public exhibition, Healthy Brains @ Leeds: Demystifying Dementia, was held at the Leeds City Museum. Visitors learnt how the brain works, discuss with scientists their research and join in the debate on future priorities for dementia research. Feedback from all activities was excellent:

“Great opportunity to get into a University lab to study interesting and relevant science in a hands on way…… well explained and accessible.”

“Great event, very important we raise awareness of dementia”

“Superb - wish we could have stayed longer”

This project has achieved its aim of engaging a wide cross section of the public with research into the causes of Alzheimer’s disease currently being undertaken at the University of Leeds. Further, the resources created will provide a long lasting legacy for the project.

Engaging with Dementia. http://www.fbs.leeds.ac.uk/blogs/dementia/


This project was funded by an Alzheimer’s Society public engagement grant. The support of the Society is gratefully acknowledged.

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PC043

**Student centered approach in Biomedical Sciences final year research project module**

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Final year research project module plays a very important role in Biomedical Sciences Programme, and is a very challenging practical task for students. I use student centered approach to achieve best teaching outcome. 1) Designing a multidisciplinary project to attract students. I particularly paid attention to using update materials to stimulate students thinking, I set the outline to guide students to select projects. 2) Inspiring students’ passion and thinking in preparing project. I deliberately included students in materials, equipment preparation and explained key updated skills to be used to increase students’ passion and to form their thinking of how to start a new topic from scratch. 3) Giving student freedom to debate and adjust experiments in practice. I encouraged students to engage and selectively allow them to make reasonable change during experiments. This facilitated students to initiate their “own researches”. 4) Providing full support and guidance for students to run their experiments smoothly. I focus on raising questions for students to self-think through regular meeting, challenging students by asking them to give short presentation, and critical praise on their experiments details including making notes. This gave students real sense of the skills application and a logical thinking and practice in research. 5) Setting high standard to help students to achieve. I continuously help students thinking how this training would fit into their studies and future career path. Students who are interested in choosing academic career start to think how they can develop their career after 4 weeks project.

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Combining art and science to facilitate teaching and learning of anatomy

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Introduction - Teaching human anatomy and physiology is an integral part of teaching for medical students, but also for many other students, for example those studying nursing and the biosciences. Teaching anatomy is complex and challenging due to the vast differences in anatomical features throughout the body; each tissue type and organ has a unique structure which closely relates to function. Although medical students have access to cadavers so they can see anatomy in situ, most students of anatomy do not have access to these, so other teaching methods are needed. Successful learning of anatomy is associated with good memory, understanding and visualisation (Pandey & Zimitat, 2007).

The Learning Pyramid (National Training Lab, n.d.) suggests that around 75% is retained where students ‘practice by doing’, compared with 20% retention for a PowerPoint presentation. Additionally, medical students have been shown to have a better retention of anatomical features where they have been encouraged to draw the anatomy themselves, rather than just looking at diagrams (Azer, 2011).

In the current pilot study of Biomedical and Medical Science students, we compared different methods of teaching anatomy and students’ perception of retention of knowledge.

Methodology - Approximately 150 students were taught anatomy of various organs either using PowerPoint presentations to show and describe them, or interactive sessions, where students draw the anatomy alongside the lecturer. A pilot group of students was randomly selected (n=15), and a brief anonymous survey was designed to gain feedback on students’ perception of knowledge retention. At the end of the academic year the full data set will be analysed and compared with exam performance. DMU ethical approval has been granted for this study.

Results - Preliminary data indicates that more students can remember how to draw gross organ anatomy following an interactive session compared to a PowerPoint presentation (87% vs. 7%, respectively; P<0.05; Fisher’s Exact Test; see Figure 1).

Additional student feedback was given by free text comments, the examples below are indicative of the type of feedback received.

“Now I can draw the kidney it really helps me understand how it works!”

“Going through step by step really helped me to remember the anatomy”

“I thought drawing in class helped me to understand the organ as a whole”

Discussion - This current pilot study shows that a simple method (learning by drawing) can be effective when teaching anatomy and how this aids understanding physiology. Almost all students reported a significant increase in their knowledge retention when they had been taught with this method. This study will be extended to include feedback from all students and analysis of exam performance, and will inform future teaching methods on anatomy and physiology modules.

We would like to thank the students at DMU for their participation in this study.

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Integration of common concepts when teaching cardiovascular and respiratory physiology

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The teaching of physiology on professional (eg MBChB, BDS) and science programmes (eg Medical and Biomedical Materials Science) has traditionally been by a systems approach in which, for example cardiovascular, respiratory and renal physiology are taught in different modules. Clearly, there are many common themes and physiological concepts that apply to all systems (Michael et al. 2009) but such curriculum delivery can lead students to compartmentalise their learning. In addition, this approach reduces the opportunities for students to recognise physiological principles common to different systems and to integrate their knowledge of the whole body. The curriculum requirements of professional bodies (eg GMC and BDA) makes a different approach more challenging but a focus on physiological concepts could be of benefit to science students and provide them with the skills to apply, analyse and synthesise their knowledge, thus allowing them to integrate their understanding of different systems and other areas of the curriculum. This way they will advance to the higher levels of Bloom’s taxonomy of educational objectives without additional delivery.

To trial this we decided to alter the delivery of teaching of cardiovascular and respiratory physiology to year 1 Biomedical Materials Science students at the University of Birmingham; this had previously been done sequentially within one module. The content was split into four themes: Mechanics, Resistance, Transport and Integration. Lectures ~ 2 per theme, covered...
common concepts such as transport by convection and diffusion (Fick’s law), and the factors governing flow and resistance (Poiseuille’s law). Each theme had a small group tutorial to allow students to discuss the material and give them the opportunity to integrate and apply their knowledge of different systems. A final interactive session challenged students to consider how these systems would respond during exercise to optimise O2 delivery; a subject that had not been covered in lectures. We also changed our summative assessments to reflect this approach.

Feedback from students suggests that they found the focus on common concepts useful and that their understanding of a physiological principle in one system was reinforced by their knowledge and understanding of the other. Tutors also felt that students had a better understanding of the common principles and were able to apply their knowledge more widely to areas not yet delivered in lectures. We believe that this approach will benefit the students when they continue their physiology education in year 2.

A focus on common themes and principles could be applied to any area in which there is a need for students to integrate and apply their knowledge. It is particularly suited to programmes for which curriculum can be developed without the constraints placed upon it by professional bodies.


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PC047

Bile acid aspiration and human Airway Epithelial Cell injury

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Background - Gastro-oesophageal reflux and aspiration may be associated with lung disease. Bile acid aspiration has been shown to be prevalent among patients with advanced lung disease, which raises the concern that recurrent microaspiration of bile acids may cause lung injury.

Objectives - To investigate the possible links between bile acids and lung injury using primary bronchial epithelial cell cultures taken from lung transplant patients, immortalized human bronchial epithelial cell line (BEAS- 2B) and Human lung carcinoma cells (NCI-H292) cells.

Methods - Primary epithelial cells (PBECs), immortalized human bronchial epithelial cell line (BEAS- 2B) and Human lung carcinoma cells (NCI-H292) were cultured. The effect of individual primary and secondary bile acids were evaluated by 48 hour challenge. Post-challenge IL-8 and IL-6 concentrations were measured using commercial ELISAs. The viability of the (NCI-H292), (BEAS- 2B) and (PBECs) cells were measured using the Cell Titerblue assay and MTT assays.

Results - Primary epithelial cells (PBECs) and Human lung carcinoma cells (NCI-H292) cells can be cultured successfully. The secondary bile acid lithocholic acid was successfully used to stimulate cultured PBECs at different concentrations from 1umol/l to 20umol/l. A concentration of lithocholic acid above 18umol/l causes 100% PBEC death. Potentially physiological challenges with bile acids led to release of IL-8 from lung transplant PBECs. Human lung carcinoma cells (NCI-H292) were stimulated with lithocholic acid and Deoxycholic acid. Lithocholic acid above 20umol/l caused 100% cell death whereas Deoxycholic acid above 50umol/l caused 50% cell death.

Conclusion - Aspiration of bile acids may cause cell damage, inflammation and cell death.

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PC048

Resistance to cytotoxic and chemotherapeutic drugs conferred by Orai3 calcium channel in breast cancer cells: the molecular mechanisms

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Cancer cells have the ability to become resistant to a variety of drugs, and apoptosis resistance of cancer cells is a major hindrance for effective therapeutic modalities. A panel of resistance markers is therefore urgently needed. Altered expression of ion channels is now recognized as one of the hallmarks of cancer [1]. Several ion channels have already been proposed as novel emerging biomarkers and targets for therapy in cancer [2]. Among them, calcium channels are of particular interest, calcium being a ubiquitous second messenger regulating a wide variety of physiological functions, including cell survival [3]. We have previously shown that Orai3 calcium channels are highly expressed in breast tumors and, to a lesser extent, in breast cancer cell lines. In addition, down-regulation of Orai3 expression decreases calcium entry and increases apoptosis specifically in breast cancer cells [4]. In this context, we wondered whether Orai3 overexpression would be able to confer resistance to breast cancer cells. The aims of this study were 1) to overexpress Orai3 in breast cancer cells, 2) to study the impact on calcium entry and cell survival, and 3) to decipher the molecular mechanisms involved in Orai3-conferred resistance.

Stable clones derived from the T47D breast cancer cell line were selected. Orai3 overexpression increases calcium entry, and decreases cell mortality after treatment with apoptosis inducers (thapsigargin, staurosporin) and chemotherapeutic drugs used for breast cancer treatment (cisplatin, 5-fluorouracil, paclitaxel). This resistance is calcium-dependent since cells overexpressing Orai3 lose their resistance to death when extracellular calcium is removed. In order to decipher the mechanism by which Orai3 can confer resistance to cell death, we conducted a high throughput screening using DNA microchips. This whole genome transcriptomic analysis revealed a down-regulation of apoptotic genes expression regulated by p53, a ‘tumor suppressor gene’-encoded transcription factor, whose expression also decreases in cells overexpressing Orai3. Furthermore, the expression of Bax, a pro-apoptotic p53 target, decreases in these cells, and the expression of anti-apoptotic Bcl-2 increases.

In conclusion, Orai3 overexpression in breast cancer cells confers resistance to cytotoxic and chemotherapeutic drugs via a down-regulation of p53 and its pro-apoptotic targets expression and up-regulation of Bcl-2 expression. These results highlight a new marker of resistance, whose presence in tumors could indicate higher resistance to drugs, pointing toward requirements for more adapted treatments.

Quinine delays healing of acetic acid induced ulcer in rats

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To-date quinine continues to play a significant role in the management of malaria especially in African countries. Many researches have reported the effects of this drug on many systems of the body, including the adverse effects. However there is dearth of information about the use of this drug on some gastrointestinal functions. Therefore, this study aims to investigate the effects of the therapeutic dose of quinine on gastric ulcer healing in rats. Albino Wistar rats of both sexes weighing 150 – 200g were used. Rats were anaesthetized with 50mg/kg thiopentone sodium (i.p), laparatomy was performed and ulcer was induced by applying 0.5ml of 80% acetic acid to the serosal surface of the glandular portion of the stomach. The abdomen was sutured back and they were returned to normal food and water. They were then divided into two groups: rats in group 1 received normal saline (1ml/kg/day, i.p), while animals in group 2 received quinine (10mg/kg/day, i.p). Treatment began 24 hours after ulcer induction. Assessment of ulcer healing was done on day 3, 7 and 10 post ulcer induction by: measurement of ulcer area, histology and gastric mucus secretion. Values were expressed as mean ± SEM and compared by student t-test. Result showed that percentage area healed in control animal by day 10 (64.3 ± 5.73%) was significantly higher (p < 0.01) than in quinine treated rats (38.8 ± 4.16%). Clearing of inflammatory cells, fibroblast proliferation, collagen deposition and re-epithelization was faster in control animals than quinine treated. Gastric mucus secretion in response to ulcer induction on day 7 was significantly higher (p < 0.01) in control rats (4.1 ± 0.37 mg alcian blue/gm glandular tissue) than in quinine treated rats (2.8 ± 0.10 mg alcian blue/gm glandular tissue) and by day 10, mucus secretion in control animals was 2.8 ± 0.11 mg alcian blue/gm glandular tissue, which was significantly higher (p < 0.01) than in quinine treated animals (2.1 ± 0.16 mg alcian blue/gm glandular tissue). We conclude that, quinine delayed gastric ulcer healing by delaying inflammatory and proliferative phases of healing and by reducing gastric mucus secretion.

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Investigation of L-Asparaginase-induced pancreatic acinar cell death

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Introduction: L-Asparaginase is an essential element of combination chemotherapy in the treatment of acute lymphoblastic leukaemia (ALL). However, asparaginase-induced acute pancreatitis is the most common reason (7-18%) for stopping the relatively successful treatment of childhood ALL (Raja et al., 2012). Acute pancreatitis is a dangerous human disease characterized by premature activation of digestive enzymes inside the pancreatic acinar cells that leads to high levels of necrosis, digestion of the pancreatic tissue and its surroundings with no specific therapy (Reference). The aim of the present study was to investigate the molecular mechanisms and the signaling pathways of cellular injury in pancreatic acinar cells induced by L-asparaginase.

Methods: Pancreatic acinar cells were isolated as previously described (Ferdek et al 2012) according to the Animal Scientific Procedures Act, 1986. Cytosolic Ca2+ ([Ca2+]c) measurements were performed with Fura-2 or Flu-4 imaging. Mitochondrial calcium ([Ca2+]m) and mitochondrial membrane potential were measured using Rhod-2 AM and TMRM, respectively. Magnesium green (MgGreen) was employed to assess cellular ATP depletion, whereas necrosis was assessed with propidium iodide staining.

Results: We found that L-asparaginase (starting from 0.1 U/ml) induces small [Ca2+]c responses in pancreatic acinar cells, while higher doses L-asparaginase (200 U/ml) leads to large and prolonged (plateau) responses of [Ca2+]c. L-asparaginase also significantly increased calcium entry and substantially reduced calcium extrusion. L-asparaginase treatment has also resulted in significant reduction of cellular ATP levels. In addition, the results showed that L-asparaginase induce substantial increase in mitochondrial calcium and decrease in mitochondrial membrane potential. Calmodulin activators CALP-3, CALPm (Gerasimenko et al, 2014) and inhibitor of calcium entry GSK-7975A (Gerasimenko et al, 2013) have protected against reduction of cellular ATP levels induced by L-asparaginase and subsequent necrosis.

Conclusions: These data suggest that L-asparaginase affects pancreatic acinar cell fate via modulating calcium signalling and mitochondrial functions. Calmodulin activators are highly effective against calcium overload, cellular ATP depletion and necrosis induced by L-asparaginase. Thus, calmodulin activators may have important therapeutic implications for improving prognosis, prevention of acute pancreatitis and improvement of childhood cancer treatments.

Comparative responses of human keratinocyte cells (HaCaT) and human lung carcinoma epithelial cells (A549) following in vitro exposure to Silicon dioxide nanoparticles (SiO2-NP)

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The use of nanoparticles have provided numerous of advantages in medicine due to their unique physicochemical characteristics such as size, charge, shape and surface reactivity [1-4]. Understanding the interaction between engineered nanomaterials and living matter has attracted increasing attention in recent years. Toxicity of nanoparticles was studied in different cell types and cell lines. Nano-SiO2 has good stability, easy dispensability, and melting degeneration, and is widely used in rubber, paints, biomedical and biotechnology fields [5]. In this study, the LDH assay and the MTT assay were applied to evaluate the cytotoxicity of in vitro Silicon dioxide nanoparticles (SiO2-NP, 20nm) on cultured cell lines. Human lung adenocarcinoma epithelial cell line (A549) were used as a lung related cell line and human keratinocyte cell line (HaCaT) as a skin related cell line representing different uptake routes. The percentage cytotoxicity of the silicon dioxide nanoparticles was measured once cultured in a 24 hour incubation period. The concentration of the SiO2 nanoparticles chosen was 10, 50, 100 and 200μg/ml. To measure the cytotoxicity of nanoparticle on cultured cell lines, we used 104 cells/100 μl of cell culture media being placed in a 96 well rounded bottom plate with the LDH assay. The extracellular lactate dehydrogenase release was measured by using a colorimetric CytoTox 96 non-radioactive assay kit and the absorbance was recorded at 492nm. The MTT assay was used to evaluate mitochondrial activity which includes cell growth and cell death. This has been performed by inserting a premixed optimized dye solution in the culture wells. The Absorbance was recorded at 570 nm, from the recorded absorbance is directly proportional to the number of live cells. In order to maintain the cell lines, they were placed in a plastic T-75cm2 tissue culture flasks grown in Dulbecco’s Modified Eagle’s Medium. Studies were performed in the absence of serum. Cytotoxicity was found in both cells the A549 and HaCaT cells and cytotoxicity increased as concentration of the silicon dioxide increased. The percentage cytotoxicity calculated was higher in HaCaT cells compared to the A549 cells. A cell count assay was plated in order to display the cell number of both the HaCaT and A549 cells. The cell count reaffirmed that cytotoxicity did occur as the cell
count decreased as the concentration of the silicon dioxide increased compared to the control. These results show that silicon dioxide nanoparticles acted differently in two different cell types and that the metabolic rate of a cell can be used to determine the nanoparticles affect. Further understanding of the mechanism involving the ROS generation could provide more information on how silicon dioxide nanoparticles increase cytotoxicity.


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**PC053**

Bile acids modulate colonic epithelial restitution via regulating CFTR expression

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Epithelial restitution is an essential process for maintenance of intestinal barrier function. Increased levels of colonic bile acids have been proposed to be involved in the pathogenesis of inflammatory bowel disease (IBD) but their roles in regulating restitution are not yet known. Here, we investigated the effects of bile acids on epithelial restitution in a model of mechanically wounded colonic epithelial cells.

T84 colonic epithelial cells, grown as monolayers on transparent permeable supports, were wounded by scratching with a pipette tip at T0. Cells were treated with either deoxycholic acid (DCA; 150 μM) or ursodeoxycholic acid (UDCA; 100 μM) and restitution was measured as wound area after 48 h expressed as %T0 values.

After 48 h, wounds spontaneously closed to 37 ± 13% of T0 values. In the presence of DCA, wound healing was reduced (wound size = 76 ± 13%), whereas UDCA enhanced healing (wound size = 12 ± 2%) (n = 5; p < 0.001). Furthermore, UDCA completely prevented inhibition of wound closure by DCA. The cystic fibrosis transmembrane conductance regulator, CFTR, has been previously shown to be important in progression of wound healing in epithelial cells. We found that DCA decreased expression of CFTR Cl- channels to 60 ± 10% of that in control cells, while a CFTR inhibitor, CFTR(inh)-172 (10 μM), attenuated wound closure (wound size = 63 ± 2%) (n = 6; p < 0.01). CFTR(inh)-172 inhibition of wound closure was also prevented by UDCA. Finally, GW4064 (5 μM), an agonist of the nuclear bile acid receptor, FXR, mimicked DCA effects on wound healing and CFTR expression.

Our data suggest that colonic bile acids differentially regulate intestinal epithelial restitution. DCA prevents wound healing by a mechanism that likely involves FXR-mediated downregulation of CFTR expression, while UDCA promotes healing and protects against the detrimental effects of DCA. Thus, therapeutic manipulation of the colonic bile acid pool may prove to be a useful approach for promoting intestinal barrier function in IBD.

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**PC054**

Regulation of proinflammatory cytokine release from monocytes by the secondary bile acids, deoxycholic and ursodeoxycholic acid

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Cytokines released by mucosal monocytes play an important role in pathogenesis of inflammatory bowel disease (IBD). Among the best characterised of these cytokines is IL-8, a potent neutrophil attractant. As normal products of bacterial metabolism, secondary bile acids are present in the colonic lumen where they are known to have many roles in regulating mucosal barrier function. Here, we investigated potential roles for the naturally-occurring colonic bile acids, deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA), in regulating IL-8 release from monocytes.

IL-8 release from U937 monocytes was induced with either lipopolysaccharide (LPS) [1 μg/mL] or the proinflammatory cytokine, TNFα [5 ng/mL] for 24 h, in the absence or presence of UDCA or DCA. Supernatants were analysed for IL-8 by ELISA. IL-8 mRNA was assessed by qPCR. Levels of phospho-p38 MAPK and phospho-p65 were measured by western blotting.

Statistical analysis was performed by one way ANOVA with the Newman Keul’s post-test.

Treatment of U937 monocytes with TNFα or LPS induced 9.8 ± 1.2 and 7.9 ± 1.6 fold increases in IL-8 release, respectively (n = 5 – 7; p < 0.001). At physiologically-relevant levels, DCA [25 μM] reduced both TNFα and LPS-driven IL-8 release to 4.6 ± 0.4 and 4.2 ± 0.6 fold of controls, respectively (n = 5 – 7; p < 0.05). In contrast, UDCA [100 μM] reduced TNFα-, but not LPS-driven, IL-8 release to 3.5 ± 0.4 fold of controls (n = 5; p < 0.001). UDCA also inhibited IL-8 mRNA expression (n = 4; p < 0.05). The cell surface bile acid receptor, TGR5, was found to be expressed in monocytes, while the nuclear receptor, FXR, was not. However, activation of TGR5 with a specific agonist, INT777 (1 – 100 μM), did not alter IL-8 release. The NFκB inhibitor, BMS-345541 (10 μM), attenuated both TNFα and LPS-driven IL-8 release (n = 4; p < 0.05). While DCA had no effect, UDCA specifically reduced TNFα-, but not LPS-, induced NFκB activation. Both TNFα and LPS stimulated phosphorylation of p38 MAPK, while a p38 MAPK inhibitor, SB203580 (10 μM), attenuated LPS, but not TNFα-induced IL-8 secretion. Interestingly, UDCA did not alter TNFα- or LPS-induced p38 MAPK activation, while DCA potentiated p38 activation in response to both stimuli (n = 4 – 6; p < 0.05).

These studies underline the importance of bile acids in regulating intestinal physiology and demonstrate a strict structural specificity of their actions. Attenuation of cytokine release from monocytes by DCA at physiologically-relevant concentrations may serve to dampen intestinal inflammation under normal circumstances, while the more specific effects of UDCA at relatively high concentrations may be important under therapeutic conditions. In summary, by virtue of their anti-inflammatory effects on monocytes, bile acids represent good targets for development of new approaches to treat IBD.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Ursodeoxycholic acid prevents monocyte-induced dysregulation of colonic epithelial barrier function: implications for therapy of ulcerative colitis

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Ulcerative Colitis (UC) is a chronic disorder that manifests as chronic, recurring, colonic inflammation. Dysregulation of epithelial barrier function, occurring as a consequence of increased apoptosis or altered tight junction (TJ) integrity, is a pivotal step in UC pathogenesis. Monocytes are key effectors of the inflammatory response in UC and regulate epithelial function through the production of cytokines and mediators. Ursodeoxycholic acid (UDCA), a naturally-occurring bile acid formed by bacterial metabolism in the colon, has well-established cytoprotective and anti-inflammatory properties and has been shown to be beneficial in animal models of UC. The aim of this study was to determine whether altered monocyte-epithelial interactions may contribute to the anti-inflammatory actions of UDCA.

U937 monocytes were treated with UDCA [100 µM] for 24 hrs prior to their addition to the basolateral side of T84 colonic epithelial cell monolayers. Barrier function was assessed as changes in transepithelial resistance (TER) or in paracellular permeability to FITC-dextran. Levels of cleaved PARP and caspase 3, and expression of the tight junction (TJ) protein, occludin, were measured by western blotting.

Co-culture with monocytes caused a 63.6 ± 3.5 % decrease in TER (n = 17; p < 0.001) and an 8.2 ± 2.0 fold increase in paracellular permeability (n = 6; p < 0.01) across T84 Cell monolayers. UDCA treatment significantly attenuated these effects. Monocyte-induced disruptions of epithelial barrier function were not due to cellular toxicity, as assessed by LDH release. Analysis of cleaved PARP and caspase 3 suggest that the effects of monocytes were not due to induction of apoptosis. However, co-culture with monocytes did cause a significant decrease in phosphorylation of occludin (n = 3; p < 0.05), suggesting that alterations in TJs may be involved. Monocyte-induced disruption of epithelial barrier function was mediated by a heat stable and cyclooxygenase-2 independent soluble factor, which we have named monocyte-derived factor (MDF) (n = 6; p < 0.01). Interestingly, studies using conditioned medium from monocytes suggest that the protective effects of UDCA are not due to inhibition of MDF release from monocytes nor are they due to a direct protective effect at the level of the epithelium.

In summary, our data suggest that monocytes disrupt colonic epithelial barrier function through production of a heat stable soluble mediator that acts, at least partly, at tight junctions to increase paracellular permeability. UDCA prevents monocyte/epithelial crosstalk by an, as yet, unknown mechanism to prevent disruptions in barrier function. In conclusion, by virtue of its ability to preserve epithelial barrier function under inflammatory conditions, UDCA is an attractive target for development of new approaches to treat UC.

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

PC056

Protein kinase D2 modulation of aldosterone-sensitive ENaC activity in renal cortical collecting duct cells

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Na+ homeostasis in the body is regulated by aldosterone actions in kidney cortical collecting duct (CCD) cells. Aldosterone signalling is transduced via binding to the mineralocorticoid receptor (MR) and has been shown to modulate the epithelial Na+ channel (ENaC) activity by rapid nongenomic and latent genomic actions at the levels of transcription, protein stability and subcellular trafficking (1). Protein kinases such as protein kinase D are important modulators of Na+ transporters in CCD cells (2). Here we report a novel mechanism for aldosterone regulation of ENaC activity via protein kinase D2 (PKD2) signalling by affecting sub-cellular trafficking of ENaC subunits in a murine cortical collecting duct (M1-CCD) cell line. ENaC current was measured as the amiloride-inhibitable transepithelial short-circuit current (Isc) in M1-CCD cell monolayers mounted in Ussing chambers. Student’s t-tests were performed alongside a one way ANOVA.

Aldosterone (10 nm) caused a rapid (<10 min) phosphorylation of PKD2 and its sub-cellular redistribution from predominantly apical membrane to the cytosol. PKD2 knock-down using shRNA in M1 cells resulted in an elevated basal Isc from 1.9 ± 0.2 µA/cm² in wild-type cells to 9.3 ± 1.4 µA/cm² in PKD2 knock-down M1 cells (n=10, p=0.002). PKD2 knock-down increased the amiloride-sensitive ENaC current (Isc,ENaC) from 1.3 ± 0.3 µA/cm² in wild-type cells to 6.0 ± 1.0 µA/cm² in PKD2 knock-down M1 cells (n=11, p=0.0001). Long-term treatment (24h) of wild-type M1 cells with aldosterone increased the basal Isc from 1.9 ± 0.2 µA/cm² to 4.6 ± 0.7 µA/cm² (n=7, p=0.008). The ENaC current showed an increase from 1.3 ± 0.3 µA/cm² in wild-type M1 cells to 3.3 ± 0.5 µA/cm² when treated for 24h with aldosterone (n=8, p=0.001). The effect of aldosterone on both the basal Isc and Isc,ENaC were abolished in the PKD2 knock-down cells.

We also investigated the activation of PKD2 by aldosterone in an autosomal dominant polycystic kidney disease in vitro cell model (WT 9-12). Expression of PKD2 under basal conditions was found to localize at the apical membrane and in the sub-apical cytosolic space (n=3). ENaCγ localization was observed to be cytosolic/basolateral membrane while the Na+/K+ATPase subunits γ3 and β1 were localized at the apical membrane. Aldosterone (10nM) treatment induced the intracellular accumulation of PKD2 (n=3) within 10 min. In conclusion, our results indicate that PKD2 normally suppresses ENaC activity and aldosterone releases this inhibition by phosphorylating and removing PKD2 from the apical membrane. We propose that protein kinase D isoforms are pivotal signalling molecules controlling both the steady-state and aldosterone-induced membrane localization and stability of ENaC and Na+ /K+ pumps. Modulation of PKD activity could have important consequences for autosomal dominant polycystic kidney disease.

Physiological and pathological calcium signalling in pancreatic acinar cell
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Pancreatic acinar cells (PAC) are secretory cells responsible for the synthesis of many digestive enzymes, the pathological activation of which can result in the inflammatory disease acute pancreatitis (AP). This non-physiological activation has been linked to toxic levels of [Ca^{2+}]i, which can be induced by various agents including bile acids, e.g. Taurolithocholic-acid-3-sulfate (TLC-S) and Sodium Cholate (NaChol). While TLC-S induced [Ca^{2+}]i increases are known to involve a nicotinic acid adenine dinucleotide phosphate (NAADP) dependent component, it is unknown for NaChol. Wild type and knockout mice of the NAADP receptor TPCN2, sacrificed humanly using a schedule 1 approved method, were used to create preparations of PAC. The effects of two NAADP antagonists (Ned-19 and BZ194) and GSK-7975A (a Ca^{2+} release-activated Ca^{2+} channel blockers) were tested on these preparations using fluorescent Ca^{2+} imaging and a necrosis/apoptosis assay.

PAC synthesise NAADP in response to Cholecystokinin (CCK); Ned-19 but not BZ194 showed the ability to inhibit [Ca^{2+}]i oscillations induced by physiological concentrations of CCK. ACh induced oscillations are NAADP dependent and showed no sensitivity to Ned-19. Ned-19 also showed the ability to partially inhibit TLC-S induced [Ca^{2+}]i increases. BZ194 had no effect on the levels of necrosis induced by TLC-S; while Ned-19 and GSK-7975A both caused reductions in necrosis induced by both TLC-S and NaChol. No significant difference was observed in the levels of TLC-S induced necrosis in TPCN2 KO mice and wild types, though Ned-19 also displayed a protective effect in the KO mice.

These results show that the NAADP antagonist Ned-19, but not BZ194, possibly in combination with GSK-7975A, might provide a pharmacological approach to managing AP.

Poster Communications

Ned-19 at a high micromolar concentration considerably inhibited calcium responses induced by physiological concentrations of CCK but not ACh
a fundamental new mechanism of inter-cellular communication; therapeutically, ECVs represent a novel vehicle by which RNA therapy can be targeted to specific cells for the treatment of kidney disease.


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

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**PC059**

**Slc26a3 (DRA)-deficient mice display dramatically low surface pH, normal mucus secretion but loss of firmly adherent mucus layer, altered colonic microbiome and low grade intestinal inflammation**

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Background: Patients with congenital chloride diarrhea (CLD), caused by loss of function mutations of SLC26a3 (DRA), display a propensity for acute and chronic intestinal inflammation. Conversely, patients with intestinal inflammation, as well as murine inflamed colon, display a defect in surface cell anion exchange, luminal alkalinisation rates, and a decrease in DRA mRNA and protein expression. Aim: In this study, we investigated the effect of genetic ablation of Slc26a3 (DRA) on the surface pH and the dynamics of mucus layer buildup in vivo by two photon microscopy, on the intestinal microbiome by 16S rRNA sequencing, and on the inflammatory state of the mucosa by qPCR and immunohistochemistry, under nonchallenged, specific pathogen-free conditions. Methods and Results: DRA-deficient mice, an animal model for congenital diarrhea (CLD), and WT littermates were anesthetized by 1.5-2% isoflurane-inhalation anesthesia and the intestinal surface pH was assayed by two photon microscopy in exteriorized vascularly perfused colon using SNARF1 free acid. DRA-deficient mice displayed strongly abnormal low surface pH and loss of fluid absorption in the mid-distal colon, but a virtually normal mucus layer buildup rate. However, they lacked a firmly adherent mucus layer. In addition, they displayed altered microbiota composition with a decrease in the firmicutes and an increase in the bacteroidetes in all parts of their colon. However, only the distal colon, which displays the thickest mucus layer and the highest DRA expression levels in WT mice, showed evidence for inflammation in the DRA KO mice, indicated by an increase in infiltrating mononuclear cells and increased TNF-alpha expression. Conclusions: Apart from absorbing Cl⁻, the anion exchanger Slc26a3 (DRA) is intimately involved in the maintenance of intestinal barrier properties such as high colonic surface pH, firmly adherent mucus layer, and the composition of the microbiome. Because the microbiota composition was different from WT in all parts of the DRA-deficient colon, but inflammation was seen only in the mid-distal colon, we speculate that the reason for inflammation is a combination of weakened barrier properties with a more aggressive microbiota in the absence of DRA expression. Intestinal inflammation is thus both a consequence of, and a cause for, disregulated ileocolonic HCO₃⁻ transport.

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**PC060**

**Loss of carbonic anhydrase IX expression impairs gastric mucosal defence against luminal acid**

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Background and Aims: Carbonic anhydrase IX is ubiquitously expressed during embryogenesis but is downregulated postnatally. CAIX expression persists in the stomach predominantly in the surface cells. We hypothesized that this exoenzyme protects the gastric mucosa against strong luminal acidity. Methods: The cellular differentiation pattern, the acid and bicarbonate secretory capacity, the ability of the surface epithelial cells to withstand a luminal acid load, the mucus layer buildup and the cytokine profiles were assessed in CAIX KO and WT mice from newborn ages to late adulthood. The experiments were performed according to the ethical guidelines that apply for animal experiments. Results: The ability of the surface cells to withstand luminal acid exposure and to generate an alkaline microclimate was significantly impaired in CAIX KO compared to WT stomach. This was accompanied by an increase in IL1ß prior to the gradual expansion of the mucus cell zone and regression of the parietal cell zone to the base of the glands. Maximal acid secretory capacity decreased proportional to the loss of parietal cells and serum gastrin levels increased, explaining the glandular hypertrophy. Mild chronic proton pump inhibition from the time of weaning dramatically reduced the parietal cell loss in CAIX KO mice. Conclusion: We speculate that the CAIX at the basolateral membrane of the gastric surface cells rapidly converts protons extruded by the surface cell basolateral membrane together with bloodborne HCO₃⁻ to CO₂ and H₂O, and thus augments interstitial buffer capacity, surface cell pH₁ regulation, and maintenance of the pH microclimate in the mucus layer. Lack of CAIX results in chronic acid damage with a gradual regression of the parietal cell zone to the lower gland area.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Supplementation of the maternal diet with palm oil affects intestinal permeability in offspring at 7 days but not 6 months

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Background and aims - Excess caloric load or a proinflammatory diet may contribute to gut barrier dysfunction, leading to increased nutrient uptake and components of the microbiota passing into the systemic circulation. These effects have been linked to the development of obesity and the metabolic syndrome (1, 2). As the maternal diet has been shown to have a significant effect on offspring development (3), we used a porcine model of a fat supplemented diet to test the effect of maternal diet on offspring gut development at two time-points.

Materials and methods - 16 sows were randomly assigned (n=8 per group) to a control diet of commercial feed (maternal control, mC) or commercial feed supplemented with palm oil (maternal high fat, mHF) from the first day of breeding, and continued with a gradually increasing amount of palm oil throughout gestation. Diets were made isocaloric by reducing the starch content in the palm oil supplemented diet. Sows farrowed naturally and their offspring were fed standard commercial diets regardless of maternal diet. All offspring were grouped based on diet and size at birth. The first group were dissected at 7 days (mC median n=6, mC small n=7, mHF median n=8, mHF small n=7), and the second at 6 months (mC median n=6, mC small n=4, mHF median n=6, mHF small n=5).

Three genes coding for proteins located on epithelial tight junctions were used as markers of gut barrier function: claudin-3 (CLDN3), occludin (OCLN), and zonulin-1 (ZO-1). Epidermal growth factor receptor (EGFr) was used as a marker of intestinal development. Quantitative PCR was used to measure mRNA abundance of the above genes in RNA extracted from the ileum. All data is presented as mean±SEM, with groups compared using Student’s t-test or Mann-Whitney U test as appropriate.

Results - Birth weight and small intestine weight relative to total body weight were unaffected by maternal diet at both timepoints. Expression of CLDN3 and OCLN was significantly reduced in mHF median offspring at 7 days compared to mC median, EGFr was significantly reduced in both mC small and mHF median offspring compared to mC median at 7 days (see figure). ZO-1 expression at 7 days was unaffected by diet or size. Expression of all four genes was not significantly different between groups at 6 months.

Conclusion - These findings demonstrate a high-fat diet during pregnancy may increase intestinal permeability in the offspring, and adversely affect intestinal development at an early age. However the changes observed at 7 days are no longer present in 6 month old pigs, suggesting that development of the small intestine taking place after 7 days, predominantly at weaning (4) may compensate for any adverse effects. Further work will explore whether other organs such as the liver are adversely affected by an increased intestinal permeability at 7 days.

7 day gene expression

PC062

Loss of Slc26a9 anion transporter results in reduced pancreatic fluid and bicarbonate secretion electrolyte in female mice

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Background: Slc26a9 is a member of the Slc26 multifunctional anion transporter family with strong expression in the lung and the stomach. In the bronchial and duodenal epithelium it may function as a chloride conductance interacting with CFTR. Polymorphisms in Slc26a9 are associated with an increased incidence of meconium ileus and diabetes in cystic fibrosis patients. Aim: We investigated the expression of Slc26a9 in the pancreas and elucidated its potential role in pancreatic ductal electrolyte and fluid secretion. Methods and Results: The mRNA expression of Slc26a9 was low in pancreatic parenchyma but 20fold higher in microdissected pancreatic ducts. No Slc26a9 mRNA expression was detected in the liver, while bile ducts displayed low Slc26a9 expression. The main pancreatic and the common bile duct were cannulated and pancreatic and biliary fluid and bicarbonate secretion assessed in isolflurane-anesthetized Slc29 knockout mice and age- and sexmatched wildtype (WT) littermates in the basal state and after intravenous stimulation with secre...
PC063

RIG-I-like receptors play a role during Crohn’s-like rat ileitis

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The precise pathogenesis of inflammatory bowel disease (IBD) is still incompletely understood. RIG-I-like receptors (RLRs) play an important role in the recognition of viruses in various tissues including the intestinal epithelium orchestration of the innate and adaptive immune defense mechanisms. Changes in the RLR signaling pathways due to host genetic predispositions may turn a physiological response into a pathological situation including development of intestinal inflammation. The aim of this research was to investigate the effect of acute ileitis on expression intensity of the RIG-I-like receptors with lymphocytes of small intestine. Materials and methods. Male Wistar rats weighing 200–250 g were housed in standard wire-mesh bottom cages at constant temperature of 25°C and 12/12 h light/dark cycles. For induction of ileitis was accompanied with the decrease in quantity of RIG-1+ lymphocytes expressing the RIG-I showed reliable increase of this parameter in lymphocytes by 10% (p>0.05) in ileum. Conclusion. We established that development of acute ileitis was accompanied with the decrease in quantity of RIG-I+ cells and it influenced the density of RIG-I-like receptors in immunopositive cells.

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PC064

Enhanced spermogram in Cinnamon verum treated male alloxan – induced diabetic Wistar albino rats

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A major global health issue is infertility and about 30% is due to male factors (Isidori et al., 2006) and diabetes mellitus interferes with reproductive function in laboratory rats (Valdes et al., 1990). Several medicinal plants lower blood glucose level in experimental animals (Khan et al., 2003). Whether use of Cinnamon verum spice can lower the hyperglycaemia and have an effect on the reproductive ability in diabetic male Wistar albino rats is unclear and the purpose of the present study. 25 male rats were randomly assigned to 5 groups. Control [(CON) 0.5 mL kg⁻¹ body weight distilled water intraperitoneally, n = 5], Diabetic control [(DC) Alloxan intraperitoneal (IP) injection of a single daily dose of 150 mg/Kg b.wt. for 3 consecutive days (Ashok et al., 2007), n = 5], Cinnamon [(CIN) 500 mg of Cinnamon verum spice solution (CVSS)/kg b.wt., per os by intragastric gavage, n = 5], and Two test groups [(250 CIN and 500 CIN) Alloxan IP injection of a single daily dose of 150 mg/Kg b.wt. for 3 consecutive days, 250 mg and 500 mg of CVSS/kg b.wt., per os respectively, n = 5]. All rats were kept in standard conditions, fed and watered ad libitum for 65 days. Blood glucose was measured weekly. Rats were humanely sacrificed on the last day and sperm collected from epididymis. Values were expressed as mean ± standard deviation and Student’s t-test was applied to test the significance of differences between groups with accepted significant level p < 0.05. Results showed that administration of both 250 mg (124.2 ± 11.2 mg dL⁻¹) and 500 mg (119.78 ± 3.8 mg dL⁻¹) of CVSS decreased blood glucose level in a dose-dependent manner (p < 0.05), improved sperm livability (82.5 ± 2.5% and 80.0 ± 5.0%) and motility (77.5 ± 2.5%, 77.5 ± 2.5%) of treated diabetic rats when compared with diabetic control rats (239.4 ± 4.5 mg dL⁻¹, 65.0 ± 3.0%, 57.5 ± 2.5%) (p<0.05). CVSS caused a 12.6% decrease in blood glucose levels of the 250 CIN diabetic rats within 1 week of administration compared to 15.1% for the 500 CIN within the same period. The maximal reduction of 42.6% was observed at 4 weeks for 250 CIN, while for the 500 CIN the maximal reduction of 31.0% was observed at 3 weeks and 23.6% at 4 weeks post administration of extract. CVSS (250 and 500 mg/kg) significantly lowered blood glucose levels of alloxan diabetic rats (p<0.05) when compared with control rats treated with distilled water. It can now be deduced from this study that CVSS while having hypoglycaemic effect consistent with the findings of Khan et al. (2003) also improves sperm motility a powerful factor for male reproductive capabilities and sperm concentration (Jensen et al., 2006) of male diabetic rats thereby enhancing fertility.
Effects of 120 minutes of treadmill running on DNA methyltransferase 3A and 3B nuclear concentrations in peripheral blood mononuclear cells

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DNA methylation, the process by which a methyl group is added to a cytosine molecule, is essential for normal transcriptional regulation, in addition to X chromosome inactivation and genomic imprinting. Initial research has shown that exercise can both acutely (Barrès et al, 2012) and chronically (Nitert et al, 2013) modify global and gene-specific levels of DNA methylation. DNA methyltransferases (DNMT) catalyse this process, however, there is a lack of literature concerning the specific molecular mechanisms by which exercise-induced epigenetic modifications occur. Interleukin 6 (IL-6) stimulation of cancer cell lines has been shown to augment DNA methyltransferase 1 (DNMT1) expression (Hodge et al, 2005), which suggests a possible pathway by which exercise is able to elicit changes in these epigenetic enzymes. Utilising 10 recreationally active males, the present study sought to elucidate the response of the de novo DNA methyltransferases 3A (DNMT3A) and 3B (DNMT3B) to 120 minutes of treadmill running at an intensity of 60% of individual velocity at VO₂max (vVO₂max), interspersed with 30 second sprints at 90% of vVO₂max every 10 minutes - a protocol previously shown to elicit a transient increase in plasma IL-6 (Walsh et al, 2010). Peripheral blood mononuclear cells (PBMCs) were incubated with plasma isolated from exercising participants or recombinant IL-6 (rIL-6), followed by nuclear protein extraction and subsequent quantification of DNMT3A and DNMT3B concentrations. Paired samples T tests showed that nuclear concentrations of DNMT3B significantly decreased (p < 0.05) from 363.9 (± 363.6) ngm protein¹ to 87.2 (± 73.3) ngm protein¹ following the experimental protocol, with no change observed in DNMT3A. ‘High’ levels (10 ngm⁻¹) of rIL-6 stimulation resulted in significantly greater nuclear concentrations of both DNMT3A and DNMT3B, compared with ‘low’ concentrations (10 ngm⁻¹). This is the first known study to characterise the response of de novo methyltransferases to an acute bout of aerobic exercise or in vitro stimulation of PBMCs with rIL-6. The conflicting results suggest that IL-6 is not the only major regulator of DNMT nuclear transport, and that other plasma mediators may exert significant influence on the nuclear concentrations of these enzymes.


Nitert MD et al. (2012). Diabetes 61, 3322-3332.


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while FTP was 278 ± 42 W. FTP and CP were not significantly different (P>0.05) and these variables were significantly correlated (r=0.96, P<0.05). Predicted 16.1 km TT performance (27.32 ± 3.29 min) was within 4.4% of, and not significant different to, actual TT performance (26.66 ± 2.17 min, P>0.05, see figure 1).

Discussion - Our findings suggest that CP and W', as derived from mobile power meters in the laboratory, may be used to accurately predict 16.1 km cycling TT performance on the road. In addition, we observed a close agreement between FTP and CP, suggesting that the FTP may provide a time efficient, single-test estimate of the CP. These observations may help inform, and improve the accessibility of, testing procedures that can be employed to predict TT performance in competitive cyclists.

In the driven oscillations point estimates were taken at 0.05 and 0.10 Hz. The enhanced input and output signals, in both age-groups, led to improvements in coherence from ~0.28-0.40 a.u. in the VLF range to >0.97 a.u. at 0.05 Hz (P<0.001); and from ~0.48 a.u. in the LF range to >0.98 a.u. at 0.10 Hz (P<0.001). With the OLBNP protocol it was conclusively shown that the expected trends of the high-pass filter was present in both age-groups. It was also revealed that the older adults had subtle changes in normalized gain (elevated at 0.05 Hz) and phase (reduced at 0.10 Hz). It is speculated that these age-related changes to TFA metrics are due to either alterations in arteriolar tone, sympathetic tone or the mechanical buffering of the compliance vessels.

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**PC068**

**Association of TTN genotype with skeletal muscle fascicle length in untrained Caucasian males**

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The titin (TTN) gene encodes the largest described protein to date and, due to its size, provides a molecular blueprint for the organisation and assembly of the muscle sarcomere (Chauveau et al., 2014). Research has demonstrated resting sarcomere length differs according to TTN isoform expression in rat skeletal muscle (Greaser and Pleitner, 2014). Differences in sarcomere length due to isoform expression may impact on muscle fascicle length, which is the primary determinant of maximal shortening velocity. Furthermore, the TTN rs10497520 polymorphism has been identified as contributing significantly to a genetic predisposition for maximal isokinetic strength at 180°s⁻¹ (Thomaes et al., 2013). Consequently, the aim of this study was to investigate the potential association between the TTN rs10497520 polymorphism and muscle fascicle length in untrained, healthy Caucasian male volunteers (n = 120; age = 20.6 ± 2.3 yr; height = 1.79 ± 0.06 m; mass = 75.1 ± 10.1 kg). Resting fascicle length of the vastus lateralis muscle was assessed in vivo using B-mode ultrasound at 50% of muscle length. Identification of fascicle length was achieved by measuring the distance from fascicular origin to insertion on the aponeurosis from a single ultrasound image using digitising software. In instances where fascicles extended beyond the ultrasound field of view, extrapolation of the fascicle and aponeuroses was necessary and fascicle length was estimated. A minimum of three fascicles were measured and a mean recorded as fascicle length. Each volunteer provided either a whole blood (n = 96) or buccal cell (n = 24) sample, from which DNA was isolated and genotyped for the TTN rs10497520 polymorphism using real-time polymerase chain reaction. Chi-square analysis was completed to assess for compliance with Hardy-Weinberg equilibrium. An independent samples t-test was used to determine any genotype-dependent differences in fascicle length. Frequency of the TTN rs10497520 polymorphism was in Hardy-Weinberg equilibrium (CC = 79.2%, CT = 20.8%, TT = 0%; X² = 1.622, P

**PC067**

**The relationship between blood pressure and cerebral blood flow during supine cycling: Influence of aging**

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We examined which of the previous paradoxical findings (impaired phase or intact gain) accurately represents the relationship between blood pressure and middle cerebral artery velocity during moderate intensity exercise. Both younger (20 to 30 years; n=10) and older (62 to 72 years; n=9) adults were examined. To enhance the signal-to-noise ratio, an experimental approach was employed that evoked non-pharmacological manipulations of blood pressure variability (via oscillatory lower body negative pressure – OLBNP) that were induced during steady-state moderate intensity supine exercise (~45-50% of heart rate reserve). Beat-to-beat blood pressure, middle cerebral artery velocity, and end-tidal PCO₂ were monitored and the cerebral pressure-flow relationship was assessed with linear transfer function analysis. The pressure-flow relationship was quantified for spontaneous data in the very low (VLF: 0.02-0.07 Hz) and low frequency (LF: 0.07-0.20 Hz) ranges.

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Endurance training induces tissue specific regulation of mitochondrial stasis

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It’s accepted that adaption to training goes beyond cardio pulmonary adaptions (Holloszy & Coyle, 1984) and greater understanding of the mitochondria’s role in energy production has provided impetus to characterise bioenergetic adaptions to athletic training. Endurance training increases mitochondrial biogenesis, but changes in mitochondrial mass following different training intensities remain poorly understood and yielded no pattern to adaptions. In our study, Swiss albino mice, (male, 45-55 g, 8 weeks) were exercised to induce a trained and overtrained state using treadmill running (Pereira et al., 2012) and rested for 0, 2 or 4 weeks. A control cohort was also included. Performance was determined using an incremental load test. Whole gastrocnemius (GC), quadriceps (Quad) and cardiac (CRD) muscle were retrieved post mortem. Relative Citrate Synthase levels were determined, as it’s an exclusive marker of the mitochondrial matrix and an accepted marker of mitochondrial mass. Relative mitochondrial genome copy number Real time PCR was determined using real time PCR. Two-way ANOVA was the statistical analysis performed. A trained state was confirmed in the first cohort the observation of a constant gradual increase in performance and stagnating levels post training. Functional over-reaching was confirmed in the second cohort, as shown by a large drop in performance at the end of training followed by a super-compensation two weeks post recovery. Mitochondrial mass in the GC muscle of trained mice was less than control (min. 20% p<0.05) throughout the recovery period, no statistically significant was observed in genome frequency. Mitochondrial mass and genome frequency in Quad muscle of trained mice increased directly after training (147% p<0.05 respectively) but returned to control levels 2 weeks post recovery. Mitochondrial mass and genome frequency in the CRD muscle of trained mice showed no significant. Mitochondrial mass in the GC muscle of overtrained mice were 30% less than control (p<0.001), which was sustained from 0-4 weeks recovery. Mitochondrial mass in the Quad muscle of overtrained mice increased (113% p<0.001) transiently and returned to control levels (p>0.05) following recovery, with no significant difference observed in genome frequency. Mitochondrial mass in the CRD muscle of overtrained mice showed no change until 4 weeks post training when a marked increase was seen (165%, p<0.001) while genome frequency showed a 1.8 fold increase (p<0.001) post training which returned to control levels (p>0.05) following 2 weeks recovery. Analysis showed different muscle groups have distinct mitochondrial responses to different training intensities. Observed trends indicate that the adaptions of mitochondrial mass to endurance training may be muscle specific and are more complex than previously thought.


With many special thanks to the Institute of technology Sligo and Orreco Ltd for their support and guidance for this work.

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

PC069

Poster Communications

Similar influence of prior time-trials performed at different altitudes on subsequent exercise in hypoxia

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We examined the influence of prior time-trials performed at different altitudes on subsequent exercise performance in moderate hypoxia and associated cardio-metabolic and neuromuscular responses. During a preliminary visit, 10 physically active subjects cycled to exhaustion at constant workload (80% of the power output associated with their maximal oxygen uptake at a simulated altitude of 2000 m: 245±42 W) for a reference time (Tlim = 947±336 s) at 2000 m (FiO2=16.3%). Thereafter, two separate (5-7 days) sessions were conducted, in a randomized order: a cycle time trial for Tlim duration (TT1), followed 22 min later (rest) by a 6-min cycle time trial (TT2) with TT1 either performed at 2000 m or 3500 m (FiO2=13.5%), while TT2 always performed at 2000 m. Expired gases together with vastus lateralis (VL) oxygenation (near-infrared spectroscopy) and electromyographic root mean square (RMS) activity for the VL and rectus femoris (RF) muscles were continuously measured. Knee extensors electromyographic and force responses to femoral nerve stim-
ulation were assessed before and -2 min after each exercise bout. During TT1, mean power output (247±42 vs. 227±37 W; P<0.001) and several physiological responses (pulse oxygen saturation: 91.0±3.0 vs. 80.2±3.1%; oxygen uptake: 44.0±7.3 vs. 39.9±5.8 ml.min⁻¹.kg⁻¹) were higher at 2000 m versus 3500 m. Despite this, VL and RF RMS activity together with VL oxygenation did not differ between conditions. During TT2, mean power output (256±42 vs. 252±36 W) and accompanying cardiopulmonary, quadriceps muscle activation and VL oxygenation responses did not differ after completing TT1 at 2000 m or 3500 m. Maximal isometric voluntary contraction torque (both conditions averaged: -7.9±8.4%; P<0.01), voluntary activation (-4.1±3.1%; P<0.05) and indices of muscle contractility (peak twitch torque: -39.1±11.9%; doublet torques at 10 Hz and 100 Hz: -38.7±10.2% and -15.4±8.9%; 10/100 Hz ratio: -25.8±7.7%; all P<0.001) were equally reduced from pre- to post-TT1, whereas VL and RF M-wave characteristics did not differ. Irrespective of the altitude of TT1, neuromuscular function remained similarly depressed at pre-TT2 and post-TT2 compared to Post-TT1. In summary, exercise capacity is impaired during a time trial conducted at 3500 m versus 2000 m, whereas neural drive to quadriceps and VL oxygenation did not differ. Neuromuscular adjustments resulting from the completion of this initial exercise bout were of similar nature with, in particular, profound alterations in muscle contractility, presumably due to excitation-contraction coupling failure (low-frequency fatigue). After 22 min of rest, there was no influence of the altitude of a prior time-trial on performance and associated cardio-metabolic responses, with also no additional muscle fatigue development, during a subsequent 6-min time trial performed in moderate hypoxia.

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

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**PC071**

Modulation of tissue composition and function by eicosapentaenoic acid and vitamin D in immobilisation

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It has consistently been demonstrated that disuse models (e.g. limb immobilisation and bed rest), result in skeletal muscle atrophy, decreases in maximal voluntary strength (1) and bone mineral density (2), and increases in intermuscular adipose content (3). Disuse models have also been associated with changes in electromyography (EMG) characteristics (4) and muscle fatigability (1). Where exercise prescription is not possible (e.g. bed rest or injury), other interventions are required to attenuate these changes associated with disuse. Non-pharmacological interventions need to be identified since polypharmacy in itself is conducive to skeletal tissue loss (5). This study set out to determine whether two potential protein-sparing modulators (eicosapentaenoic acid [EPA] and vitamin D [VitD]) would modulate the deleterious effects of immobilisation. The non-dominant arm of 24 healthy participants, aged 23.0±5.8 years, was immobilised in a sling for a period of 9 waking hours a day over two continuous weeks. Participants were randomly assigned to one of three groups: placebo (PLA) (n=8), EPA (n=8) or VitD (n=8). Body composition (DEXA), arm girth (anthropometry), muscle co-contraction (EMG) and muscle fatigability (Cybex and EMG) were measured before, at the end of the immobilisation period and two weeks after re-immobilisation. The effect of immobilisation and supplement group were assessed by either repeated measures ANOVA (parametric data), with post-hoc Bonferroni corrected 2-tailed t-tests or Kruskal Wallis test (non-parametric data), with post-hoc Mann-Whitney U tests. All data are presented as mean ± standard deviation. There were significant decreases in upper and lower arm girth, lean mass and bone mineral content (BMC) post-immobilisation in the PLA group (P<0.05). Despite no significant effect of group, EPA and VitD supplementation showed trends towards attenuating the decreases in upper/lower arm girths, −1.3±0.4% PLA, −0.6±0.5% EPA, −0.9±1.0% VitD, p=0.18 and −0.8±0.8% PLA, −0.4±0.5% EPA, −0.3±0.4% VitD, p=0.21, respectively and BMC, (−2.3±1.5% PLA, −0.3±1.0% EPA, −0.7±1.9% VitD, p=0.47) observed in the PLA group. The EPA supplementation group demonstrated a non-significant attenuation of the decrease in lean mass observed in the placebo group, (−3.6±3.7% PLA, −1.9±2.8% EPA, −4.0±2.8% VitD, p=0.95). There was no significant change in muscle fatigue parameters or EMG co-contraction values with immobilisation and no effect of supplementation group (P>0.05). All parameters had returned to baseline values at the re-immobilisation phase of the study. The results suggest that both EPA and VitD may generally attenuate the changes in muscle size and bone parameters associated with immobilisation. These findings may be applicable to both sporting (e.g. off-season detraining) and clinical (injury/surgery induced short-term immobilisation) populations.


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**PC072**

Disproportionate increase in cardiac muscle relaxation during exercise in hypoxia


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In vitro cardiac myofibre contraction and relaxation are linearly associated across a wide range of muscle fibre (or sarcomere) lengths, contraction frequencies and β-adrenergic activation (Janssen, 2010, see Fig. 1A). Whether linearity of cardiac contraction-relaxation coupling is maintained when in vivo O₂ availability is reduced and the heart muscle itself experiences a greater demand for coronary flow remains to be elucidated. Therefore, in a within-day repeated-measures experiment, sixteen healthy males (age: 22 ± 4 years, VO₂peak: 45.5 ± 6.9 ml/min/kg) exercised at i) 2% above individual anaerobic
threshold (IAT) and ii) 40% of normoxic peak power in room air (40% peak\textsubscript{peak}/NORM and iii) in hypoxia (40% peak\textsubscript{peak}/HYP, \textit{FiO}\textsubscript{2} = 12%). Left ventricular (LV) contraction and relaxation were determined from speckle-tracking ultrasound-derived twist and untwisting rate, respectively. From rest to exercise at 40% peak\textsubscript{peak}/HYP, LV untwisting rate was disproportionally increased compared with LV twist (y = -8.7x) in accordance with the aforementioned literature (Janssen, 2010, see Fig. 1A). However, during exercise at 40% peak\textsubscript{peak}/HYP, LV untwisting rate was disproportionally increased compared with LV twist (y = -18.1x, see Fig. 2B). This phenomenon occurred while whole-body O\textsubscript{2} consumption was similar between 40% peak\textsubscript{peak}/NORM and 40% peak\textsubscript{peak}/HYP (mean ± SD: 22.3 ± 4.9 and 22.2 ± 4.6 ml kg\textsuperscript{-1} min\textsuperscript{-1}, respectively, \textit{P} > 0.05) but rate pressure product, a surrogate of cardiac O\textsubscript{2} consumption, was significantly increased in hypoxia (21124 ± 2313 and 26823 ± 3309 bpm mm H\textsubscript{1}, respectively, \textit{P} < 0.001). The disproportionate increase in LV untwisting rate was not associated with the preceding twist rate, frequency of contractions or altered LV volumes. In conclusion, the present data provide in vivo evidence of an acute uncoupling of LV contraction and relaxation as represented by twist and untwisting rate. We hypothesise that LV contraction and relaxation may be regulated at least in part separately, according to the peripheral whole-body vs. coronary O\textsubscript{2} demands. This finding may help to explain pathological conditions in which systolic and diastolic function are affected differentially, for example in “diastolic heart failure” (Zile et al., 2004), also termed “heart failure with preserved ejection fraction” (Anderson & Borlaug, 2014).


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PC073

Two minutes of all-out sprint exercise per week improves aerobic capacity

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Aerobic capacity (\textit{VO}\textsubscript{max}) consistently manifests as the strongest predictor of future morbidity and mortality, superseding other more widely recognised risk factors such as hypertension (1). We recently collected data from 11 inactive individuals which suggested that \textit{VO}\textsubscript{max} can be improved with as little as two minutes of all-out sprint exercise per week (2); however, this finding requires confirmation in a larger cohort. In the present study, thirty-four sedentary but otherwise healthy men \textit{n} = 16; age: 33 ± 9 y; BMI: 25.1 ± 2.1 kg m\textsuperscript{-2}; \textit{VO}\textsubscript{max}: 39.2 ± 8.6 ml kg\textsuperscript{-1} min\textsuperscript{-1}) and women \textit{n} = 18; mean±SD: age: 36 ± 9; BMI: 24.0 ± 3.5 kg m\textsuperscript{-2}; \textit{VO}\textsubscript{max}: 31.7 ± 4.6 ml kg\textsuperscript{-1} min\textsuperscript{-1}) performed 18 supervised training sessions over 6 weeks on a cycle ergometer. The 10-min exercise sessions consisted of unloaded pedalling and one (first session) or two (all other sessions) brief ‘all-out’ sprints against a resistance of 5% body mass (10 s in week 1, 15 s in weeks 2-3 and 20 s in the final 3 weeks). \textit{VO}\textsubscript{max} was assessed during an incremental ramp test to volitional exhaustion (Lode Excalibur Sport, Groningen, the Netherlands) with continuous breath-by-breath measurements of \textit{VO}\textsubscript{2} taken using an online metabolic cart (ParvoMedics TrueOne 2400, Utah, USA). \textit{VO}\textsubscript{max} was taken as the highest 15-breath rolling average value achieved during the test. In all tests two or more of the following criteria were met: a plateau in \textit{VO}\textsubscript{2} despite increasing intensity, RER > 1.15, heart rate within 10 beats of age-predicted maximum, and/ or volitional exhaustion. Despite the low total training time commitment (30 min/week of which no more than 2 min were at high intensity) and relatively low ratings of perceived exertion (RPE 14±2), two-way ANOVA revealed an increase in \textit{VO}\textsubscript{max} in both men (+9%; \textit{mean±SD}: 3.22±0.50 vs 2.95±0.56 l min\textsuperscript{-1}, \textit{p}<0.01) and women (+10%; \textit{mean±SD}: 2.28±0.36 vs 2.08±0.30 l min\textsuperscript{-1}, \textit{p}<0.01) following training, with no difference between sexes. In conclusion, herein we confirm that two minutes of all-out sprint exercise per week is sufficient to increase aerobic capacity in previously sedentary men and women.


We would like to thank all our dedicated participants for their time and effort. Richard Metcalfe was funded by a University of Bath PhD studentship.

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PC074

Acute exercise and an elevated intraabdominal obesity: implications for cerebrovascular function

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Introduction: An increase in body fat percentage and more so an elevated visceral adiposity has been demonstrated to decrease cerebrovascular function and an increase the risk of stroke. Acute exercise is a challenge to the brain and may help highlight subtle consequences in cerebrovascular function (1). The aim of the study was to examine if elevated intraabdominal obesity impairs cerebrovascular function and to what this is further compounded by acute exercise.
Results: Obese had a higher VAT (81 vs. 140 P<0.05) and WC (8.7 cm) compared to the non-obese controls (7.2 cm; 28 vs. 45 years). Correlations were observed between CVR and VAT (r = -0.46, P<0.05), and between CVR and TF (r = 0.47, P<0.05). There was no effect of acute exercise on cerebral vascular function and cerebral vascular function was not further impaired by obesity.

Conclusion: The findings demonstrate that an increased intraabdominal obesity was associated with impaired cerebral vascular function which was not further compounded through acute exercise. The mechanisms and the long term consequences associated with body fat distribution on cerebral haemodynamic function still warrants further investigation.

Table 1: Cerebral haemodynamic function

<table>
<thead>
<tr>
<th>Measure</th>
<th>Non-Obese (n=13)</th>
<th>Obese (n=10)</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCAv (cm/s)</td>
<td>65±11</td>
<td>66±11</td>
<td>59±14</td>
<td>60±19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVR (mmHg/ml/cm)</td>
<td>1.32±0.27</td>
<td>1.17±0.25</td>
<td>1.59±0.39*</td>
<td>1.50±0.35*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVC (cm/s/ml/dl)</td>
<td>0.79±0.16</td>
<td>0.89±0.20</td>
<td>0.69±0.15*</td>
<td>0.70±0.16*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>83±11</td>
<td>75±9</td>
<td>82±13*</td>
<td>85±18*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: Values are mean±SD; * (P<0.05) vs. non-obese.

Figure 1: Correlation between cerebrovascular conductance and visceral adiposity


Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
**Cigarette smoking interferes with dietary nitrate metabolism and its effects on blood pressure and exercise tolerance**

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Introduction - Cardiovascular diseases (CVDs) are the leading cause of mortality globally with cigarette smoking a major risk factor for the development of CVDs and the leading preventable cause of mortality worldwide. Increasing inorganic nitrate (NO\textsubscript{3}\textsuperscript{−}) intake can lower CVDs risk factors with the efficacy of NO\textsubscript{3}− dependent on its uptake into the salivary circulation and subsequent reduction to nitrite (NO\textsubscript{2}−) and nitric oxide (NO) (Kapil et al., 2014). Thiocyanate (SCN\textsuperscript{−}), a competitive inhibitor of salivary NO\textsubscript{3}− uptake (Edwards et al., 1954), is abundant in cigarette smoke. Therefore, this study tested the hypothesis that dietary NO\textsubscript{3}− supplementation would increase salivary and plasma [NO\textsubscript{2}−] and [NO\textsubscript{3}−], lower BP and improve exercise tolerance to a lesser extent in cigarette smokers (S) than non-smokers (NS).

Methods - Nine (5 males) healthy S and eight (4 males) healthy NS controls reported to the laboratory for initial baseline assessment (CON) and following 6 day supplementation periods with 140 mL/day\textsuperscript{−1} NO\textsubscript{3}−-rich (8.4 mmol NO\textsubscript{3}−/day\textsuperscript{−1}; NIT) and NO\textsubscript{3}−-depleted (0.08 mmol NO\textsubscript{3}−/day\textsuperscript{−1}; PLA) beetroot juice in a cross-over experiment. During each laboratory visit, resting blood pressure (BP) was assessed, saliva and venous plasma samples were collected, and a cycling incremental test to exhaustion was completed.

Results - Plasma and salivary [SCN\textsuperscript{−}] were elevated in S compared to NS in all experimental conditions (P<0.05). Relative to CON, salivary [NO\textsubscript{3}−] (3.5 ± 2.1 vs. 7.5 ± 4.4 nM), plasma [NO\textsubscript{2}−] (484 ± 198 vs. 802 ± 199 μM) and plasma [NO\textsubscript{3}−] (218 ± 128 vs. 559 ± 419 nM) increased with NIT in both S and NS, but the magnitude of these increases was lower in S (P<0.05). Salivary [NO\textsubscript{2}−] was similarly increased above CON with NIT in S and NS (P<0.05). Systolic BP was lowered with NIT (100 ± 10 mmHg) relative to CON (107 ± 7 mmHg) and PLA (103 ± 8 mmHg) in NS (P<0.05), but not S (P>0.05). Peak aerobic power (AP\textsubscript{peak}) and oxygen uptake (VO\textsubscript{2peak}) were not significantly impacted by NIT in S (P>0.05). In NS there was no difference in VO\textsubscript{2peak} with NIT (P>0.05), but AP\textsubscript{peak} was higher in NIT compared to both PLA and CON (P<0.05).

Discussion - These findings suggest that the metabolism of dietary NO\textsubscript{3}− is compromised in S leading to attenuated blood pressure reductions and exercise tolerance gains relative to NS. These observations may provide novel insights into the cardiovascular risks associated with cigarette smoking and suggest that this population is less likely to improve cardiovascular health if they conform to global initiatives to increase fruit and vegetable consumption.


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**Ice cooling jacket effect on cardiovascular responses to treadmill exercise in untrained young human adults**

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Hyperthermia has been shown to be a limiting factor to endurance exercise performance. Pre-cooling is an approach used to combat the debilitating effect of heat stress induced fatigue. The efficacy of pre-cooling before prolonged submaximal exercise is demonstrated with a variety of pre-cooling modalities amongst which is wearing of ice cooling jacket/vest. Most studies demonstrated an attenuated heart rate response under steady conditions during endurance exercise after pre-cooling. None of these studies examined blood pressures changes that may occur following exercise after pre-cooling. Therefore, we sought to recognize, quantify, and compare changes that may occur in blood pressures and heart rate after pre-cooling (wearing of ice jacket) following isotonic treadmill exercise. Twelve (n=12) healthy nonathletic young male adults recruited randomly, volunteered to participate in this study after obtaining written consent. The Mean ± S.E.M age, weight, height and B.M.I are 22.0±0.8 years, 60.3±3.0 kg, 1.7±0.2 m, and 21.0±1.0 kg/m\textsuperscript{2} respectively. On the first day of experiment, participants performed treadmill exercise to exhaustion using the Bruce Protocol. Two-reading blood pressures and heart rates were taken before exercise, at exhaustion and five minutes into recovery. On the second day, participants wore ice jacket for about 40 minutes before exercise. Values were recorded as means± SEM compared using unpaired T test at 95% significance. Our findings revealed statistically significant reduction in the heart rate (bpm) at exhaustion (p<0.05) from 101.3 ± 6.5 to 98.1 ± 5.7 and during recovery (p<0.05) from 95.3 ± 5.0 to 79.4 ± 3.6 with no significant changes in the blood pressures before exercise, at exhaustion, and during recovery after pre-cooling. Thus, findings from this study are in support of previous studies and it revealed cardiovascular responses that may occur after pre-cooling. In conclusion, wearing of pre-cooling jacket attenuates heart rate with no significant change in the blood pressures following treadmill exercise in untrained young male adults.

Keywords: Pre-cooling, heart rate, blood pressure, treadmill exercise


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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Resting serum concentration of high-sensitivity C-reactive protein (hs-CRP) in healthy young sportsmen and untrained male adults

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There is an inverse relationship between regular physical activity and concentration of serum inflammatory markers, with variations in resting CRP in trained and untrained subjects (Kasapis and Thompson, 2005). The effect of acute and prolonged exercises has been studied on inflammatory markers (serum CRP) with dearth of information and controversies on the resting serum values of high sensitivity CRP (hs-CRP) in various sportsmen and untrained subjects. Therefore, we sought to identify and compare possible variations that may exist in serum levels of high sensitivity CRP in groups of sportsmen (6) and in untrained human subjects. Eighty one (n=81) healthy male participants (21 control (physically inactive), 10 track athletes, 10 karate athletes, 10 football, 10 volleyball, 10 basketball, and 10 baseball players) volunteered to participate in this study after obtaining written consent and ethical approval. Mean±SEM age, weight, and height of the participants are 22.0±0.8 years, 64.1±2.2 kg, and 1.74±0.3 cm respectively. Participants rested while in sitting position for about 30 minutes during which blood pressures and heart rates were taken. 5 mls of venous blood was withdrawn from the antecubital fossa of the participants (aseptically) into lithium heparin bottles between 8:00 and 10:00 am after overnight fasting. Serum was collected, stored at -250°C after centrifugation (at 3000 rpm for 5 minutes), and later assayed using enzyme linked immunosorbent assay (ELISA) kits for hs-CRP. Values of hs-CRP from the 7 groups were recorded as Mean±SEM age, weight, and height of the participants are 22.0±0.8 years, 64.1±2.2 kg, and 1.74±0.3 cm respectively. Participants rested while in sitting position for about 30 minutes during which blood pressures and heart rates were taken. 5 mls of venous blood was withdrawn from the antecubital fossa of the participants (aseptically) into lithium heparin bottles between 8:00 and 10:00 am after overnight fasting. Serum was collected, stored at -250°C after centrifugation (at 3000 rpm for 5 minutes), and later assayed using enzyme linked immunosorbent assay (ELISA) kits for hs-CRP. Values of hs-CRP from the 7 groups were recorded as Mean±SEM and compared using One-way ANOVA set at 95% significance. From our findings the values of CRP (mg/L) was 1.0±0.2 in the control, 2.6±0.7 in football, 3.6±2.1 in track athletes, 2.4±0.5 in basketball, 2.2±0.5 in volleyball, 2.4±1.3 in baseball, and 1.7±0.5 in karate with no significant differences (p>0.05) in all the experimental groups. Thus, this data reveal average normal range (<10mg/L) of resting values of hs-CRP in the sportsmen and untrained control group with an insignificant variations in the values. In conclusion, resting serum levels of hs-CRP varies insignificantly but falls within normal in healthy trained and untrained young male adults.

Key notes: Resting hs-CRP, physical activity, healthy, young males.


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

Sensitivity and specificity of manual versus automated methods of anaerobic threshold detection in patients undergoing colorectal surgery; implications for clinical outcomes

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Background: Cardiopulmonary exercise testing (CPX) is used to determine cardiorespiratory fitness in patients prior to major surgery given its association with post-operative survival. Typically an automated anaerobic threshold (AT) value of <11.0 ml O₂.kg⁻¹.min⁻¹ (Older et al., 1999) has been employed as an objective biomarker of increased perioperative risk. In the present study, we compared to what extent differences between automated versus manual (gold-standard) methods of AT detection have the theoretical potential to influence surgical risk stratification.

Methods: A randomised sample of 213 patients scheduled for elective colorectal surgery who underwent CPX testing were retrospectively examined. Manual AT results were calculated using the gold standard ‘V-slope’ method (Beaver et al., 1986) and confirmed by two independent clinicians. Automated AT results were compiled using default settings in Breeze software (Medgraphics, UK). Ventilatory equivalent for CO₂ (VE/VCO₂) slope and respiratory exchange ratio (RER) were also recorded at both Manual and Automated ATs. Following confirmation of distribution normality (Shapiro-Wilk tests), data were analysed using a combination of paired samples t-tests and Chi-Squared tests. Data are expressed as mean ± SD and significance established at p < 0.05.

Results: Pulmonary oxygen uptake (V̇O₂) at the AT was 11.0 ± 3.0 versus 12.5 ± 3.8 ml.kg⁻¹.min⁻¹ for the Manual and Automated methods respectively (p < 0.05). One hundred and twelve ATs <11.0 ml O₂.kg⁻¹.min⁻¹ were reported for the Manual versus 70 for the Automated method (p < 0.05). Fifty two false negatives were reported for the Automated method (sensitivity 55%, specificity 91%).

Conclusions: Automated detection of the AT overestimates V̇O₂ by 13% and is associated with a high rate of type II errors (false negatives). This could result in some patients transcending risk stratification boundaries thus leading to incorrect decision making and inappropriate surgical risk stratification. Despite the ease of use of automated software based AT predictions, clinicians should be encouraged to use the manual and gold standard V-slope method for a more accurate assessment of patient cardiorespiratory fitness.

Table 1. Sensitivity, specificity, positive and negative predictive values associated with automated detection of the anaerobic threshold

<table>
<thead>
<tr>
<th>Automated detection</th>
<th>Manual detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT &lt;11.0 ml O₂.kg⁻¹.min⁻¹</td>
<td>AT &lt;11.0 ml O₂.kg⁻¹.min⁻¹</td>
</tr>
<tr>
<td>63</td>
<td>89</td>
</tr>
<tr>
<td>Sensitivity 55%</td>
<td>Sensitivity 91%</td>
</tr>
</tbody>
</table>

Type I error (false positive rate) = 9%, Type II error (false negative rate) = 45%

Beaver et al. (1986). J Appl Physiol 60(6), 2020-2027
Effects of acute inspiratory hypoxia on the transcerebral exchange kinetics of large neutral amino acids in healthy volunteers

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Large neutral amino acids compete for transport across the same saturable carrier across the blood brain barrier; an increase in the arterial levels of aromatic relative to branched-chain amino acids may thus cause brain dysfunction through increased intracerebral formation of so-called ‘false’ neurotransmitters that inhibit central noradrenergic pathways (Berg et al, 2010). A reduction in Fischer’s ratio ([valine+leucine+isoleucine]/[phenylalanine+tyrosine]) is therefore thought to cause encephalopathy in fulminant hepatic failure and sepsis. Because these conditions are often associated with arterial hypoxaemia, it is unknown whether this contributes to changes in the transcerebral exchange of large neutral amino acids.

Eleven healthy males aged 27 (mean; SD 4) years were examined in normoxia and following 9h passive exposure to hypoxia (12.9% O₂). Global cerebral blood flow (CBF) was measured using the Kety-Schmidt technique, and arterial-to-jugular venous differences of large neutral amino acids were determined at both conditions by high-performance liquid chromatography. Cerebral delivery and net exchange of amino acids were then calculated by multiplying CBF with the arterial concentrations and arterial-to-jugular venous differences of amino acids, respectively. Data are reported as median (interquartile range), and conditions were compared by Wilcoxon’s signed rank test.

Hypoxia was associated with an increase in the arterial levels and cerebral delivery of both the aromatic amino acid phenylalanine, and the branched-chain amino acids leucine and isoleucine (Table). Fischer’s ratio was thus unaffected (normoxia: 4.8 [4.5-4.9]; hypoxia: 4.6-5.0; NS), and a net cerebral uptake of leucine and isoleucine was maintained during hypoxia with no changes in the cerebral net exchange of phenylalanine (Table). Although inspiratory hypoxia increases the cerebral delivery of the aromatic amino acid phenylalanine, the concurrent increased cerebral delivery of branched-chain amino acids renders the transcerebral exchange kinetics of large neutral amino acids unchanged. Our data thus suggest that arterial hypoaxaemia is not critical to the changes in the transcerebral exchange kinetics of large neutral amino acids that occur in fulminant hepatic failure and sepsis.

<table>
<thead>
<tr>
<th>Aromatic Amino Acids</th>
<th>Normoxia</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>45.7</td>
<td>45.7</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>37.3</td>
<td>37.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>119</td>
<td>119</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>56.4</td>
<td>56.4</td>
</tr>
<tr>
<td>Valine</td>
<td>221</td>
<td>221</td>
</tr>
</tbody>
</table>

Poster Communications

PC080

Difference between normoxia and hypoxia, *p<0.05, **p<0.01; difference between arterial and venous amino acid concentration, †p<0.05, ‡p<0.01.


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

Serum brain-derived neurotrophic factor response to acute aerobic exercise: Implications of plasma volume correction

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Background and Aims: The neurotrophin brain-derived neurotrophic factor (BDNF) is widely discussed as a potential molecular mediator associated with post-exercise enhancement of mood and cognitive function in humans (Chang et al., 2012). In support, emerging evidence suggests a dose-response relationship between exercise intensity and the peripheral concentration of circulating BDNF (Knaepen et al., 2010). However, exercise can also contract plasma volume (PV) due to the combination of increased capillary hydrostatic and interstitial osmotic pressures forcing fluid from the intravascular to the extravascular space. Thus, to what extent the increase in circulating BDNF is due to exercise per se or is simply a reflection of haemoconcentration remains to be established.

Methods: To examine this, 23 physically active participants (19 males/4 females) aged 27 (mean) ± 7 (SD) years old were assessed at rest and during a standardised incremental semi-recumbent cycling test to volitional exhaustion (Bailey et al., 2013). Exactly 30 min following cannulation, blood samples were obtained without stasis from the cephalic vein. Haemoglobin (Hb) was assessed via photometry and haematocrit (Hct) by ultracentrifugation with plasma volume (PV) shifts calculated according to established methods (Dill and Costill, 1974).

Following centrifugation, serum was flash frozen in liquid nitrogen and stored at -800C. BDNF was measured with an enzyme-linked immunosorbent assay using BDNF monoclonal antibodies (Kolbeck et al., 1999). Breath-by-breath online respiratory gas analysis was employed for the determination of maximal oxygen uptake (VO₂max) and associated cardiorespiratory parameters. Given that BDNF data were not normally distributed (Shapiro Wilk tests), differences were analysed using Wilcoxon Matched Pairs Signed Ranks tests and relationships via a Spearman’s Rank Correlation. Significance was established at P < 0.05.

Results: All participants achieved a maximal effort according to established criteria recording a VO₂max of 35 ± 8 ml/kg/min (P < 0.05). Exercise was shown to increase serum BDNF (P < 0.05, see Figure A) and decrease PV by 15 ± 5% (P < 0.05). Adjustment for this apparent haemoconcentration atten-
ated the exercise-induced increase in BDNF by ~42% ($P < 0.05$, see Figure B). In contrast, we failed to observe a relationship between exercise-induced changes in PV and BDNF ($r = 0.08$, $P > 0.05$).

Conclusions: The present findings emphasise the significance of adjusting for PV contraction when assessing the “true” magnitude of the peripheral BDNF response to acute exercise.

Figure. Implications of plasma volume correction for the exercise-induced increase in serum BDNF

Values are mean ± SD; PV+/−, plasma volume shifts non-adjusted/adjusted; *different compared to Rest ($P < 0.05$); †different compared to PV− ($P < 0.05$).


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

PC082

Pre-exercise citrulline malate ingestion has no effect on gastric emptying rate of a carbohydrate-electrolyte solution during moderate intensity exercise

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Water and nutrient delivery during exercise is determined by the combined rates of gastric emptying and intestinal absorption. Exercise at an intensity that exceeds 70% VO$_{2\text{max}}$ results in a reduction in gastric emptying rate, particularly when exer-

cise is of an intermittent nature[1]. It has been suggested that this observation is likely to be due to the reduction in splanchnic blood flow that has been observed during exercise[2]. It has also been suggested that this reduction in splanchnic blood flow is the main mechanism for a number of gastrointestinal complaints observed during exercise[2]. Citrulline, a precursor to L-arginine, increases nitric oxide production and pre-exercise ingestion has been shown to increase blood flow in gut microcirculation and reduce levels of enterocyte damage[3]. The aim of this study was to determine whether pre-exercise citrulline ingestion influenced gastric emptying rate during moderate intensity exercise.

Ten healthy male participants aged 21 ± 1 years undertook two experimental trials in a randomised, crossover, double blind, placebo controlled study. Following an overnight fast, participants ingested 100 mL of water with either 6 g of citrulline malate or alanine before a 30 minute rest period. Participants then ingested 600 mL of a commercially available sports drink containing 100 mg $^{13}$C sodium acetate, for measurement of gastric emptying rate. They then undertook a 60 minute period of cycle ergometry exercise at an initial intensity sufficient to illicit 65% of age predicted maximal heart rate. Breath samples were collected pre-exercise and at 10 minute intervals throughout exercise to assess gastric emptying rate using the $^{13}$C acetate breath method. Data was analysed using t-tests and two factor ANOVAs where appropriate.

Half emptying time ($T_{1/2}$) of the solution amounted to 45 ± 7 and 47 ± 12 minutes ($P = 0.559$) during the alanine and citrulline malate trials respectively. Time of maximal emptying rate ($T_{max}$) amounted to 35 ± 5 and 35 ± 6 minutes ($P = 0.822$) during the alanine and citrulline malate trials respectively. Two factor repeated measures ANOVA on breath delta over baseline data showed no main effect of supplement ($P = 0.888$), a main effect of time ($P < 0.001$) and no interaction effect ($P = 0.915$).

Citrulline supplementation has been demonstrated to improve blood flow of the gut microcirculation[3] however the results of this study suggest that 6 g citrulline malate 30 minutes prior to moderate intensity exercise has little effect on gastric emptying rate during exercise. Future studies should focus on the intensity of exercise and/or the dosage of citrulline malate provided.


The authors would like to acknowledge the assistance of Mr Dave Maskew during the course of this study

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Exploring a novel role for zinc in modulation of sarcoplasmic reticulum calcium release in skeletal muscle

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Aberrant Ca\(^{2+}\) release from sarcoplasmic reticulum Ca\(^{2+}\) stores due to dysfunction in the type-1 ryanodine receptor (RyR1) is implicated in the pathophysiology of muscular dystrophy (1). Recently it has been suggested that dynamic alterations in intracellular Zn\(^{2+}\) and Ca\(^{2+}\) homeostasis play a key role in the pathogenesis of dystrophies (2). The role of physiological levels of Zn\(^{2+}\) in modulating RyR1-channel gating has never been considered. We therefore set out to study the effects of Zn\(^{2+}\) on RyR1-channel function. RyR1 channels were prepared from guinea pig skeletal muscle and incorporated into planar phosphatidylethanolamine lipid bilayers under voltage-clamp conditions using previously described techniques (3). Single channel recordings were acquired under standard experimental conditions of 250 mM HEPES, 80 mM Tris, pH 7.2 (free [Ca\(^{2+}\)] = 10 \(\mu\)M) at the cytoplasmic face (cis-chamber) and 250 mM Glutamic acid, 10 mM HEPES, pH 7.2 with Ca(OH)\(_2\) (free [Ca\(^{2+}\)] = 50 \(\mu\)M) at the luminal (trans-chamber) face of the channel. The cis-chamber was held at 0 mV relative to ground. Channel open probability (Po) was determined over 3 minutes, and a Student’s t-test was used to determine statistical significance between mean values. Sequential addition of physiological concentrations of Zn\(^{2+}\) increased the frequency of channel openings. The mean channel open time was 1.7 \(\mu\)s in the absence and 2.1 \(\mu\)s in the presence of 10 nM Zn\(^{2+}\) (free [Zn\(^{2+}\)] = 0.018 to 0.162 \(n\)M), and 2.7 \(\mu\)s in the presence of 100 nM Zn\(^{2+}\) (free [Zn\(^{2+}\)] = 0.014 to 0.142 \(n\)M) and 5.3 \(\mu\)s in the presence of 1 mM Zn\(^{2+}\) (free [Zn\(^{2+}\)] = 0.012 to 0.142 \(n\)M) and 15 \(\mu\)s in the presence of 10 mM Zn\(^{2+}\) (free [Zn\(^{2+}\)] = 0.014 to 0.142 \(n\)M). Our data reveal that Zn\(^{2+}\) is a high affinity effector of RyR1 that increases channel Po by modulating the sensitivity of the channel to cytosolic Ca\(^{2+}\). We suggest that perturbation of Zn\(^{2+}\)-homeostasis will lead to aberrant Ca\(^{2+}\)-release through inappropriate activation of RyR1 and that this will contribute to the pathophysiology of debilitating muscular wasting disorders such as muscular dystrophy.


This work is supported by the Royal Society of Edinburgh, Teneveroucot Scotland & the British Heart Foundation.

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

Lack of correlation between maximal oxygen uptake and heart rate recovery in healthy adult volunteer subjects

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In addition to use in the assessment of performance in endurance sports, the measurement of cardiorespiratory fitness (CRF) has wide clinical application as a prognostic indicator of mortality (Blair et al., 1996). However, while maximal oxygen uptake (VO\(_{2\text{max}}\)) is generally considered to represent the gold standard index of CRF, its measurement is infeasible in a healthcare setting. On the other hand, heart rate recovery (HRR) and heart rate variability (HRV) are readily measurable parameters that are considered to reflect CRF and autonomic regulation of the heart and have prognostic value as indicators of mortality (Dekker et al., 1997; Nishime et al., 2000). This study aimed to investigate the correlation between VO\(_{2\text{max}}\) and HRR and HRV as potential surrogate measures of CRF. Approval was obtained from local ethics committee. 48 healthy adult volunteers (24 male and 24 female), of varying levels of training, were recruited with informed consent from the student population of Bristol. VO\(_{2\text{max}}\) was measured by a stepped workload protocol on a bicycle ergometer (25 W every 2 min) until volitional exhaustion. HRV was measured as the coefficient of variation of the RR intervals from a 5 min pre-exercise test electrocardiogram recording with the subject supine. HRR was measured as the recovery of heart rate within the first minute following cessation of exercise. Blood lactate levels were measured sequentially using a Lac-tate Pro 2 analyser from blood samples obtained using sterile lancets during exercise. Data, presented as means\pm standard error of the mean, were subject to Shapiro Wilks normality test and compared by either unpaired t-test or Mann-Whitney U test and correlations investigated by calculation of either Pearson’s or Spearman’s correlation coefficient. P<0.05 was used as the limit of statistical confidence. While there were no differences in resting heart rate (HR, male 68.5\pm1.6 bpm, female 73.9\pm2.2 bpm), maximal HR (male 189.4\pm1.9 bpm, female 191.9\pm1.8 bpm), HRR (male 37.7\pm1.9 bpm, female 35.2\pm1.9 bpm) or HRV (male 96.2\pm7.0 ms, female 82.3\pm5.0 ms), males showed significantly greater maximal power output (male 319.8\pm10.0 W, female 226.0\pm6.5 W, P<0.001) and VO\(_{2\text{max}}\) (male 53.3\pm1.6 ml/kg/min, female 40.5\pm1.5 ml/kg/ min, P<0.001) than females. VO\(_{2\text{max}}\) was not significantly correlated with either HRR (Spearman’s r=0.013, P=0.930) or HRV (Pearson’s r=0.091, P=0.538). Thus, there was no evidence of a direct relationship between VO\(_{2\text{max}}\) and the heart rate indices of autonomic control of cardiac function within this group of relatively trained subjects. Care is therefore required in the use of HRR and HRV as surrogate measures of cardiorespiratory fitness. Further investigation into the correlation between HRR, HRV and VO\(_{2\text{max}}\) in patient groups with elevated mortality may be warranted.


Development of a minimally invasive tracer technique to quantify acute muscle protein synthesis: internal comparison between D$_2$O and L-[ring-^{13}C$_6$]-phenylalanine in humans


MRC-ARUK Centre of Excellence for Musculoskeletal Ageing Research, Clinical, Metabolic and Molecular Physiology, School of Medicine, University of Nottingham, Derby, Derbyshire, UK

Stable isotopically-labelled amino acids (AA) are a crucial tool for developing our understanding of the control of human muscle protein synthesis (MPS) in health, ageing and catabolic disease(s) [1]. Typically, intravenous (I.V) infusions of heavy carbon/nitrogen/hydrogen AA-tracers alongside tissue [muscle] sampling and mass spectrometric (MS) analyses permit calculation of MPS as a fractional rate of protein synthesis (FSR). Yet, despite providing highly accurate dynamic readouts of musculoskeletal metabolism, these traditional AA-tracer methodologies are subject to limitations. These approaches, require I.V infusions and venous cannulation(s), which can be challenging in some populations (e.g. frail elderly), and are costly. Recently, we developed less invasive methods and showed that oral provision of deuterium oxide (D$_2$O) tracer was efficacious for quantifying MPS over 2-8 days [2]. The sensitivity of our approach to quantify short-term MPS (i.e. over hours) remained unknown. Here, we hypothesized that our newly developed precision MS approaches coupled to appropriately D$_2$O dosing strategies would permit accurate quantification of MPS over periods of hours. We recruited nine males (24±3y, BMI: 25±3kg.m$^{-2}$, ±SD) into an internally controlled comparison of D$_2$O vs. AA tracers. Having orally consumed 400 ml D$_2$O ~18 h earlier, on the study day subjects received I.V infusions of L-[ring-^{13}C$_6$]-phenylalanine (0.3 mg.kg$^{-1}$ prime, 0.6 mg.kg.$^{-1}$h$^{-1}$, continuous) to permit bi-tracer quantification of MPS under: i) basal [postabsorptive] and, ii) stimulated [postprandial] conditions i.e. consumption of 20g EAA. Muscle biopsies were taken from m. vastus lateralis at 0h, 3h and 6h (i.e. 0-3 h, basal MPS, and 3-6 h, impacts of EAA anabolic stimulus), with blood and saliva samples being taken regularly throughout the study. The FSR of myofibrillar protein fractions were quantified by GC-C-IRMS ($^{13}$C$_6$) and GC-pyrolysis-IRMS (D$_2$O) techniques, using intracellular phenylalanine and body water as surrogate precursors; respectively. Postabsorptive rates of MPS were equivalent between tracers i.e. D$_2$O: 0.050±0.007 and $^{13}$C$_6$: 0.065±0.004 %h$^{-1}$ (±SEM). Similarly, increases in MPS following EAA consumption were both significant (P<0.05, two-way ANOVA) and indistinguishable between tracers: 0.088±0.008 and 0.089±0.006 %h$^{-1}$ using D$_2$O and $^{13}$C$_6$; respectively. Moreover, despite moderate intra-individual tracer-dependent differences in FSR (NB: not different from other cross tracer comparisons), there was a significant Pearsons correlation between D$_2$O and $^{13}$C$_6$ phenylalanine derived FSR (r=0.438, P=0.035 one-tailed), whilst Bland-Altman plots showed only a small mean bias of 0.0083 between the two methods. As such, we have developed, and validated, a less invasive D$_2$O method for the acute quantification of MPS.

Poster Communications

PC085

Highly active antiretroviral therapy alters sperm parameters and testicular antioxidant status in lean and diet-induced obese male Wistar rats

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Highly active antiretroviral therapy (HAART) is considered the most effective treatment for individuals with HIV-1 infection (Ahmad et al., 2011), although its effect on male reproductive function is still debatable. Epidemiological studies have revealed that the desire to raise children exists in about one-third of HIV patients and two-thirds of the patient population are usually overweight or obese, (Crum-Cianflone et al, 2008; Kushnir and Lewis, 2011).

This study was designed to investigate the in vivo effects of HAART on some sperm functional parameters and the testicular oxidative status in lean and diet-induced obese (DIO) rats. Rats (males, 180-200g, n= 40) were assigned equally into 4 groups and treated orally for 16 weeks. Group I and II were the vehicle control groups that received 1.0 ml/kg/day distilled water by gavage for the latter 6 weeks. Group I served as the lean group fed with standard rat chow, Group II was the DIO group fed with rat chow supplemented with sucrose and condensed milk while Groups III and IV were lean and DIO groups respectively that were treated with HAART (antiretroviral drug combination of tenofovir, emtricitabine and efavirenz at a dose of, 17, 26 and 50mg/kg/day for the latter 6 weeks). At the end of the experimental period, animals were anaesthetized (160mg/kg i.p.) and the testicular antioxidant status in lean and DIO groups were analysed. Sperm analysis was done on the sperm collected from the caudal epididymis while the testes and epididymis were removed. Sperm analysis was done on the sperm. This study was designed to investigate the in vivo effects of HAART on some sperm functional parameters and the testicular oxidative status in lean and diet-induced obese (DIO) rats. Rats (males, 180-200g, n= 40) were assigned equally into 4 groups and treated orally for 16 weeks. Group I and II were the vehicle control groups that received 1.0 ml/kg/day distilled water by gavage for the latter 6 weeks. Group I served as the lean group fed with standard rat chow, Group II was the DIO group fed with rat chow supplemented with sucrose and condensed milk while Groups III and IV were lean and DIO groups respectively that were treated with HAART (antiretroviral drug combination of tenofovir, emtricitabine and efavirenz at a dose of, 17, 26 and 50mg/kg/day for the latter 6 weeks). At the end of the experimental period, animals were anaesthetized (160mg/kg pentobarbital i.p.) and the tests and epididymis were removed. Sperm analysis was done on the sperm collected from the caudal epididymis while the testes was homogenized for antioxidant enzyme and lipid peroxidation assays. Values were expressed as mean ± S.E.M. compared by ANOVA. When the lean and DIO treated groups were compared with their respective control, results showed that HAART significantly decreased sperm motility (94.13±0.60 % vs. 81.56±3.23 %; 83.06±1.91 % vs. 65.25±4.76 %, p<0.05) and viability (97.00±2.44 % vs. 81.86±2.25 %; 89.29±1.43 % vs. 78.43±2.08 %, p<0.05). Testicular glutathione (50.17±4.18 vs. 18.83±1.85 μg/mg protein, p<0.05), catalase (143.5±14.61 vs. 112.80±10.94 μg/mg protein, p<0.05) and superoxide dismutase (7.92±0.16 vs. 6.23±0.23 μg/mg protein, p<0.05) were significantly decreased while Thioabarbituric acid reac-
tive substances (TBARS) level (2.0±0.17 vs. 2.84±0.24 nmol/L tissue, p<0.05) was significantly increased only when the DIO HAART group was compared with DIO control group. Thus, the decrease in sperm qualities associated with HAART might be as a result of increased testicular oxidative stress pronounced in obese animals. These data implies that obesity is a confounding factor and interferes with the treatment regiments effectiveness.


This study was supported by a grant from the Harry Crossley Foundation and funding from the Vice Dean Research’s strategic Fund (Stellenbosch University)

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

PC087

Pancreatic functions in high salt-fed female rats

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Background and Aim: Salt consumption has been increased worldwide and the association of high salt diets with enhanced inflammation and target organ damage was reported. Little data were available about the effect of high salt diet on exocrine function of pancreas, while the relation between high salt intake and insulin sensitivity were controversial. This study was designed to investigate the effect of high salt diet on exocrine and endocrine pancreatic functions, and to elucidate the possible underlying mechanism(s).

Materials and Methods: 20 adult female Wistar rats were randomly divided into 2 groups; control group; fed standard rodent diet containing 0.3% NaCl, and high salt fed group; fed 8% NaCl for 8 weeks. On the day of sacrifice, rats were anaesthized by i.p. pentobarbitone (40 μg/kg B.W.). Nasoanal length was measured and fasting glucose was determined in blood samples obtained from venipuncture of the tail vein. Additional blood samples were obtained from the abdominal aorta under anaesthesia for determination of plasma sodium, potassium, amylase, lipase, aldosterone, insulin, TGF-β1 and IL6. Pancreata of both groups were histologically studied.

Results: Compared to control group, 8 week-high salt fed group showed: significant elevation in body weight, body mass index, Lee index, plasma sodium, TGF-β1 and IL6, however, plasma aldosterone, amylase, lipase and insulin levels were significantly decreased. A non-significant increase in plasma potassium and non-significant changes in fasting blood glucose and HOMA-IR were detected between groups. Pancreatic fibrosis was observed in test group.

Conclusion: High salt diet for 8 weeks caused pancreatic fibrosis evidenced by decline of both exocrine and endocrine functions of pancreas in Wistar rats.

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

PC088

Amelioration of hyperglycaemia and oxidative stress in alloxan-induced diabetic Wistar rats treated with probiotic and vitamin C

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Hyperglycaemia-induced oxidative stress plays a crucial role in the development and progression of diabetes mellitus (DM). Albeit, insulin has remained an important component treatment plan for DM management in patients with type 1 diabetes mellitus (T1DM), it fails to prevent the long-term complications. Experiment aimed at investigating ameliorative effects of probiotic and vitamin C (vit-C) treatments on the hyperglycaemia and oxidative stress in alloxan-induced diabetic rats. Wistar rats (male, 100-180g, n=48) were allowed to acclimatize for two weeks, animal experiments adhered to the Guide for the Care and Use of Laboratory Animals (NRC, 2011). T1DM was induced in overnight-fasted rats by a single i.p. injection of alloxan (150 mg/kg, dissolved in a cold physiological saline (0.9 % NaCl) solution. Blood glucose (BG) concentration of the rats was measured 72 h after alloxan administration. BG concentration in all groups were recorded following 12-h fast each day at 8:00 AM before feeding the rats using glucometer (On Call®Plus, 30175, Germany), and glucose test strips. Animals with BG concentration ≥14 mmol/L were considered diabetic (Lenzen, 2008). At the end of the experiment, rats fasted overnight were killed by jugular venisection after light chloroform anaesthesia. 5ml of blood from each rat collected into dried tubes and centrifuged at 2000 × g for 15 min to obtain serum. Activities of catalase and glutathione peroxidase were determined spectrophotomerically, according to Beers and Sizer (1952), Paglia and Valentine (1967), respectively using the serum and tissues homogenates. Malondialdehyde concentration in serum and tissues of rats was determined, according to Placer et al. (1996). Six groups of animals (n=8, per group) received the following treatment regimens for 4 weeks: 1) Oral administration of normal saline; 2) alloxan (150 mg/kg, i.p.); 3) alloxan (150 mg/kg) + insulin (4 U/kg, s.c.); 4) alloxan (150 mg/kg) + probiotic (4.125 × 10^6 CFU/100 ml per os); 5) alloxan (150 mg/kg) + vit-C (100 mg/kg, i.m.); 6) alloxan (150 mg/kg) + probiotic (4.125 × 10^6 CFU/100 ml per os) + vit-C (100 mg/kg, i.m.). Experiment lasts 4 weeks. Values are means ± S.E.M., compared by repeated-measures ANOVA. Decrease in BG concentration (12.55±0.89, P<0.001; 13.03±0.74, P<0.001; 11.02±0.69, P<0.001; 10.97±0.76 mmol/L, P<0.001) was obtained in diabetic treated groups compared to control. Treatment with probiotic + vit-C decreased BG concentration (10.97±0.76 mmol/L, P < 0.01) than the untreated diabetic group (14.18 ± 0.68 mmol/L). Probiotic + vit-C, reduced lipid peroxidation in the serum, brain and kidney (Figures 1, 2, and 3, respectively) but increased activities of antioxidant enzymes. In conclusion, probiotic + vit-C may be effective in ameliorating hyperglycaemia, and oxidative stress in alloxan-induced diabetic rats.
PC089

Cellular mechanisms of saline extract of alligator pepper (Zingiberaceae afromomum melegueta) for specific protection against fetal macrosomia

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Introduction: Aqueous extract of Alligator pepper is known to reduce gestational weight gain and litter size in Sprague Dawley rats. This study was done to determine the underlying hormonal and cellular mechanisms of action.

Methods: It was a controlled cross sectional intervention study with 45 female Sprague Dawley rats, which after acclimatization for two weeks, were allowed to mate with male rats for three days. Thereafter, the pregnant female rats were randomly allocated into three groups A, B and C with 15 rats in each group. These three major groups were further randomly divided into three subgroups so that each subgroup had 5 female rats. Rats in Group A were injected with 2ml of normal saline intraperitoneally on day 4. Rats in groups B and C were injected intraperitoneally with 6.7mg/Kg body weight and 13.4mg/kg body weight of saline extract of Alligator Pepper respectively on day 4. All rats were euthanized by cervical dislocation on days 7, 14 and 21 respectively following chloroform anesthesia. Blood was collected by intra-ventricular aspiration and assayed for insulin levels using Insulin ELISA. The uteri of the rats were dissected to reveal the embryonic fetuses in their gestational uterine cavity. Glucose levels were estimated with glucometer on days 7, 14, and 21. Blood was collected by intra-ventricular puncture and assayed for insulin levels. Observed differences between control and experimental groups were subjected to tests of significance.

Results: Alligator pepper treated pregnant rats had significantly higher serum glucose levels than control group. Low dose and high dose Alligator pepper depressed serum insulin levels in the experimental group on day 7 and days 7 and 14 respectively.

Conclusion: Intraperitoneal injection of saline extract of Alligator pepper prevents first and second trimester hyper-insulinemia in pregnant Sprague Dawley rats.


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

PC090

Sex dependent effects of agomelatine on reproductive functions in rats

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Agomelatine is an antidepressant with a novel mechanism of action. It is a melatonergic agonist for MT1 and MT2 receptors and a serotonin (5-HT)2C receptor antagonist. Unlike selective serotonin reuptake inhibitors (SSRIs), agomelatine has been suggested not to have sexual dysfunction. Melatonin is suggested to have effects on hypothalamic–pituitary–gonadal (HPG) axis. Our previous study showed that melatonin directly affected GT1-7 cells, immortalized GnRH neurons, increasing intracellular free calcium levels ([Ca2+]i). However, the effects of agomelatine on reproductive functions have not been sufficiently studied in animal models. Therefore, in this study, we aimed to explore the effects of agomelatine in male and female rats. For the experimental studies, male and female Sprague Dawley rats were used. Each group consisted of 10 rats. The animals started to receive daily oral agomelatine (10 mg/kg) on post-natal day 21. The control group received only vehicle. Puberty onset was monitored by examination of vaginal opening and preputial separation in female and male rats, respectively. Changes of body weight and food intake were daily monitored. The female rats were decapitated under general anesthesia with xylazine (80 mg/kg)/ketamine (12 mg/kg) cocktail on the first diestrus following 90 days. The male rats were maintained until 120 days for analyzing sexual behaviour and antidepressant efficacy, which was assessed using the forced swimming test (FST). Agomelatine advanced vaginal opening in the female rats (48.9±1.1 days vs 43.1±0.7 days for control rats, p<0.001) whereas it delayed puberty onset in male rats (45.8±0.5 days vs. 50.2±0.9 days for control rats, p<0.001). Pubertal weight was significantly higher in agomelatine-treated male rats (165.5±3.74 g vs 144.29±3.53 g for control rats, p<0.001) whereas there was no any significant change in female rats. None of the parameters related to sexual behaviour showed significant differences between agomelatine-treated and control male rats except intromission frequency (IF). Agomelatine decreased IF (18.9±1.5 vs 26.5±2.3 for control rats, p<0.05), which indicates a facilitator role of this antidepressant on male sexual behaviour. Agomelatine was very active in the FST at 10mg/kg and induced a significant decrease (p<0.01) in duration of immobility (67.3±4.9 vs. 103.7±9.5 for control rats). The swimming time was significantly increased by agomelatine (145.7±7.8 vs. 113.7±5.4
for control rats, $p<0.01$). The present findings suggest that agomelatine shows a strong antidepressant effect in male rats without any adverse influences on sexual behaviour, and its effects on pubertal maturation seem to show sex-dependent differences.


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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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**PC091**

**Oxidative stress increases soluble (pro)renin receptor in human cultured erythroid cell line, YN-1**

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(Pro)renin receptor ((P)RR) is a specific receptor for renin and prorenin (Nguyen et al. 2002). When prorenin binds to (P)RR, prorenin becomes enzymatically active in converting angiotensinogen to angiotensin I. In addition, (P)RR has various biological functions independent of the renin-angiotensin system. The binding of (pro)renin to (P)RR stimulates the intracellular signals for proliferation and/or hypertrophy of cells. (P)RR is associated with vacuolar-type H⁺-ATPase, a large multi-subunit, membrane-associated protein complex which keeps the acidic environment of intracellular compartments and of the extracellular space. Soluble (P)RR (s(P)RR) is generated by the cleavage of full length (P)RR consisting of 350 amino acids with a single transmembrane domain. (P)RR is expressed in various types of cells including erythroid cells. (P)RR is related to the pathophysiology of vascular complication of diabetes mellitus and hypertension. Moreover, plasma levels of s(P)RR were elevated in patients with obstructive sleep apnea syndrome (Nishijima et al., 2014). However, the mechanism which controls plasma s(P)RR levels has not been clarified. The aim of the present study is therefore to clarify effects of oxidative stress on generation of s(P)RR in erythroid cells. Human cultured erythroid cell line, YN-1 (Kaneko et al., 2012) was cultured in Iscove’s modified Dulbecco’s medium containing fetal bovine serum. The cells were treated with H₂O₂ (10, 100, 1000 μM) for 24h. (P)RR expression was analyzed by western blot analysis using the rabbit polyclonal (P) RR antibody specific for its extracellular domain (Hirose et al. 2009). The effect of erythropoietin (EPO, 10 ng/ml) on s(P)RR levels was also studied. Western blot analysis (n=4) showed that expression levels of s(P)RR (28 kDa) were increased about 21-fold by the treatment of 1000 μM H₂O₂ compared with control. EPO (10 ng/ml) alone did not affect expression levels of s(P)RR. Co-treatment of EPO (10 ng/ml) and H₂O₂ (1000 μM), however, suppressed the H₂O₂-induced increase in s(P)RR expression level to about 33%. Expression levels of furin protein were not changed by any treatments, suggesting that H₂O₂-induced increase in s(P)RR expression level was not mediated by the expression level of furin. These findings raised the possibility that oxidative stress increased expression levels of s(P)RR and that EPO played an protective role against oxidative stress.


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**PC092**

**Effect of gum arabic on oxidative stress and total antioxidant capacity in sickle cell disease**

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SCD is primarily a disorder of red blood cells (RBCs), which are a significant source of free radicals in biological systems. Hb S polymerization creates pro-oxidant reactive environment, which contributes to haemolysis, vaso-occlusive processes and endothelial dysfunction. Oxidative stress represents the imbalance between enhanced generation of reactive oxygen species and low cellular content of antioxidants. Oxidative stress is one of the factors that modulates the phenotypic expression of SCD. Gum Arabic is edible, dried gum exudate from the stems and branches of Acacia senegal and Acacia seyal, that is rich in non-voscous soluble fiber. US FDA recognized it as one of the safest dietary fibers. Clinically, GA has been shown to be beneficial in patients with CRF. GA found to have antioxidant and lipid peroxidation lowering effects in vitro. It was found that GA protected against lipid peroxidation in dose dependent manner. In this study we investigated the effect of GA as an anti oxidant among sickle cell patients.

Method: 47 patients with hemoglobin SS aged 5-42 years, who are on regular follow up in Military hospital. Patients received GA 30g/day for 12 weeks. Total Anti Oxidant Capacity(TAC), Malondialdehyde(MDA) and hydrogen peroxide (H₂O₂)level were measured before and after the intervention. Total antioxidant capacity (TAC ), malondialdehyde (MDA)and hydrogen peroxide (H₂O₂)were measured colorimetrically. (MDA) levels and (H₂O₂) were considered as indicator of lipid peroxidation. Data were analyzed using SPSS. 20. Paired samples T test was used to compare between pre and post intervention results . P values equal or less than 0.05 were considered significant. Ethical clearance was obtained from the Institutional Review Board at ALNeelain University and from State Ministry of Health ethical research committee. Written informed consent was obtained from patients or from parents when the patient is less than 18 years old.

Results: Serum TAC improved significantly after taking GA [P:V:0.000 (95% CI 0.02587 to 0.07073)]. Regarding oxidative stress biomarkers Serum MDA level decreases significantly[P:V:0.024(95%CI-2.183 to -0.1629)]. Also plasma H₂O₂ decreased from baseline [P:V:004[ 95CI-1.820 to -0.3698]]

Conclusion: Gum Arabic daily intake showed to increase TAC and decrease oxidative stress which could be of great health benefits for sickle cell anemia patients

Chirico EN, Pialoux V. Role of oxidative stress in the pathogenesis of sickle cell disease. IUBMB Life 2012 Jan;64(1):72-80.


We would like to acknowledge Dr Omer Eissawi and Dr Hyder Alwad from Military hospital for their great help in recruiting the patients and doing the clinical follow up during the study period. We also acknowledge Miss Nada Altayb from Alneelain research centre for the technical support.

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The effect of polyamidoamine dendrimers on epidermal growth factor receptor signaling in vitro and in vivo


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Polyamidoamine (PAMAM) dendrimers are cationic, branch-like spherical polymers being investigated for a variety of applications in nanomedicine including nucleic acid drug delivery. However, little is known about their toxicology in terms of their interactions with signal transduction pathways both in vitro and in vivo. We previously showed that a commercially available fragmented (or activated) generation (G) 6 PAMAM dendrimer, Superfect (SF), stimulated epidermal growth factor receptor (EGFR) tyrosine kinase signaling - an important signaling cascade that regulates cell growth, differentiation, migration, survival and apoptosis- in cultured human embryonic kidney (HEK-293) cells (Akhtar et al, 2013). Here, we firstly studied the in vitro effects of Polyfect (PF), an intact G6 PAMAM dendrimer, on EGFR tyrosine kinase signaling via extracellular-regulated kinase 1/2 (ERK1/2) in cultured HEK-293 cells and then compared the in vivo effects of a single i.p. administration of PF or SF (at 10mg/kg) on EGFR signaling in the kidneys of normal and diabetic male Wistar rats. Diabetes was induced by a single 55mg/kg i.p injection of streptozotocin and animals were sacrificed by cervical dislocation after 4 weeks. Dendrimers were administered 24 h before sacrifice. In cultured human embryonic kidney (HEK-293) cells, Polyfect, exhibited a dose- (0.4 to 40 microgram/mL) and time (1h to 24h) -dependent inhibition of EGFR phosphorylation in a manner similar to that observed for AG1478, a selective EGFR tyrosine kinase inhibitor. For example, treatment of cells with PF at 40 microgram/mL dose for 24h significantly reduced EGFR phosphorylation to 12.5 + 2.1% of vehicle-treated controls (p>0.05; N=6; Mean + s.d. shown; typically mean values were compared using analysis of variance followed by post hoc test (Bonferroni)). Administration of PF or SF to non-diabetic or diabetic animals (N=4-6) with subsequent study of EGFR phosphorylation in rat kidneys 24 h post-administration by Western blotting showed that PF inhibited EGFR phosphorylation by about 40% in both sets of animals whereas SF stimulated phosphorylation by 12.5 + 2.1% of vehicle-treated controls (p>0.05; N=6; Mean + s.d. shown; typically mean values were compared using analysis of variance followed by post hoc test (Bonferroni)). Administration of PF or SF to non-diabetic or diabetic animals (N=4-6) with subsequent study of EGFR phosphorylation in rat kidneys 24 h post-administration by Western blotting showed that PF inhibited EGFR phosphorylation by about 40% in both sets of animals whereas SF stimulated phosphorylation by 12.5 + 13% relative to control in non-diabetic and 141 ± 10% in diabetic animals. Interestingly, the opposing effects of SF and PF on EGFR phosphorylation could be significantly reversed by the antioxidants apocynin (100 mM), catalase (2000 units/ml) or tempol (1 mM) in HEK-293 cells implying that both the stimulatory and inhibitory effects involved an oxidative stress.
dependent mechanism. These results show for the first time that PAMAM dendrimers can modulate the important EGFR-ERK1/2 cellular signal transduction pathway both in vitro and in vivo and may have important clinical implications for the safe use of these polymers in nanomedicine.


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PC094

GLP-1 reverse pancreatic insulin resistance in HFD feeding mice
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Objective: To investigate the protective mechanism of GLP-1 on β cell function by examining the GLP-1 effect on pancreatic insulin signaling pathway of high fat diet mice.

Methods: High fat diet (HFD, 20 weeks) fed mice were administered with vehicle or GLP-1 via osmotic pumps implanted subcutaneously (4 weeks). Blood samples were collected from survival mice from either retro-orbital sinus or tail vein for insulin or glucose measurement respectively. In the end of the experiments, the mice were euthanized by carbon dioxide for sample collection. The islet structure was demonstrated by HE staining. Immunofluorescence antibodies targeting insulin and glucagon were used to examine α and β cell distribution and insulin and glucagon abundance was measured by ELISA using pancreatic homogenates. The molecules involved in insulin signaling pathway (IRc, IRS-1, IRS-2, PI-3 kinase) were examined with immunohistochemistry or immunoblotting and their arbitrary densities as indices of the protein expressions were calculated and analyzed.

Results: The normal structure of islet was deformed in chronic HFD mice shown by HE staining and the islet area was significantly diminished by HFD (HFD vs. Control, p<0.05). The normal pattern of α and β cell distribution were disturbed by HFD. The expressions of IRc and IRS-1 from signaling pathway in the pancreas were significantly reduced or inhibited, insulin and glucagon contents were however increased (HFD vs. Control, p<0.05). But all of the defects induced by HFD were reversed by GLP-1.

Conclusion: Insulin signaling pathway in pancreas is suppressed as part of systematic insulin resistance induced by HFD but was reversed by GLP-1 which may play a beneficiary effect on β cell function.

Fig.1 Insulin resistance in HFD mice

Fig.2 The effect of GLP-1 on insulin signaling pathway


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Effect of regular ingestion of Gum Arabic (Acacia Senegal) on the BMI and lipid profile; an intervention case control study

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Introduction - Gum Arabic is a safely consumable water soluble fiber which is used in food and drug industry. The beneficial effects of gum Arabic in renal disease, colon cancer and body weight management has been documented in many reports. Increasing the intake of water soluble fibers has long been recognized as dietary intervention in patient with obesity, DM, Hypertension and other conditions to decrease the complications of hyperlipidemia.

Gum Arabic is known to bind to bile acids and decrease their absorption in the terminal ileum thereby decreasing lipid absorption and stimulating new bile acids synthesis by the liver which consumes cholesterol. The study was conducted to evaluate the effectiveness of regular intake of an appreciable dose of Gum Arabic (Acacia Senegal) on body mass index (BMI) and the lipid profile in healthy individuals.

Methods: This study was an Intervention case control study. The study population was a total of 53 young and healthy females (age 17-21 years old) recruited by volunteering from the students of the Faculty of the medicine, University of Khartoum the volunteers were blindly and randomly allocated into two groups. An intervention group (n=32) and control group (n=21). The intervention group had to consume 30 grams of gum Arabic (15g dissolved in 400 ml of water twice per day) on daily bases all through the follow up period which is 8 weeks. At both the start and end of the follow up period data was collected from volunteers, being:

Measurements of starting and ending body weight; height; skin fold thickness and fasting blood samples for lipid profile analysis. Both groups had to follow their normal life style regarding feeding habits and daily activity. The study was ethically approved and all volunteers consented to participate.

Results: There was an observed reduction in the mean body weight and BMI in the study group and not in the control group but the difference before and after was not significant. On the other hand the intervention group showed a significant improvement of their lipid profile in the form of a significant reduction in serum cholesterol level (160.7 +- 25 to 106.7 +/- 14, p = 0.001), triglyceride level (28.2 +- 21 to 52.3 +/- 10, p = 0.002) and LDL level (118.9 +/- 24 to 72 +/- 15, p = 0.001) and a significant increase in HDL Level (24.2 +/- 5 to 30 +/- 7, p = 0.001).

Conclusion: Regular ingestion of Gum Arabic improved the lipid profile of the study group by decreasing cholesterol and LDL levels and increasing the HDL level with minimal frequent side effects in the form of GIT disturbance in the first few weeks

The possibility of using Gum Arabic as an effective dietary intervention to prevent complications of hyperlipidemia in risk groups such as Obese, DM and hypertensive patient is likely and should be investigated.

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Effect of ascorbic acid on long term cold exposure induced changes in thyroid activity in Sprague Dawley rats

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Background: Our body responds by heat production and conservation when exposed to prolonged cold environment. Efficient thermogenesis is achieved by thyroid hormones. They are associated with the oxidative status of the body due to their role in metabolism. Ascorbic acid protects body from oxidative damage and neutralizes free radicals.

Objective: To determine the effect of ascorbic acid supplementation on long term cold exposure induced changes in thyroid activity in Sprague-Dawley rats.

Methodology: Nine weeks old healthy, male Sprague-Dawley rats, weighing 200±25 grams were included in this study. The study was approved by the ethical committee of RARE Rwp. Sample Size: Ninety healthy Sprague-Dawley rats (N=90) were randomly divided into three groups with thirty rats in each group.

Control group (n = 30)
These rats were fed on standard diet and kept at room temperature at 22±3°C.

Cold Exposed Group (n = 30)
Rats of this group were fed on standard diet. They were exposed to cold environment between 8-14°C for 1h/day for one month by keeping the cages in ice-filled tubs and recording the temperature by thermometer.

Cold exposed with ascorbic acid supplementation group (n = 30)
Rats of this group were fed on standard diet. They were given ascorbic acid (Vitamin C Ascorbic acid MERCK, research grade Cat No. 500074) supplement in a dose of 500mg/L mixed in drinking water. They were exposed to cold environment between 8-14°C for 1h/day for one month by keeping the cages in ice-filled tubs and recording the temperature by thermometer.

Data collection: After one month of study, the rats were sacrificed and intracardiac blood sampling was done. The blood samples were analyzed for serum total tri-iodothyronine (T3), thyroxin (T4) and thyroid stimulating hormone (TSH) by using chemiluminescent immunometric assay on Siemens immulite 2000 analyzer.

Results:
Serum T3 levels were significantly different in control group, cold exposed group (47.35 ± 2.21 vs. 51.72 ± 6.81 vs. 50.92 ± 5.73, p-value = 0.004) and cold exposed with ascorbic acid supplementation group.
Serum T4 levels were also significantly (p-value = 0.002) different in control group (1.92 ± 0.47), cold exposed group (2.41 ± 0.58), and cold exposed with ascorbic acid supplementation group (2.09 ± 0.52).
Serum TSH levels were also found highly significant (p-value = 0.000) in (0.16 ± 0.03) control group, cold exposed group (0.38 ± 0.13) and (0.29 ± 0.04) in the cold exposed with ascorbic acid supplementation group.

Conclusion: Ascorbic acid prevents the cold induced changes in thyroid hormone levels.

Maternal sleep deprivation affects morphometry and cardiovascular functions of Wistar rat offspring

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Available evidence suggests a link between altered birth morphometry and increased risk of cardiovascular disease in adulthood. Sleep deprivation during pregnancy has been associated with adverse pregnancy outcomes, but its effects on the cardiovascular function of offspring are yet to be fully elucidated. This study examined the effects of maternal sleep deprivation on birth morphometry and cardiovascular function of offspring of Wistar rats. Thirty pregnant female Wistar rats were randomly assigned to three treatment groups (n=10) from GD 1-7, 8-14 and 15-21. Half of each group (n=5) was sleep deprived (SD) on their treatment days, while the other half served as control for that group (n=5). Sleep deprivation was induced by the modified multiple platform technique. Pup birth weight, head circumference, abdominal circumference and crown-rump length were measured at parturition. At six months postnatal life, blood pressure, heart rate, blood flow and blood volume were assessed using a computerized non-invasive blood pressure system (Kent Scientific, USA). Data were summarized as mean ± standard error of mean and analyzed using Student’s t-test and differences in means were based on 95% confidence interval and p<0.05. Systolic (144±2.7 vs. 109±3.0 mmHg, p<0.05) and diastolic (122±2.8 vs. 78±5.7 mmHg, p<0.05) pressures were higher in the GD1-7SD compared with GD1-7control. GD8-14SD had increased crown rump length (47.5±0.07 vs. 46.8±0.07 mm, p<0.05), systolic blood pressure (119±1.58 vs. 110±1.83 mmHg, p<0.05) and blood flow (34.6±4.7 vs. 14.0±1.4 mmHg, p<0.05) compared with GD8-14 control. Birth weight (5.3±0.1 vs. 5.7±0.2 g, p<0.05), systolic (76±6.3 vs. 113±3.7 mmHg, p<0.05) and diastolic (59±8.7 vs. 95±3.5 mmHg, p<0.05) pressures the GD15-21SD were reduced while crown-rump length (48.9±0.1 vs. 47.1±0.5 mm, p<0.05), was increased compared with GD15-21control. The results suggest that maternal sleep deprivation during gestation days 8-14 and 15-21 affected birth morphometry. Irrespective of the period of gestation involved, maternal sleep deprivation adversely affected elements of cardiovascular functions of offspring of Wistar rats.

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

PC097

Poster Communications

A ketone ester drink alters levels of circulating lipids and glucose

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Introduction: Recently we developed a novel ketone ester (KE) to deliver nutritional sources of ketones without starvation, intravenous infusion or dietary manipulation [1]. Previous investigation of ketosis has been confounded by the metabolic sequelae required to produce ketones endogenously. Exogenous provision of ketones from the infusion of salts demonstrated rapid alterations in glucose and fatty acid metabolism [2] consistent with the evolutionary role of ketone bodies (KB) to conserve glucose stores. Furthermore ketones regulate their own production via negative feedback on lipolysis. It is unclear whether similar effects are pertinent to nutritional ketosis delivered by KE. The aim of this study was to characterize changes in blood metabolites; p-hydroxybutyrate (BHB), acetoacetate (AcAc), glucose, non-esterified fatty acids (NEFA), triacylglycerol (TAG) and insulin following a KE drink.

Results: Following favorable ethical review, healthy, non-obese volunteers (n = 16) completed 2 identical study visits. Following an overnight fast subjects consumed 395 mg/kg BW KE mixed with flavoured water. Blood samples were obtained via an intravenous catheter at baseline (BL) and at regular intervals post-drink. Samples were analysed for metabolites using enzymatic methods, and ELISA. Values are mean ± SEM. A 2 factor ANOVA for repeated measures, corrected with post-hoc Dunnett’s tests were used to determine significance. BHB increased following KE, from 0.2 mM (±0.02 mM) at BL to 3.3 mM (±0.02 mM) 1h later. AcAc levels lagged behind BHB, increasing 2-fold from 0.6 mM (±0.1 mM) to 1.2 mM (±0.1 mM) at 1.5h. BHB:AcAc ratio peaked (3:1) at 1h. NEFA and TAG significantly fell following KE, reaching a nadir at 3h. (NEFA = 0.6 mM (±0.1 mM) to 0.2 mM (±0.03 mM) and TAG = 1 mM (± 0.1 mM) to 0.7 mM (± 0.1 mM)). Glucose decreased following KE from 5.6 mM (± 0.1 mM) at BL to 3.9 mM (± 0.1 mM) 1h post-KE. Following KE, Glucose, NEFA and TAG fell remained significantly lower than BL (p<0.05). Insulin was 4.54 μU/mL at BL; rose significantly 0.5h post KE (12.5 μU/mL) (p<0.05 vs BL) and rapidly returned to basal levels.

Conclusions: KE consumption rapidly increased circulating BHB to levels equivalent to several days of total fasting [3] with normal glucose and insulin levels and without increasing NEFA. Nutritional ketosis therefore represents a novel metabolic state with which to re-examine the effects of ketone bodies on human health and disease.


PC098

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PC099

Knockdown of citrate synthase impairs fatty acid oxidation and induces a shift towards glycolysis in muscle cells

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Type 2 diabetic patients often show low rates of complete fatty acid oxidation [1]. Citrate synthase (CS) is a key enzyme of the mitochondrial Krebs cycle. It has been suggested that an inborn defect in mitochondrial citrate synthase might be of crucial importance in limiting substrate flux through the Krebs cycle and thus causing a reduction in fatty acid oxidation of muscle cells in type 2 diabetes patients [2,3].

The main aim of our study was to test the hypothesis that low CS expression and activity impairs fatty acid oxidation in C2C12 muscle cells. Lentiviral shRNA (shRNA1) which targets Cs mRNA was used to lower CS expression and activity in C12C12 myotubes. CS activity was reduced by ~50% compared to the control C2C12 myotubes (CON) treated with the lentiviral vector that did not contain any shRNA. For glucose oxidation cells were incubated with HBS media containing 5.5 mM glucose with [14C]glucose at 2 µCi/ml for 1 h. For palmitate oxidation, cells were incubated with HBS media containing 0.8 mM palmitate conjugated to 2% bovine serum albumin BSA with [14C] palmitate at 2 µCi/ml for 2 h. A Seahorse XF Analyzer was used with standard protocols [4]: ‘Glycolysis Stress Test’ and ‘Mitochondria Stress Test’. Extracellular acidification rate (ECAR) - indicating glycolysis and oxygen consumption rate (OCR) - indicating oxidative phosphorylation, were measured in response to these tests in both CON and shRNA1. shRNA1 treated cells showed a decrease (P<0.05) in intracellular citrate concentration compared to CON cells when incubated with 0.8mM palmitate for 2 hours (shRNA1 49.60±46.67, CON 127.20±65.32 ng/mg) as well as after 24 h incubation with palmitate and glucose (P<0.05) (shRNA1 113.1±95.12, CON 389.9±135.5 ng/mg). shRNA1 cells also showed reduced palmitate oxidation (P<0.05) under conditions where cells were exposed to glucose and palmitate for 2 hours (shRNA1 1.56±0.88, CON 2.82±0.97 pmol/mg/min) and also under the same conditions after 24 hours incubation (P<0.05) (shRNA1 0.71±0.07, CON 1.3±0.22 pmol/mg/min). In the Mitochondria Stress Test, shRNA1 cells had reduced OCR compared to CON (shRNA1 20.2±1.8, CON 25.4±4.6 pmol/ mg/min). In the Glycolysis Stress Test, the ratio of ECAR/OCR was increased in shRNA1 cells compared to CON (shRNA1 0.34±0.23, CON 0.27±0.17 ratio).

Our results show that shRNA mediated silencing of CS gene reduced citrate levels and palmitate oxidation in muscle cells, especially after 24 h incubation with palmitate and glucose. Furthermore, shRNA1 cells had an increase in the ratio of glycolysis compared to OCR. Thus, low expression levels of Cs are associated with impairment in palmitate oxidation in muscle cells and an apparent shift towards glycolytic metabolism.


Nicholls DG et al. 2010 “Bioenergetic profile experiment using C2C12 myoblast cells” J Vis Exp. vol. 46, pp. 2511

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PC100

Defining the autonomous role of the vitamin D receptor in skeletal myogenesis

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Purpose: Age-related loss of skeletal muscle mass (sarcopenia) and the associated loss of muscle function (dysapenia) is a major concern for the healthcare system. Indeed, as the sheer number of elderly individuals rises due to increases in longevity, as a result of improved healthcare and the post-war “baby-boom”, age related frailty is an increasingly onerous problem of complex etiology. Epidemiological studies have linked Vitamin D deficiency to, age-related declines in muscle function and regenerative capacity in addition reduced muscle protein expression of the Vitamin D receptor (VDR) (1). Moreover, bona fide confirmation of VDR expression in muscle cells has only recently been substantiated. In recent studies, muscle VDR expression has been positively linked to tissue regeneration (2) and also to be under the control of expression by exogenous vitamin D3. In this study, we hypothesized that the VDR has a mechanistic role at multiple levels of myogenic regulation.

Results: In order to study the autonomous role of VDR we generated C2C12 skeletal muscle cells harboring sustained shRNA lentiviral-mediated knockdown of the VDR (VDR-KD). Knockdown was confirmed such that VDR-KD cells exhibited <85% of VDR expression vs. scrambled sequence shRNA (SCR) transfected cells. VDR-KD cells proliferated at a slower rate than SCR cells (<27%, P<0.01) displaying a reduction in DNA synthesis (assessed via BRDU incorporation) (-31±7%, P<0.05). Furthermore, altered cell-cycle activities, as assayed by flow cytometry, were also evident in VDR-KD cells, with a greater proportion of the cell population being G0/G1 phase (+12%, P<0.05). Following induction of differentiation total alkaline soluble protein was measured at multiple days, with VDR-KD populations shown to be consistently (~20±3%) less than SCR controls. During differentiation SCR populations exhibited no significant increases in total DNA content, whereas VDR-KD content continued to increase (SCR +26±6% N.S. total DNA vs. VDR-KD +325±26% P<0.05). Myosin protein expression throughout differentiation was markedly reduced in VDR-KD vs. SCR cells (<9±2%, P<0.001). VDR-KD cells impaired differentiation characteristics, producing fewer myotubes (SCR 24±1/mm2 vs. VDR 18±1, P<0.01, N=6) with a greater diameter compared to SCR controls (+10±2% P<0.05, N=6). In addition a greater myonuclei number was observed.
In VDR-KD myotubes (SCR 10±0.3 nucleus/myotube vs. VDR 25±1, P<0.05).

Conclusion: The VDR plays a role in the myogenic regulation of myoblast proliferation and differentiation suggesting a fundamental role of the VDR in the control of myogenesis, and perhaps mass and function. These findings imply an autonomous role of the VDR and may explain potentially formative links between vitamin D, the VDR, and impairments of muscle mass/ function and metabolism in ageing and any associated vitamin D deficient conditions.


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

PC101

Influence of pre & post treatment with Moringa oleifera leaf extract on testicular damage in cadmium exposed male Wistar albino rats - a comparative study

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In today’s industrialized society we are exposed to various environmental hazardous materials. One of these highly toxic metal is Cadmium (Cd), is known to affect various mammalian organs like, kidney, liver, lung, pancreas, prostate, ovaries, placenta etc, including tests1. It induces oxidative stress which is one of the most important causes leading to male infertility. Herbal remedies like, Moringa tree has a great therapeutic value & is found to be effective both in prevention and treatment modalities, to combat toxicity of these materials 2. This study is conducted to evaluate the influence of Pre & Post treatment with Moringa oleifera leaf extract (MoE) on testicular damage in cadmium exposed male Wistar Albino rats. All procedures were performed as per the guidelines established by the Institutional Animal Ethics Committee. Wis- tar Albino rats weighing between (180-200) g were broadly divided into 5groups, each having six (6) animals, as follows: Group i - control group, received normal saline only. Group ii - experimental control group, pre- treated (oral) with M. oleifera leaf extract, 100 mg/kgbw for 10 days. Group iii - received a single oral dose of cadmium chloride (10 mg/kgbw), Group iv - pre- treated with M. oleifera leaf extract (100 mg/kgbw) for 10 days, followed by cadmium chloride (10 mg/kgbw) given orally for one day. Group v: Received a single dose of cadmium chloride (10mg/kgbw) followed by Moringa oleifera leaf extract (100mg/kgbw) for 10 days. Group vi received the combination treatment of cadmium chloride (10mg/kgbw) and MOE.

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PC102

Comparative effects of chronic and acute administration of ethanolic extract of kolanut (cola nitida) seed on glucose tolerance of rat

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Kolanut (Cola nitida) seed is rich in caffeine which could be as high as 51% (Salahdeen et al; 2015). In view of the reported insulin insensitivity caused by caffeine (Urzua et al; 2012), this study investigated the effects of acute and chronic administrations of ethanolic extract of Kolanut (EEK) seed on glucose tolerance of rats. Male albino Wistar rats (250 - 300gm) and divided into three groups (I, II and III) with 8 rats per group were studied. Group I was orally administered distilled water (control). Group II had acute oral administration of EEEK (6mg/kg), while group III was orally administered EEEK (6mg/kg) for eight weeks. Blood samples (0.2ml per sample from distal end of the tail) and liver biopsies were taken to determine plasma glucose and serum insulin levels, liver glycogen, liver glycogen synthase and phosphorylase activities. Blood glucose was determined by glucose oxidase method. Oral glucose tolerance test was carried out on each rat. Plasma glucose concentrations were plotted as a function of time and areas under glucose curve (AUGs) calculated. Glycogen level was determined by anthrone method. Radioimmunoassay kit (diagnostic product) was used to determine the serum insulin level. Values are mean ± S.E.M, compared by ANOVA and Student t-test.

AUG increased by 35% for acute group and decreased by 28% for chronic group. Peak serum insulin also increased by
18% for acute group and was reduced by 15% for the chronic group. There were no significant changes in liver glycogen and activities of glycogen synthase and phosphorylase in acute condition. However, in chronic group, the liver glycogen and the activity of glycogen increased significantly. There was also a significant decrease in phosphorylase activity. The results suggest that the effects of EEK following acute and chronic administrations are not the same. While acute intake produced glucose intolerance, chronic intake improves glucose tolerance.


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PC103

Blood pressure association with segmented abdominal fat

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Visceral obesity is strongly associated with both cardiovascular and chronic kidney diseases. A potential mechanism by which obesity could promote hypertension and kidney diseases is through accumualtion of fat in the renal sinus (RS) (Chughtai, et al. 2010; Foster, et al. 2011). Aim of the study was to evaluate association between CT measured abdominal fat segments, serum renin and blood pressure (BP). The study included 210 subjects (F/M 110/110) aged 37.30 ±4.10yr. CT images were captured and volumetric RS fat, retroperitoneal (RP), intraperitoneal (IP) and subcutaneous (SC) fat volumes were measured at the level of right kidney (RP), IP and SC was segmented according anatomical landmarks published previously (Chughtai et al. 2010). Fat depots were measured using the 3D-Doctor software. Total volumetric kidney segmentation was performed (we excluded major branches of the renal artery and vein as well as any visible branches of the renal collecting system). In order to exclude the kidney size effect on RS fat volume, ratio of RS fat volume to corresponding total kidney size was calculated for each participant. Serum renin level was detected by xMAP technology. Experimental procedures were approved by the Ethical Committee of the Institute of Experimental and Clinical Medicine, University of Latvia. All procedures performed in this study were in accordance with the ethical standards of the institutional and international research committee and with the 1964 Helsinki declaration. Informed consent was obtained from all (n = 210) study participants.

Volumetric CT measurements showed asymmetric RS fat deposition. Left RS accumulate significantly (p<0.001; Wilcoxon Signed Rank test) higher amount of adipose tissue compared to the right RS both for men and women - left RS fat volume (2.76 (1.49 to 4.62) cm³ and 1.39 (0.57 to 2.99) cm³ for men and women, respectively; right RS fat volume 1.07 (0.46 to 2.45) and 0.39 (0.10 to 0.95) for men and women, respectively (nonparametric data, presented as median and interquartile range. Multiple regression (Forward method; Square root (sqrt) transformation was used for skew data; Dependent variable: systolic/diastolic (BP); Independent variable: all abdominal fat segments) revealed that only IP fat volume increase were positively associated with systolic (βmen=0.45; βwomen=0.62 p<0.001) and diastolic (βmen=0.41; βwomen=0.61 p<0.001) BP. Standardized coefficient β significantly increase when sqrt renin were entered in model. Interestingly - renin in this model had inverse effect (decrease of serum level of renin together with increased IP volume ensured systolic BP increase). It has recently been demonstrated that adipocyte angiotensin (ANG) plays a central role in the development of hypertension. Angiotensinogen in adipocytes are converted to ANGI by cathepsin instead of renin (Frogolet et al. 2013). Additionally, adipocytes secrete aldosterone that can induce sodium retention and BP increase. BP increase due to previously mentioned mechanism possibly inhibit renal renin secretion.

These data suggest that RS fat accumulation does not have effect on systolic and diastolic BP in middle-aged subjects. IP fat accumulation show positive association with systolic and diastolic BP increase possible due to adipocyte secreted ANG or aldosterone. Chughtai HL, Morgan TM, Rocco M, Stacey B, Brinkley TE, Ding J, Nicklas B, Hamilton C & Hundley WC (2010). Renal sinus fat and poor blood pressure control in middle-aged and elderly individuals at risk for cardiovascular events. Hypertension 56 901-906.


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PC104

Influence of BMI on motor performance in Santal children of Purulia district, West Bengal, India

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Children’s motor development is closely associated with their adiposity status. Association between adiposity in terms of BMI and motor performance has been established in children and adults by few researchers. These results were observed in children from nontribal population, BMI related differences in motor skills have not been studied in tribal children and specifically in Santal children. The present study was undertaken to assess the adiposity status by measuring body mass index (BMI) and to investigate the influence of BMI on motor performance in 5-12 years aged Santal children. This study was conducted on 816 Santal children. The adiposity status
of each child was assessed by BMI-for-age z-score based on WHO reference data. Motor development was measured using the Bruininks-Oseretsky Test of Motor Proficiency (BOT-2). Mean BMI values of Santal children remained around the 50th percentile values of WHO reference data. The normal weight (NW) children scored higher in some individual motor subtests (bilateral coordination, balance, running speed and agility, upper limb coordination, and strength) and in total BOT-2 score (p<0.05) compared to that of underweight (UW) and overweight (OW) children. A significant association was observed between distribution of children in BOT-2 z-scores and overweight (OW) children. A significant association was observed between distribution of children in BOT-2 z-scores and overweight (OW) children. A significant association was observed between distribution of children in BOT-2 z-scores categories and BMI-for-age z-score categories (X²=71.36, df=3, p<0.01). Stepwise regression showed that BMI even has a significant impact on motor performance of Santal children. Motor performance showed a curvilinear relationship with BMI as the motor scores found lower in both UW and OW children compared to NW children. The results also indicated that gross motor skills are more affected with the change of BMI in comparison to fine motor skills.

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Nitric oxide--mediated reduction of functional vesicle pool size

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Nitric oxide (NO) signalling is implicated in several neurodegenerative diseases through induction of high NO release. However, its exact contribution to degeneration remains elusive due to the complexity of downstream targets. High levels of NO can induce post-translational modifications which are associated with neuronal degeneration (Steinert et al., 2010; Nakamura et al., 2015). NO reactivity with superoxide anions to form cytotoxic peroxynitrite which in turn leads to 3-Nitrotyrosination with largely detrimental changes in protein function. Additionally, NO signalling alters protein function via S-nitrosylation. To date, little is known as to what extent these post-translational modifications contribute to or exacerbate neuronal dysfunction. We use glutamatergic synapses as a model system to identify novel nitrergic signalling pathways to correlate protein modifications with functional changes.

The Drosophila neuromuscular junction was used to characterise NO effects employing electrophysiological methods. Two-electrode-voltage-clamp (TEVC) analyses were carried out in HL-3 solution using sharp electrodes (~30 MΩ). Data denote mean±SEM (n-number) with *p<0.05 indicating statistical significance (t-test, ANOVA). TEVC data showed little NO (~0.1 μM) effects on miniature excitatory junctional currents (mEJC) or decay kinetics but induced a reduction in mEJC frequencies (Ctrl: 1.6±0.1 Hz (49) vs NO: 1.0±0.1 Hz* (24)). Furthermore, evoked EJC amplitudes and quantal content were strongly reduced following NO exposure for >40 min (Ctrl eEJC: 119±7 nA (22) vs NO: 62±8 nA* (14); Ctrl QC: 189±12 vs NO: 104±12*) suggestive for a reduction in presynaptic release. The above NO effects were detected following inhibition of the soluble guanylyl cyclase (sGC). Importantly, enhancing presynaptic S-Nitrosoglutathione reductase (GSNOR) enzyme activity, by overexpression (OE), prevents nitrergic effects. Cumulative postsynaptic current analysis (500 ms 50 Hz train) further showed a reduced number of release-ready vesicles following NO exposure (Ctrl: 276±21 (22) vs NO: 108±19* (14)) which was also confirmed by estimating the number of release sites using fluctuation analysis. Furthermore, pool sizes could be modulated in a positive or negative manner by enhancing or reducing GSNOR activity, respectively (Ctrl: OE: 212±25, RNAi: 139±23*; +NO: OE: 276±44, RNAi: 130±18*). Together, our data suggest that NO can modify synaptic signalling possibly via inducing post-translational protein modifications. This data interpretation is supported by the notion that sGC inhibition is ineffective but modulation of neuronal GSNOR activity impacts on synaptic physiology implying presynaptic actions of NO in a sGC-independent manner. The data extends our understanding of NO signalling, potentially leading to the identification of putative targets for therapeutic intervention(s) in disease.

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Olfaction starts at the olfactory cilia of the olfactory receptor cells (ORCs). This site mediating the olfactory transduction exhibits a fine cylindrical structure having 100-200 nm diameter and length of several tens of micrometers. All molecular factors needed for the signal conversion are equipped in such a fine tubing; namely, receptor protein, G protein, adenylyl cyclase, cyclic nucleotide-gated (CNG) and Ca²⁺-activated Cl⁻ (Cl(ClC)Ca) channels. Cytoplasmic cAMP and Ca²⁺ play roles for the second messengers within the cilia. In the present study, we examined the effect of off-flavors generated in wide varieties of foods/beverages on the transduction current using the isolated newt ORCs and human sensory test. The experiments were performed under the latest ethical guidelines for animal/human experimentation at Osaka University. Ciliary current responses were obtained by the photolysis of cytoplasmic caged cAMP under the whole-cell recording configuration (voltage clamp, Vb=-50 mV) (1).

We show with human psycho-physical tests that TCA (known for a powerful off-flavor inducing the cork taint of wines) reduces flavors of wines with very low concentration (10 ppt, ~47 pm). In parallel, it was shown that TCA suppressed the order of potency of CNG suppression by TCA analogues or precursors was identical to that of the human detection of the corresponding off-flavors. For instance, suppression ratio of TCA equivalent to 2,4,6-tribromoanisole (TBA), which is much greater than 2,4,6-trichlorophenol (TCP; precursor of TCA), suggesting that TCA suppression of CNG channels is related to cork taint. TCA exerted a much more potent suppressive effect on CNG channels (100-1,000-fold) than other known olfactory
masking agents called geraniol that have been widely used in perfumery. It was also more potent than a well-known specific CNG channel blocker, L-cis-diltiazem. Furthermore, TCA suppression of transduction current was detected even with atto-molar (aM) concentration (2). In the presentation, we show experimental evidence to discuss the molecular mechanism expressing such super-efficiency. The findings not only reveal a likely mechanism of flavor loss of foods/beverages, but also suggest certain molecular structures as possible olfactory masking agents and powerful channel suppressors. Takeuchi H & Kurahashi T (2002). J Physiol 541, 825–833. Takeuchi H et al. (2013). PNAS 110, 16235-16240.

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PC107

Subconvulsive dose of kainic acid transiently increases the locomotory activity of adult Wistar rats

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Kainic acid (KA) is a potent neurotoxic substance valuable in research of temporal lobe epilepsy. We tested how subconvulsive doses of KA influence the behavior of adult Wistar rats (Rattus norvegicus). Animals were treated intraperitoneally with 5 mg/kg of KA and observed in Laboras open field test for one hour. Following behavioral parameters were quantified: time of locomotion, time of immobility, time of rearing, average speed of locomotion and distance travelled. Week after the KA treatment animals were tested again in Laboras open field test. On 8th day, animals were transcardially perfused under deep thiopental anaesthesia (40mg/kg). Rat’s brains were sliced and stained with Fluoro-Jade B to detect possible neuronal degeneration. Treatment with KA increased the time spent by locomotion (p<0.01), exploratory rearing (p<0.05) and distance traveled (p<0.01), with the approximate onset thirty minutes since the KA administration. One week after the KA treatment, behavioral parameters were already the same as of the control group. Histology in terms of Fluoro-Jade B staining did not reveal any obvious neuronal damage in hippocampus. These data suggest, that subconvulsive KA dose changes the behavioral parameters only transiently. Clarification of timing of KA induced changes may help to understand mutual relationship between non-convulsive seizures and behavioral/cognitive consequences.

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PC108

Riding the waves of astrocytic calcium: the role of astrocytes in experience-dependent plasticity

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There is increasing evidence that astrocytes partner neurons in synaptic communication and plasticity. They sense the same synaptic inputs and respond with intracellular [Ca$^{2+}$] elevations, which correlate with gliotransmitter release (1). While there is evidence for astrocytic involvement in neuronal plasticity (NP) in vitro (2), the impact of astrocytes on NP in vivo is less established.

Deflection of a single whisker leads to a substantial neuronal firing in its corresponding cortical representation (principal input, P) which dominates responses elicited by the stimulation of immediately adjacent whiskers (surround input, S). This pattern of dominance can be altered, a phenomenon known as experience-dependent plasticity (EDP), through whisker deprivation.

Removal of all but one whisker (single whisker experience (SWE)), leads to an expansion of the cortical representation of the intact whisker into the territory of the immediately surrounding whiskers in layers 2/3, while the responses of the deprived inputs get depressed. We compared the magnitude of responses to whisker deflections collected from the barrel cortex of C57BL/6J wild type and IP$_3$-R2 knockout mice, either undeprived (WT:n=8, KO:n=5) or deprived (SWE for 18 days) with 5-9 days of whisker regrowth (WT:n=11, KO:n=9). In IP$_3$-R2 KO mice spontaneous and G-protein-coupled receptors-mediated increases in astrocytic [Ca$^{2+}$] elevations have been shown to be impaired (3-5). Mice were anaesthetised with urethane (1.5 g/kg body weight, i.p.) and extracellular data was collected in vivo by recording layer 2/3 cells in the barrel cortex while stimulating P and S (50 deflections of 1º were delivered for 10 ms at 1 Hz per each whisker). The Mann-Whitney U-test was used to compare data which is expressed as spikes per stimulus ±SEM.

Undeprived WT and KO animals did not differ concerning the magnitude of whisker-evoked responses (WT, P:1.43±0.27, S:0.36±0.05; KO, P:0.97±0.06, S:0.24±0.05; p>0.05). Comparison of the WT undeprived and WT SWE animals showed a significant difference in the S responses (WT undeprived, S: 0.36±0.05; WT SWE, intact whisker S (IWS): 0.92±0.14, deprived whisker S in the intact representation (DWS): 0.09±0.03; p<0.05) as did a comparison of the undeprived and deprived KO mice (KO: undeprived S:0.24±0.05; KO SWE IWS:0.80±0.13, DWS:0.05±0.01; p<0.01, Fig 1). In both WT and KO animals IWS responses were potentiated while the DWS responses were depressed when compared to their respective undeprived S (p<0.05).

Comparison of the SWE deprived WT and KO mice revealed no significant differences (P-deprived/P-intact/IWS/DWS for WT SWE: 0.72±0.17, 1.57±0.21, 0.92±0.14, 0.09±0.04 and for KO SWE: 0.71±0.17, 1.54±0.26, 0.80±0.13, 0.05±0.01 respectively; p>0.05, Fig 2). These results show that the impairment of the astrocytic IP$_3$-R2 mechanism does not affect neuronal EDP.
PC109

Isolation of a glutamate receptor variant associated with stretch-sensitive peripheral nerve endings

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Increasing evidence suggests glutamate has an important modulatory role in mechanosensory endings in the peripheral nervous system in addition to its well-established role in the central nervous system. Annulospiral stretch-sensitive nerve endings in skeletal muscle – muscle spindles – express a novel glutamate receptor with an atypical pharmacological profile most closely matching the phospholipase D (PLD)-coupled glutamate receptor. Although reported in the hippocampus by a number of groups, the receptor has never been isolated or sequenced. We previously reported spindle expression of a ~102 kDa probable mGluR5 splice variant and an unidentified ~110 kDa protein capable of binding our unique biotinylated ligand – ZCZ-180. Here we report the use of spindles extracted from the rat deep masseter muscle – an enriched source of nerve endings – to further investigate and isolate the novel glutamate system.

Adult male Sprague Dawley rats (weight 200 – 450g) were euthanised by CO₂ overdose (ASPA 1986, 63/2010/EU) and deep masseters removed. For 1D gels and affinity chromatography, spindles were extracted as previously described. For immunohistochemistry, muscles were fixed overnight in 4% formaldehyde, teased then squashed and incubated in primary antibody for 48 hr followed by secondary antibody for 1 hr. Hydrophobic spindle protein was separated on 1D gels. Bands were selected by molecular weight and analysed by mass spectrometry. A band at ~100 kDa did not match any known proteins on the Mascot database. However, a subset of unassigned peptides showed strong sequence homology with glutamate receptors following NCBI-based sequence alignment, suggesting sufficiently representative protein isolation. Functional clustering analysis using IPA (Ingenuity Pathway Analysis) software identified PLD as a potential regulatory hub, suggesting the protein is likely to signal through PLD. Protein isolation was cross-validated by affinity chromatography. Eluate had immunoreactivity to mGluR5 antibody at ~102 kDa confirming successful isolation of one protein by two separate strategies. Immunohistochemistry showed the mGluR5-like protein was not localised to the annulospiral nerve endings, but in nociceptive fibres parallel to spindles, suggesting the mGluR5-like protein is not involved in mechanosensation.
An mGluR5 variant was isolated by two separate strategies. Although we initially suspected the expression of a novel heterodimer in spindles, the mGluR5-like protein is not localised to the mechanosensory portion of spindles, suggesting it is not involved in the stretch response. Future work will focus on localising and sequencing the ZCCZ-binding protein in our quest to isolate the receptor protein.


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**PC110**

**Activated mammalian target of rapamycin (p-mTOR) and S6 ribosomal protein (p-S6RP) are expressed in the trigeminal subnucleus caudalis and up-regulated in a model of inflammatory oro-facial pain**

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In spinal cord, the mammalian target of rapamycin complex 1 (mTORC1) is implicated in chronic pain (Obara et al., 2011) but its contribution to trigeminal pain is unknown. Using immunohistochemistry, we investigated whether phospho-mTOR (p-mTOR) and phospho-S6 ribosomal protein (p-S6RP), a downstream substrate of mTORC1, are expressed in the dorsal horn (DH) of trigeminal subnucleus caudalis (Vc), a region involved in oro-facial nociception. Furthermore, changes in p-mTOR and p-S6RP expression in Vc were quantified in a Complete Freund’s Adjuvant (CFA) model of inflammatory oro-facial pain as compared to saline-injected controls. In perfusion-fixed tissue, p-mTOR and p-S6RP were co-stained with the astrocytic marker GFAP, the neuronal marker NeuN, and the microglial marker Iba1. All procedures were carried out under anaesthesia (pentobarbital, 50 mg/kg i.p.) and accorded with UK Home Office legislation. In naïve rats, p-mTOR and p-S6RP were constitutively expressed in the deep and superficial DH laminae of Vc. Following subcutaneous injection of CFA (50 µl) into the rat left whisker pad, labelling for p-mTOR was up-regulated in the ipsilateral superficial DH laminae of Vc at 24 h and 72 h post-CFA injection (p<0.001 and p<0.05, respectively) and in the deep laminae of Vc (p<0.01) 24 h post-CFA injection. Also, at 24 h post-CFA injection p-S6RP was up-regulated in the superficial laminae of Vc in CFA-treated rats (p<0.05).

Immuno-labelling for p-mTOR showed co-localization mainly with NeuN but not GFAP or Iba1 24 h post-CFA injection. In conclusion, p-mTOR and p-S6RP are expressed in neurones of the trigeminal brainstem and are up-regulated in Vc in a model of inflammatory oro-facial pain. This suggests participation of the mTORC1 signalling pathway within the trigeminal nociceptive system and a potential contribution to chronic oro-facial pain.

Obara I, T ochiki KK, Geranton SM, Carr FB, Lumb BM, Liu Q, Hunt SP, 2011, Pain, 152, 2582-2595

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**PC111**

**Role of BK and TRPM8 channels in adipose-derived mesenchymal stem cells**

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Mesenchymal stem cells (MSCs) have high proliferative capacity and ability to differentiate into different lineages, making them a good model to use in regenerative medicine (1, 2). Proliferation and cell differentiation are very complex processes involving diverse players that include ion channels (1). It is known that changes in intracellular calcium concentration are associated with proliferation and differentiation of MSCs (3, 4). Consequently, ion channels involved in calcium sensing and calcium homeostasis must have a role in such processes. The present study evaluates expression of TRPM8 and BK channels in adipose-derived human (hMSCs) and rat (rMSCs) mesenchymal stem cells. MSCs were characterized by the expression of specific surface markers (rat: CD90+, CD45+ and CD29+, human: CD90+, CD105+ and CD34+) and by the capacity to differentiate into adipogenic and osteogenic lineage. Western blot, immunocytchemistry and flow cytometry assays were performed to determine protein expression. Cell viability was determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) and propidium iodide assays. Flow cytometry assays were made by triplicate and reported as mean±SD. More than 95% of rMSCs and hMSCs express specific MSCs surface markers (n=3). Both type of MSCs were able to differentiate into osteogenic and adipogenic lineage (n=3). We measured the expression of BK channel α and accessories β subunits in hMSCs (α: 29.3±2.0, β1: 83.3±11, n=3) and rMSCs (α: 34.3±31.6, β1: 64.6±29.3; n=5; β2: 90%, n=1; β4: 91.5±9.2; n=2) by flow cytometry. Remarkably, we found TRPM8 channel expression in hMSCs (96.8±1.5; n=4) and rMSCs (66.7±40.7, n=3). No changes in MSCs cell viability were observed after treatment with agonist and antagonist compounds of BK channel (NS1619 and Iberiotoxin; p<0.001; n=3) or TRPM8 channel agonist (Menthol; p<0.001; n=3). Changes in TRPM8 expression were found during differentiation being high at the initial stages and decreasing gradually until the process ends (n=3). Herein, we report for the first time TRPM8 channels expression in mesenchymal stem cells and changes in channel expression during MSCs differentiation suggesting a role of TRPM8 channels in the process.


Ding F et al. (2012). Tissue cell 44: 358-364

Dose dependent aversive effects of chemogenetic Locus coeruleus activation

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The Locus coeruleus (LC) is the principle noradrenergic nucleus in the CNS. Dysfunction of the noradrenergic system has been associated with pathologies such as major depression, Alzheimer’s disease and neuropathic pain. We have therefore been interested in developing means to produce long term activation of the noradrenergic system. We describe here the use of an engineered excitatory receptor-ionophore (PSAM) to manipulate LC activity (Magnus et al. 2011).

We developed a lentiviral vector (lenti-PRS-EGFP2aPSAM-HA) with catecholaminergic promoter (PRS) to allow expression of a traceable (HAtag) excitatory ion channel (PSAM-HA) in NAergic LC neurons. Expression was evaluated using immunofluorescence for EGFP and dopamine beta hydroxylase (DBH) to test the existence and strength of such relationship. The results are in accordance with the findings of other authors on structural disorder. To our knowledge, this is the first study to test the existence and strength of such relationship. The authors are grateful to the Ministry of Education and Science, Republic of Serbia (Grants 175059 and 41027).

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Correlation between chromatin textural features and nuclear envelope circularity in hippocampal dentate granule cells

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Textural analysis performed using Gray Level Co-occurrence Matrix (GLCM) analysis is a contemporary mathematical algorithm applicable in quantification of cell and tissue structure. So far, it has been successfully applied in various biological and medical fields, including neurosciences. Some of the chromatin textural features may change as the result of various physiological and pathological processes, such as ageing, apoptosis and immune responses. On the other hand, the relationship between GLCM parameters and nuclear shape remains unknown. The aim of our research was to test the existence and strength of correlation between chromatin structural characteristics and nuclear envelope shape in a sample of hippocampal dentate granule cells.

Brain tissue was obtained from 10 healthy male Swiss albino mice, and stained using Feulgen method for DNA visualization (figure 1). Digital micrographs of dentate gyrus structures were made in 8-bit gray scale format. Chromatin analysis on a sample of 100 dentate granule cells (10 per animal) was performed in ImageJ software and its plugins (National Institutes of Health, Bethesda, MD) directly from the micrographs. For each chromatin structure GLCM entropy (chromatin disorder), angular second moment (indicator of uniformity) and inverse difference moment (homogeneity) were determined. Nuclear envelope circularity as an indicator of nuclear envelope shape was calculated based on the nuclear area and perimeter. There was a statistically highly significant (p<0.01) negative correlation between circularity and angular second moment. Angular second moment increased as the circularity decreased and vice versa. No such relationship (p>0.05) was detected between circularity and other GLCM parameters. These results indicate that the two-dimensional roundness of nuclear envelope is strongly related to the uniformity of chromatin structure. This is one of the first studies to test the connection between nuclear shape and chromatin structure in this manner. The detected correlation may be explained by the specific events occurring in nuclear lamina in physiological conditions.

The authors are grateful to the Ministry of Education and Science, Republic of Serbia (grants 175059 and 410277)

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

PC116

Kinetics of changes in sympathetic and parasympathetic activity during exposure to elevated ambient temperature are different

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Incomplete understanding of heat stroke pathophysiology makes an efficient treatment of this deadly condition difficult. There is a general agreement that heat stress leads to a hyperadrenergic state, however information about the parasympathetic activity is virtually absent. To further clarify the role of the autonomic nervous system in heatstroke, 4-month old Wistar-Kyoto male rats (n=8) were studied. There is a general agreement that heat stress leads to uncontrolled tachycardia and coincided with the rapid decline of HF HRV to 1.2 (0.1) ms⁻². A similar pattern was seen in changes of the sBRS: an increase from preheating values of 1.3 (0.3) ms/mmHg, to 1.9 (0.4) ms/mmHg, followed by rapid decline to very low values of 0.25 (0.09) ms/mmHg, P<0.05. Vascular sympathetic activity based on LF SPV increased evenly during warm air exposure from 3.8 mmHg² to a maximum of 13.8 mmHg² (P<0.05) attained during the heatstroke when parasympathetic activity was barely measurable. Thus, exposure to the hot environment activated both branches of the autonomic nervous system. However, parasympathetic activation was only temporary and its decline could be related to the uncontrolled tachycardia and transition to heatstroke.

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PC117

Voltage-gated cation channels in undifferentiated CAD neuroblastoma cells

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The CAD cell line was established from tyrosine hydroxylase positive tumours induced in mice carrying the Simian virus 40 large T antigen, and it has been suggested that these ‘immortal’ cells may provide a model of catecholaminergic neurones (Wang and Oxford, 2000). However, electrophysiological studies showed that these cells were depolarized (V_m ~40 mV) and unable to generate action potentials unless V_m was deliberately held at a hyperpolarized value (Wang and Oxford, 2000). The aim of the present study was to establish the physiological basis of this depolarized phenotype. As anticipated (Wang and Oxford, 2000), step depolarization to a series of test potentials (V_test) normally evoked transient, inward currents that were followed by a slowly developing but sustained outward current (Fig 1A). Raising [K⁺]₀ to 145 mM by iso-osmotically replacing external Na⁺ abolished the initial transient current (Figure 1B, C) and caused a rightward shift in the steady state current – voltage relationship (Fig 1D). Moreover, under these conditions repolarization at the end of each test pulse evoked a transient inward ‘tail current’ (I_tail, Fig 1B) that is carried by K⁺ entering the cell through channels that had opened during the preceding depolarization. Analysis of the I_tail – V_test relationship showed that the half maximal activation of these K⁺-permeable channels occurred at ~30 mV (Fig TE). This observation was surprising since sustained
outward current does not normally become apparent until the cells are depolarized past ~20 mV (Fig 1D). Indeed, under control conditions, depolarization to -50 to -20 mV evoked small (2 - 3 pA pF⁻¹) but sustained inward currents (Fig D). Since these observations are inconsistent with the hypothesis that the outward current flows via K⁺ selective channels, subsequent experiments sought to establish the ionic selectivity of the ion channel population underlying the sustained outward current. Cells were therefore depolarized to 70 mV in order to open the voltage-gated conductance fully and then stepped to a series of test potentials. Analysis of the current quantified at steady state is plotted against Vm. The voltage-gated channels that underlie the sustained outward current thus display relatively poor K⁺ vs Na⁺ selectivity.

Rather than being dominated by highly selective K⁺ channels, the membranes of depolarized CAD cells thus display relatively poor ionic selectivity. These cells thus appear to express voltage-gated channels that allow sustained, inward Na⁺ current and this could well explain why these cells are normally depolarized (Wang and Oxford, 2000). Our data therefore suggest that CAD cells do not retain the biophysical features of mammalian neurones.

Absence seizures (ASs) are characterized by sudden, transient behavioral arrest with impairment of consciousness and a distinctive bilaterally synchronous spike-and-wave discharge (SWD) in the EEG. These seizures arise in the thalamocortical (TC) network, but the firing dynamics of its neurons during ASs are not known. Studies in anesthetized animals and in vitro have yielded contradictory results, suggesting predominant electrical silence and regular T-type calcium channel (T-channels) dependent burst firing, respectively. However, no studies have previously recorded the activity of thalamic neurons in a freely moving model of absence epilepsy, and genetic studies that proposed a significant role for T-channels in ASs have limited direct relevance to thalamic neurons since global T-channel knockout or overexpression was used. In this study, we obtained ensemble recordings from TC and reticular thalamic nucleus (NRT) neurons in freely moving rats (the Genetic Absence Epilepsy Rats from Strasbourg, GAERS), and intrathalamically applied a selective T-channel antagonists (TTA-P2) via reverse microdialysis.

GAERS were fully anesthetized (2.5% isoflurane inhalation) and implanted with EEG electrodes and a silicone probe in the ventrobasal (VB) thalamic complex, in accordance to UK legislation and local ethical guidelines. In some animals, microdialysis probes were also implanted bilaterally either in the VB or the NRT. Following a week of recovery, TC neurons (n=139) were observed to mostly either be silent (54±3%, mean±SEM, of spike-and-wave complexes, SWC) or fire single spikes (30±7%) sparsely but synchronously during ASs. T-channel mediated bursts were rare (16±2%) (n=370075 SWCs from 4214 SWDs in 6 GAERS). Reverse microdialysis of 300 micromolar TTA-P2 throughout the VB did not block ASs (94±7% compared to vehicle injection, ANOVA followed by Bonferroni test, p<0.05, n=8 GAERS), suggesting that T-channel activity is not necessary for the generation or propagation of ASs. In NRT neurons (n=25), T-channel mediated bursts were frequently observed during ASs (25±8% of SWCs), and block of these channels with TTA-P2 in the NRT suppressed ASs (80±11% decrease compared to vehicle injection, ANOVA followed by Bonferroni test, p<0.01, n=8 GAERS).

These results demonstrate that VB TC neurons predominantly express a mixture of single spikes and silence during ASs and suggest that T-channel mediated bursts in TC neurons are not required for the expression of ASs. Consequently, despite the synchronous output of the VB, intra-thalamic T-channel dependent mechanisms are not a viable candidate for AS pacemakers.

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Poster Communications

PC118

Lack of burst firing and T-type calcium channel involvement in thalamocortical neurons during absence seizures in freely moving models

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The effect of electroencephalogram peak frequency changes on increasing of theta band power in Parkinson’s disease

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The previous EEG studies have shown the increasing of θ-power in patients with Parkinson’s disease (PD). However, nowadays the mechanisms of that phenomenon are still not clear. The better understanding of it could bring the light on nature of non-motor dysfunctions in PD and help to find new diagnostic methods and ways of predictions of PD progression.

The aim of this research was to discover the EEG-activity during resting state in PD patients depending on the lateralization of motor symptoms. PD patients with right-sided (RPD, n=30) and left-sided (LPD, n=30) motor symptoms onset as well as 30 healthy volunteers have participated in the investigation. Both volunteers and patients were of age 45-65; stage of PD according to Hoehn-Yahr scale was 2-3. EEG was recorded during 3 minutes in the eye-closed resting state. The Mann-Whitney criterion was used to compare the independent samples data.

We revealed the increasing of spectral power of θ2 band (6-7.5 Hz) and amplitude of the peak frequency in this band in PD patients compared to controls. Also the enhancement of θ1 band (4-6 Hz) in LPD and α1 band (7.5-9.5 Hz) in RPD were registered. We suggest two hypotheses that can explain the increasing of θ-power. The first one suspects the increasing of activity of specific generators of θ-oscillations in the brain. The other hypothesis suggests the shift of the frequency of main resting state rhythm (normally equal to 10 Hz) to lower part of the spectrum which may affect the θ band due to Gibbs effect.

We used Independent Component Analysis (ICA) to remove the components with dominant α-oscillations from whole EEG signal. After this the difference in θ power between controls and PD patients disappeared in most of the sites. However the difference in θ remains significant in temporal brain areas. These data testify that the main reason of θ-power increasing in PD patients is the slowing of the resting state rhythm. In addition, some increasing of the activity of ;q-generators in parietal structures may not be fully excluded. The difference of clinical significance of both mechanisms indicate the importance of them considering the assessment of the level of the integrity of general brain activity in PD patients.

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Diabetes UK

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PC121

Non-invasive assessment of retinal ganglion cell function using the electroretinogram: Data from healthy twin pairs

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The electroretinogram (ERG) represents the summed electrical response of the retina to light stimuli, and can be recorded non-invasively from human subjects. The photopic negative response (PhNR) is a negative component of the waveform, elicited in response to full-field stimuli in photopic conditions, and has been shown to reflect retinal ganglion cell function, which is of interest both clinically and in research. The aim of this study was to perform a classic twin study of the PhNR, to obtain normative data and to explore relative genetic and environmental contributions to variation in the response. 106 healthy monozygotic (MZ) and dizygotic (DZ) twin pairs were recruited from the TwinsUK cohort, with local research ethics committee approval. Mean (SD) age was 62.5 (11.3) years. 93% were female and the majority were Caucasian (reflecting the cohort’s demographic). ERGs were recorded in response to full-field stimuli in photopic conditions, which was defined as the average over a time window of 20 ms prior to flash delivery. Approximately 150-180 repetitions of the stimulus were performed in photopic conditions (in the presence of a 30 cd m⁻² white background), with responses averaged (from the right eye). Frames were excluded in cases of significant noise or blink artefact. Heritability was calculated using maximum likelihood structural equation modeling (OpenMx package). Responses were included from 196 twins (51 MZ and 47 DZ pairs). The mean (SD) amplitude was -14.6 (4.8) microvolts, and the range was -0.4 to -33.6 microvolts. Correlation coefficients within twin pairs were 0.32 for MZ pairs and 0.15 for DZ pairs, and the point estimate of heritability was 30.2%. Our study provides normative data for the PhNR when recorded with the fibre positioned low in the conjunctival fornix (though the range may not be generalizable outside the demographics of our cohort or with different methods of recording). This study is the first to explore the PhNR in twin pairs: the intra-pair correlation coefficient for MZ twins was double that for DZ twins; our findings suggest that 30% of the variation in the PhNR, as measured in this study, may be due to genetic factors.

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PC122

Tumor necrosis factor administered into the brain increases arterial blood pressure independently of nitric oxide synthase

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Introduction: Acutely acting tumor necrosis factor (TNF) in the circulatory system exerts a hypertensive effect via activation of nitric oxide synthase (NOS) [1]. However, long term administration of TNF triggers hypertension accompanied by decreased NOS expression [2]. Increased production of nitric oxide (NO) in the central nervous system results in a decrease of blood pressure and inhibition of NOS leads to hypertension [3]. Aim: In the present study we aimed at finding out if acutely administered TNF into the cerebral ventricles affects arterial blood pressure and heart rate and whether TNF actions are dependent on NOS activity. Methods: We carried out the study on adult male Sprague-Dawley rats. All animals were implanted with brain cannula for intracerebroventricular (ICV) infusions and arterial catheter for recording blood pressure. All surgical procedures were carried out under ketamine (100 mg/kg b.w.) and xylazine (10 mg/kg b.w.) anesthesia. Hemodynamic parameters were recorded in conscious freely moving rats at baseline and during hourly ICV infusion of saline (5 µl/hr), TNF (200 ng/5µl/hr), L-NAME (NO synthase inhibitor; 1 µg/5µl/hr), or TNF together with L-NAME in four separate groups of animals (each n=6). Mean arterial blood pressure (MABP) and heart rate (HR) were derived from raw blood pressure signal. One-way and repeated measures ANOVAs were used for statistical analysis. Results: ICV infusion of TNF caused significant increase in MABP and HR. Inhibition of NOS with ICV infusion of L-NAME resulted in significant increase of MABP without changes in HR. TNF administered together with L-NAME led to significant increase of MABP and HR. All treatments resulted in significant elevation of MABP in comparison to control animals. Coadministration of TNF and NOS inhibitor did not augment changes of hemodynamic parameters. Conclusion: Centrally administered TNF causes increase of MABP independently of brain NOS activity.


Effect of micro infusion of orexin B on ethanol consumption and expression of glutamate in the nucleus accumbens of male wistar albino rats

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Alcoholism is one of the most costly diseases afflicting population worldwide. The nucleus accumbens is a basal forebrain structure situated deep in the grey matter. 1. It is one of the major component of the reward system.2. The present study was designed to elucidate role of Orexin B in ethanol consumption and glutamate levels in the nucleus accumbens. All procedures were performed as per the guidelines established by the Institutional Animal Ethics Committee (IAEC/KMC/57/2009-2010). Eighteen male rats were divided into 3 groups i.e. Control, Treated 1(3nm/kg) and Treated 2(30nm/kg) (n=6). The rats were anaesthetized with a cocktail of ketamine (60 mg/kg BW) and xylazine (10 mg/kg BW) injected intraperitoneal route. The nucleus accumbens lesion was performed according to the stereotaxic coordinates as prescribed in the Paxinos and Watson 3. Post infusion, in overnight fasted rats, alcohol and food intake was measured meticulously after 1 hour, 2 hours, 4 hours, 6 hours, 12 hours and 24 hours. Further glutamate levels in the specific brain nuclei were estimated. The animal was decapitated and the brain tissue homogenate was used for the analysis. Glutamate was quantified using a commercially available ENZY\textsuperscript{TM}-CHROM GLUTAMATE ASSAY KIT (Bioassay systems, USA; catalogue number: EGT-100) according to the manufacturer’s protocol. Data were expressed as mean±SEM (ANOVA; Student-Newman Keuls test.), P<0.05 was considered as statistically significant. The high dose (HD) of Orexin B increased ethanol consumption (10%) significantly at the end of 1, 2, 4, 12, 24 hrs. In low doses (LD) of Orexin B (3nm/μl) there was significant increase in alcohol intake, 1.42 and 24 hrs.[C(3.1±0.48, 2.9±0.18, 3.7±0.34, 5.4±1.19, 16.62±1.20)LD(6.8±0.70, 3.7±0.38, 4.8±0.35, 7.16±1.36, 20.85±1.53) HD(10.1±0.6, 5.28±0.35, 6.64±0.42, 8.76±0.91, 26.85±0)]. Food intake was also increased significantly at the end of 1 and 4 hrs(p<0.001)[C(2.3±0.36, 0.69±0.20, 0.34±0.05, 6.9±0.71, 10.26±1.05)LD(5.39±0.500, 83±0.27, 1.31±0.08, 5.47±0.11, 10.31±1.44)HD(6.2±0.4, 0.9±0.14, 2.18±0.09, 8.62±0.60, 17.96±0.99)]. Glutamate levels of nucleus accumbens were significantly decreased with the infusion of Orexin B into the same nucleus (p<0.001) as well as BLA (p<0.01). [NACC(72.5±0.45,55.49±0.48)] BLA(57.44±0.30, 63.2±1.21). This result suggests that infusion of Orexin B into NAcc bilaterally, led to increase in alcohol and food and decrease in glutamate may be due short-term consumption of ethanol.

Key words: Orexin Nucleus accumbens, ethanol, lesion.


Manipal University.

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The presence and function of TMEM16A encoded calcium activated chloride channels in rat coronary artery

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Failure of Coronary blood vessels to adequately supply cardiac myocytes with oxygen underlies ischaemic heart disease and ultimately myocardial infarction. Thus it is important to determine the factors that regulate coronary blood flow in order to understand the pathophysiology of such conditions and, conceivably, identify new therapeutic targets for their treatment. Calcium-activated chloride channels, encoded by TMEM16A, play a key role in depolarising vascular smooth muscle cell (VSMC) membrane potentials, thus causing influx of Ca2+ through voltage gated Ca2+ channels and ultimately VSMC contraction. The aim of this study was to determine whether TMEM16A was expressed in rat coronary arteries and whether its modulation by novel TMEM16A specific blockers affected coronary artery function.

Male wistar rats were killed in accordance with Schedule 1 of the United Kingdom Animals Act (1986) or Danish regulations. 1st and 2nd order left anterior descending (LAD) coronary artery segments or septal coronary artery segments were isolated. A gelNorm experiment was carried out to find out the shared most stably expressed genes across all vessels compared to which the abundance of the genes of interest would be normalised. TMEM16A mRNA was detected in coronary arteries although at levels lower than rat pulmonary arteries. To study the role of TMEM16A in vascular physiology isometric tension recordings were carried out on LAD and septal segments. Cumulative application of U46619 (1nM – 3μM) produced concentration dependent contractions that were attenuated with prior application of TMEM16A specific blockers (T16inh-A01 and MONNA). 10μM T16inh-A01 caused a shift in EC50 of U46619 from 66nM±9 in vehicle controls to 285nM±59 (n=10, p<0.001) while 10μM MONNA caused a shift from 66nM±9 to 317nM±107 (n=8, p<0.005). Shifts in EC50 were also seen with 3μM T16inh-A01 (n=7, p<0.001) and 1μM MONNA (n=8, p<0.01), while 1μM T16inh-A01 and 0.3μM MONNA both had no effect on the contraction (both n=7). Values are mean EC50±S.E.M, compared by 1-way ANOVA to vehicle controls. In Langendorff perfused rat heart preparations increasing concentrations of T16inh-A01 (10μM -10μM) produced a concentration dependent increase in coronary flow of ~60% (n=4).

We have shown the presence of TMEM16A in rat LAD and Septal coronary arteries and demonstrated that TMEM16A-specific blockers attenuated U46619 induced contractions and increased coronary flow.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
In vivo assessment of endothelial function in small animals using an infrared pulse detector

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Aim: Endothelial dysfunction is the earliest change in atherosclerosis. Flow-mediated vasodilatation (FMD) is used to assess endothelial function in humans. However, it is not easy to do this assessment in small animals. We designed an instrument that uses infrared sensors to assess FMD in vivo using a rodent model.

Methods: Twenty-four adult male Wistar Kyoto (WKY) rats were randomly divided into three groups. The animals were anesthetized with intraperitoneal injection of urethane (1.2 mg/kg; Sigma-Aldrich, St. Louis, MO, USA). To stabilize body temperature, each animal was placed on a heated electric blanket with a warming lamp overhead. FMD was measured under continuous infusion of normal saline followed by intra-arterial infusion of either acetylcholine (Ach; n=8), sodium nitroprusside (SNP; n=8), or \(N\)-nitro-L-arginine methyl ester (\(\text{L}\)-NAME; n=8). Results: The dilatation indices (DIs) of all three groups were similar before the application of the vasoactive agents, (1.82 ± 0.46, 1.81 ± 0.44, and 1.91 ± 0.40, p=0.877, by one-way ANOVA). The DI was significantly increased during infusion of Ach (2.97 ± 1.03 vs. 1.82 ± 0.46, p=0.015), unchanged during the infusion of SNP (1.81 ± 0.44 vs. 1.98 ± 0.40, p=0.574), and attenuated during the infusion of \(\text{L}\)-NAME (1.91 ± 0.40 vs. 1.42 ± 0.35; p=0.028).

Conclusions: The results of the present study correlated well with those of previous human studies, suggesting that the method can be used for in vivo evaluation of endothelial function in small animals.

The effect of drugs on flow-mediated dilatation.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dilatation indices at baseline</th>
<th>Dilatation indices during infusion of drug</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine (n=8)</td>
<td>1.82 ± 0.46</td>
<td>2.97 ± 1.03</td>
<td>0.015  (^{5})</td>
</tr>
<tr>
<td>Sodium nitroprusside (n=8)</td>
<td>1.81 ± 0.44</td>
<td>1.98 ± 0.40</td>
<td>0.574  (^{4})</td>
</tr>
<tr>
<td>(\text{L})-NAME (n=8)</td>
<td>1.91 ± 0.40</td>
<td>1.42 ± 0.35</td>
<td>0.028  (^{3})</td>
</tr>
</tbody>
</table>

\(^{4}\) There is no individual difference in DI before the infusion of these drugs by one-way ANOVA.

\(^{5}\) Because of individual differences in the response to vasoactive agents, the changes during and before infusion of drugs were evaluated by non-parametric Mann-Whitney U test. Statistical significance was accepted for p < 0.05.

Pulse wave amplitudes were recorded before and after reactive hyperemia. The reactive hyperemic vasodilatation index was defined as the ratio of the mean amplitude during one minute period (period b), one minute after the release of the clip to the mean amplitude during one minute (period a) before occlusion of the left femoral artery.


This work was supported by grants from the Ministry of Science and Technology, Taiwan (100-2221-E-303-001-)

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“N” refers to number of biological replicates, “n” to the number of cells tested. mRNA and protein expression for TRPV4 was detected in RMECs (N=3). In immunocytochemistry studies TRPV4 channels were found to be mainly localised to the cytoplasm and cell nuclei, although some plasma membrane staining was also observed (N=3). The TRPV4 agonist GSK1016790A (100nM) elevated [Ca$^{2+}$], in RMECs (from a baseline R340/380 of 0.53 ± 0.02 to a peak of 2.41 ± 0.03; n=13,N=3), an effect that was blocked by pre-incubating the cells with the TRPV4 antagonist HC067047 (1μM) (p<0.001; n=8,N=3). HC067047, and a second TRPV4 inhibitor, RN1734, blocked in vitro sprouting angiogenesis in a concentration-dependent manner (IC50s for these drugs were 2.62μM ± 0.04707 and 3.2μM ± 0.06124, respectively), but had no effect on cell proliferation (Brdu ELISA) or cell migration (scratch-wound assay) (N=3, p>0.05 in both cases vs normal/DMSO controls). These drugs did, however, block tubulogenesis (HC067047 20μM, RN1734 15μM; N=2 per treatment). OIR was induced in C57BL/6 mice as previously described (1). Mice were anesthetised at P15 with ketamine (60mg/kg) and xylazine (6mg/kg, both IP) and given 1μl intravitreal injections of TRPV4 inhibitors/control agents. At P17 animals were killed by cervical dislocation in accordance with Schedule 1 of the UK Scientific Procedures Act, 1986. Isometric tension recordings were conducted on segments of second order branches of the mesenteric artery. Vessel segments were mounted on a wire myograph, normalised, and set to a resting tension of about 2mN. Bath application of MANS peptide (100nM-100μM), a cell-permeable MARCKS inhibitor, evoked concentration-de
sels preconstricted with U46619 (a thromboxane mimetic), endothelium-dependent relaxation was determined using carbachol (CCCh, 0.1 nM-10 μM) and endothelium-independent relaxation using the NO donor, sodium nitroprusside (SNP, 0.1 nM-10 μM). Data are expressed as mean ± SEM and were analysed by t-test. Significance was accepted when p<0.05.

Femoral endothelium-dependent vasodilatation was blunted in VDD compared to control group fetuses (PECtrl C, 6.55 ± 0.19, n=7; VDD, 5.79 ± 0.09, n=9; p<0.05), with no effect on maximal response. In contrast, femoral endothelium-independent vasorelaxation response to SNP was enhanced in VDD compared to control group fetuses (PECtrl C, 6.27 ± 0.47, n=5; VDD, 8.031 ± 0.23, n=8; p<0.01), with no effect on maximal response.

Our data suggest that in pregnancies of reduced dietary vitamin D there is a disruption in the contribution of endothelial and smooth muscle mechanisms in the regulation of fetal femoral artery tone. This could involve altered expression of endothelial nitric oxide synthase or smooth muscle guanylate cyclase (1). Such adaptations in vascular control may impact endothelial nitric oxide synthase and smooth muscle mechanisms in the regulation of fetal hind limb blood flow and muscle growth, and have additional ramifications for adult cardiovascular and metabolic health.


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**Intracellular pressure during neutrophil chemotaxis**

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Leukocyte migration through confined spaces contributes to immunity and may involve trailing surface contraction via actomyosin, which can push nuclei through narrow gaps (Lämmermann et al. 2008; Wolf et al. 2013), but the forces so exerted are uncertain. This study investigates intracellular pressure during such migration, by counting blebs on neutrophils. Blebs are transient surface swellings, apparently raised by intracellular hydrostatic pressure generated by actomyosin contraction in the cell cortex (Charras et al. 2008). Neutrophils (from human blood obtained by finger puncture) migrated at 22-28 °C into micropipettes containing solutions of the chemoattractant N-formyl-L-methionyl-L-leucyl-L-phenylalanine (0.1 μM) at various constant hydrostatic pressures applied using a water manometer, while viewed via a water immersion objective. Values appear as mean ± S.E.M. As neutrophils migrated into tips of radius 1.37±0.03 μm (n=144), blebs sometimes formed on their leading and/or trailing surfaces, the former implying that pressure inside the front of the cell exceeded that in the micropipette (Charras et al. 2008). Leading blebs appeared at manometer pressures between 0-0.60 kPa, but not between 0.62-1.28 kPa (Figure 1). Micropipette pressures presumably corresponded closely to these manometer readings, because other experiments showed that flows along micropipettes containing single migrating neutrophils were much lower than those along open lumens. At 0.80-0.95 kPa, single neutrophils migrated inwards along micropipettes (of radius 2.97±0.29 μm) at 0.142±0.004 μm s⁻¹ (n=5), apparently driving single flow marker beads (of radius 1.75±0.04 μm) suspended 2-30 μm ahead at 0.134±0.008 μm s⁻¹ (n=5; P>0.10; paired t-test). In open micropipettes at 0 kPa, beads moved inwards too quickly for speed measurement, driven by capillarity. Together, the present observations indicate that some neutrophils generated intracellular pressures of up to at least (Charras et al. 2008) 0.50-0.60 kPa, while partly impeded during chemotaxis. This resembles previous evidence from servo null micropuncture that increased intracellular pressure may correlate with different forms of protrusion in amoebae (Yanai et al. 1996) and fibroblasts (Petrie et al. 2014) and is consistent with hypotheses that such hydrostatic pressure assists some cell movements.


PC132

Effect of remote intermittent ischemia on contralateral extremities skin microcirculation

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Body’s endogenous protective capabilities are challenged in different stress situations and one of challenge is adequate blood flow changes which could causes oxygen concentration large fluctuations and ischemia/reperfusion injury. Studies confirm that interorgan protection against injury could be promoted by previous short ischemic events (Przyklenk et al. 1993); even more this adaptation could be remotely promoted (Hausenloy & Yellon 2008). Remote ischemic preconditioning (RIPC) stimulus, nonlethal limb ischemia can be achieved by applying a supra-systolic blood pressure by cuff to limb. RIPC is used in cardioprotection (Hausenloy & Yellon 2008), however there exists evidence that protective linkage exists between other vascular beds (Enko et al. 2011). The mechanism, which exerts protection in a remote organ, is currently unclear (Hausenloy & Yellon 2008). The aim of study was to determine whether RIPC induces local regulatory activity changes in contralateral upper limb’s skin microcirculation. Eleven healthy young participant’s, age (24±3, yr), height (1.71±0.10, m), mass (68±8, kg) and BMI (23±2, kg/m2), without systemic or peripheral vascular diseases. Data were collected continually by single point laser Doppler imaging (moorLDI2) at the forearm’s dorsal non-glabrous skin 10 cm distally from elbow joint. After 10 min baseline was RIPC applied to contralateral limb by inflating a blood pressure cuff placed on the upper arm to 200 mmHg for 5 min and deflating the cuff for 5 min; a cycle was performed four times, followed by 10 min recovery. To evaluate local regulatory factors was used wavelet transformation, the frequency intervals of 0.0095–0.021, 0.021–0.052, and 0.052–0.145 Hz corresponding represents endothelial, sympathetic, and myogenic activity (Hodges & Del Pozzi 2014) of the microcirculatory regulation. All data are non-parametrically distributed and presented as medina (25%; 75%). To compare samples before (B) and after (A) RIPC was used Wilcoxon Signed Rank Test. Systemic circulation parameters were influence by RIPC, statistically significant changes in mean arterial pressure (B=79.8(75.0; 88.3) vs. A=79.5(74.7; 82.7) mmHg; P=0.005) and heart rate (B=57(54;61) vs. A=55(50; 60) BPM; P=0.001) was observed, but it were not physiologically significant. Blood perfusion was not changed by RIPC (B=42(31;52) vs. A=51(37;57) PUI; P=0.054) and there was not discovered significant change in endothelial activity (B=0.69(0.24;1.89) vs. A=1.02(0.42;2.20) PUI/Hz; P=0.269). However, significant change of fluctuations were observed in sympathetic (B=1.68(0.58;2.99) vs. A=2.70(0.99;4.84) PUI/Hz; P=0.035) and myogenic (B=2.42(1.00;2.92) vs. A=2.91(1.49;6.60) PUI/Hz; P=0.012) activity. RIPC induces different vascular regulatory remodulation in contralateral microvascular bed by changing sympathetic and myogenic activity of the microcirculatory regulation.


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Similarity and differences in action of high-energy photon and proton irradiation on vascular function in rats

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The patients with neoplasm receiving photon or hadrons therapy (PT/HT) can develop unwanted side cardio-vascular effects. Up to date there have been no studies that directly compare acute effects of PT and HT on vascular function. That is why comparison of these effects is the main goal of this study. Experimental design comprised outward macroscopic potassium currents (IKo) measurements provided in single rat aortic smooth muscle (SM) cells using whole-cell patch clamp technique, isolated aortic rings endothelium-dependent acetylcholine (Ach)-induced vascular relaxations (contractile recordings), Langendorff-perfused hearts and non-invasive systolic arterial blood pressure (BP) measurements. Photons were delivered using 60Co gamma-rays (0.8 Gy min-1, TGT ROCUS M). The particle irradiation was done using 60-MeV energy proton beam accelerated in the U-240 isochronous cyclotron. Particle beam parameters were established on the basis of modeling data using semiimperical model of Ziegel, Biersack and Littmark (1985). We take into account the average values of the tissue density and element composition in the irradiated area. Rats were exposed to a total absorbed dose of 6 Gy in both kinds of irradiation impact and then were euthanized on 9 day following the irradiation. Both PT and HT led to an increased SM sensitivity to alpha-agonist arterenol while amplitudes of Ach-induced relaxations had decreased. PT decreased the maximal Ach-induced relaxation (Rmax) on 9th day post-irradiation from 91±2% in a control to 80±3 vs 53±3% under HT (pD2 was 7.8±0.2 in a control, 7.0±0.02 and 6.9±0.04 under PT and HT, respectively, n=14, P<0.05). It is important to note that PT lead to decrease in Rmax on the 30th day post-irradiation up to 51±3%, i.e. this value is close to that seen on 9th day after HT. After 9d ICo, which is mainly due to current through Maxi-K+ channels, from cells underwent PT and HT have been reduced from control 32±2 pA/pf to 18±1 pA/pf and to 10±1 pA/pf at +70 mV, respectively (n=7, P<0.05). The voltage-dependens of activation (V1/2) was
shifted to more positive voltages and the activation kinetics were slower in the HT treated cells compared with PT and control. The HT was without significant effect on systolic BP in rats (128±8 mm Hg vs 122±8 mm Hg in a control) while PT produced significant hypertensive development on the 9th day of post-irradiation (158±6 mm Hg vs 126±8 mm Hg in a control, n=7, p<0.05). Both HT and PT were without effect on cardiac contractility in Langendorff-perfused rat hearts. Thus, effects of PT and HT on vascular function are in substantially the same direction, except arterial blood pressure, but their intensity differs significantly. The data obtained provide new relevant information on the impact of the different radiation species on vascular tissues.

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Lysosome–ER coupling supported by two pore channel 2 is required for Nicotinic acid adenine dinucleotide phosphate-induced global calcium waves in pulmonary arterial myocytes

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The mechanism by which nicotinic acid adenine dinucleotide phosphate (NAADP) triggers intracellular Ca2+ release has been hotly debated. Two-pore segment channels (TCP1–3, TCPN1–3 for gene name) were recently identified as endolysosome-targeted ion channels (1-3) that support NAADP-evoked Ca2+ signals from acidic stores. Although TCP3 is expressed by other mammals, only TCP1 and TCP2 are expressed by humans, rats and mice (1, 3). The relative capacity of TCP1 and TCP2 to support coupling, by calcium-induced calcium release (CICR), between acidic stores and the sarco/endoplasmic reticulum has yet to be assessed in detail outside recombiant systems. We sought to address this question using methods described previously (1) and in doing so took advantage of our most recent observation (4), that when stably expressed in HEK293 cells, human (h)TCP1 is preferentially targeted to endosomes, hTCP2 to lysosomes, and rabbit (r)TCP3 to both endosomes and lysosomes. When applied by intracellular dialysis from a patch-pipette (voltage-clamp mode, -40 mV holding potential), 10 nM NAADP evoked robust, global calcium transients in both acutely isolated rat pulmonary arterial myocytes and in hTCP2-expressing HEK293 cells, the Fura-2 fluorescence ratio (F340/F380) increased from 0.35 ± 0.04 to 1.29 ± 0.08 (n=10) and from 0.31 ± 0.02 to 1.22 ± 0.08 (n=40), respectively. In both cell types, NAADP-evoked calcium transients were markedly attenuated by thapsigargin (1μM), but abolished by bafilomycin (1μM) and nifedipine (10μM). Qualitatively, similar results were obtained in relation to NAADP-evoked calcium signals in HEK293 cells that stably expressed the endolysosome targeted rTCP3 (F340/F380 increased from 0.38 ± 0.04 to 1.59 ± 0.24, n=11). By contrast, NAADP evoked highly localised (spatially restricted) calcium transients in hTCP1-expressing HEK293 cells, which remained unaffected in the presence of thapsigargin (F340/F380 increased from 0.30 ± 0.03 to 0.58 ± 0.06, n=8), but were blocked by bafilomycin (1μM) and nifedipine (10μM). Furthermore, NAADP failed to evoke global calcium signals in pulmonary arterial myocytes isolated from Tpcn2 knockout mice, despite the continued expression of TCP1. We conclude that NAADP induces global calcium waves in pulmonary arterial myocytes via lysosome-SR junctions and in a manner supported by TCP2, but not TCP1.


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PC135

Simvastatin improve cerebral endothelial function and reverse brain functional microvascular rarefaction in a hypertension model

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Motivation: Statins are widely used in the treatment of dyslipidemia, which is usually associated with cardiovascular abnormalities including hypertension. Microvascular rarefaction and endothelial dysfunction are aggravating factors of hypertensive end-organ damage. Thus, this study was designed to investigate the acute effects of simvastatin (SIM) on cerebral microcirculation and endothelial function in spontaneously hypertensive rats (SHR).

Methods: Male Wistar normotensive rats (WKY) and SHR were divided into 3 groups of 8 animals each: WKY-CTL and SHR-CTL treated with 0.9% saline solution, and SHR+SIM treated with SIM 30 mg/kg/day during 3 days by gavage. Systolic blood pressure (SBP) was measured by a tail-cuff plethysmography system. Rats were anaesthetised with (50 mg/kg sodium pentobarbital, i.p.). We investigated brain functional capillary density (FCD) and vascular reactivity using intravital fluorescence videomicroscopy after IV injection of FITC labeled dextran. We assessed pial arterioles endothelium-dependent vasodilation responses to acetylcholine (Ach, 1μM) administration. Vascular responses were expressed as percentage changes from the baseline arteriolar diameters. Values are means ±S.E.M, compared by ANOVA and Bonferroni’s Test, p values <0.05 were considered significant. All protocols were approved in accordance with the internationally accepted principles for the Care and Use of Laboratory Animals (license # L-48/12).
Results: SIM administration reduced SBP in SHR (SHR-CTL 203±3 vs. SHR+SIM 172±6 mmHg; p<0.001). Cerebral FCD was reduced in hypertensive rats compared with normotensive rats (SHR-CTL 337±61 vs. WKY-CTL 421±35 capillaries/mm²; p<0.05). The administration of SIM during 3 days induced a significant increase in cerebral FCD in hypertensive rats (SHR+SIM 530±31 capillaries/mm²; p<0.05). ACh induced arteriolar vasodilation in WKY rats (WKY-CTL +6.6±1.2%) but arteriolar vasoconstriction in SHR (SHR-CTL -1.4±1.3%; p<0.05); SIM restored ACh-induced arteriolar vasodilation (SHR+SIM +11.5±3.1%; p<0.05). Microvascular endothelial dysfunction in SHR was associated with a down-regulation of endothelial nitric oxide synthase (eNOS) expression in the brain (SHR-CTL 0.76±0.1 vs. WKY-CTL1.25±0.2 eNOS/GAPDH (AU); p<0.05). Treatment of SHR with SIM normalized the brain expression of eNOS (SHR+SIM 2.13±0.7 eNOS/GAPDH (AU); p<0.05).

Conclusion: Acute treatment with simvastatin reversed cerebral microvascular rarefaction and restored brain microvascular endothelial function in hypertensive rats. In addition to cholesterol-lowering effects, vascular pleiotropic effects of statins could turn out to be a new therapeutic approach for improving microcirculatory function in hypertensive patients.

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intestinal complications that might come with the presence of HIV virus in patients.


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The use of inert bulking agents to reduce pig bladder spontaneous contraction

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Spontaneous contractions of the bladder wall, enhanced in patients with overactive bladder (OAB), are abolished by removal of mucosa that overlies detrusor smooth muscle and a potential source of diffusible contractile mediators. Minimising communication between bladder layers may therefore reduce spontaneous activity and one approach is to inject inert bulking agents below the mucosa. We tested the hypothesis that ‘inert injectables reduced spontaneous bladder contractions’.

Pig bladders from a local abattoir were stored in fresh Tyrode’s solution. Intact bladder wall segments (1x2cm) were dissected and superfused with Tyrode’s solution (37°C). One end of the preparation was clamped to an anchor and the other to an isometric force transducer. Strips were exposed to 1μM carbachol (CCh, muscarinic agonist) or 0.3μM α,β-methylene ATP (ABMA, purinergic desensitiser) for 10min. Spontaneous contractions were measured before and during drug interventions. Preparations were then injected below the mucosa with polyethylene glycol (PEG) or coaptite (CA, 0.2 ml) and no material (null injectate) and drug exposure repeated. Data were analysed with a purpose-made algorithm and normalised to values before injections (control). For CCh and ABMA responses, contraction amplitude was measured. For spontaneous contractions, amplitude, force integral, and frequency, were all measured over a 10min period. Data sets are medians [25, 75% interquartiles] and compared using Mann-Whitney U-tests against the control. The null hypothesis was rejected at p<0.05.

Time-dependent increases of CCh and ABMA contractions were recorded - null injection vs control (CCh: 156.2 [126.2, 250.8]%: n=11; ABMA: 142.3 [133.9, 224.9]%: n=11). This increase was abolished with PEG but not by CA injection. For CCh contractions: PEG 119.6 [102.6, 130.0]%: n=11; CA, 133.2 [118.1, 216.6]%: n=10. For ABMA contractions: PEG 99.4 [66.1, 109.0]%: CA, 139.7 [89.2, 228.7]%.

Spontaneous contractions also changed in a time-dependent way (null injection vs control): Contraction amplitude increased significantly (172.3 [79.5, 304.5]%: as did force integral (128.6 [65.2, 228.4]%), but frequency was unchanged (106.7 [83.7, 134.0]%: n=10). With PEG injection time-dependent changes were abolished: amplitude (109.4 [47.5, 115.4]%); frequency (113.7 [92.3, 150.2%]); force integral (68.6 [41.9, 127.2]%)(n=9). CA even reduced spontaneous contraction amplitude (77.0 [21.2, 112.5]%: and force integral (55.7 [17.7, 92.9]%)); although frequency was slightly increased (133.3 [123.9, 161.3]%: n=9).

Bladder spontaneous contractions are reduced by inert bulking agents, with coaptite more successful than PEG. This approach offers an alternative approach to pharmacological therapeutics in reducing symptoms of OAB.

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Endothelial dysfunction of rat aorta after exposure to mercury is dependent on reactive oxygen species and nitric oxide

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Exposure to mercury is known to increase cardiovascular risk but the underlying mechanisms are not well explored. The present study was undertaken to investigate the modulatory role of mercury on vascular function and to elucidate the mechanisms behind the observed effects.

Thoracic aorta from Wistar rats (300g, n=50) treated with either perse mercury chloride (10−5M, 10−4M) or methyl mercury chloride (10−5M, 10−4M) or acutely mercury chloride (single dose of 5 mg/kg; ip (1)) or methyl mercury chloride (single dose of 5 mg/kg; po (2)) or chronically mercury chloride (1.25x10−6 M/L in drinking water for 30 days (3)) or methyl mercury chloride (0.5 mg/kg/day, orally for 30 days (2)) were used.

Experiments were performed in accordance with recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India. Zero mortality was observed and there was no change in mean body weight after 30 days. Decreased acetylcholine (ACh) induced vasodilatation in perse mercury chloride (10−5M; R max (52.7±2.8%:08.2±1.2%*), methyl mercury chloride (10−5M; R max (52.7±2.8%:11.5±2.9%*), acutely mercury chloride (R max (52.7±2.8%:36.1±2.2%*), chronically mercury chloride (R max (52.7±2.8%:26.3±1.2%*) and methyl mercury chloride (R max (52.7±2.8%:34.8±2.1%*) exposed rats was observed suggesting endothelial dysfunction. However, an increased ACh induced vasodilatation was observed in perse mercury chloride (10−4M, R max (52.7±2.8%:73.9±1.8%*), methyl mercury chloride (10−4M; (52.7±2.8%:79.29±1.3%*)) and acutely methyl mercury chloride (R max; (52.7±2.8%:70.4±2.9%*), exposed rats indicating modulation of the endothelial function. The superoxide anion scavenger SOD + catalase augmented the ACh responses in vessels from mercury-treated rats suggesting mercury induces oxidative stress (Fig.1). The NOS inhibitor, L-NAME significantly reduced the ACh induced vasodilatation in perse mercury chloride (10−5M, 10−4M) or acutely methyl mercury chloride (10−5M), methyl mercury chloride (10−4M) and acutely methyl mercury chloride, in comparison to mercury chloride (10−5M), methyl mercury chloride (10−4M), acutely mercury chloride, chronically mercury chloride and methyl mercury chloride exposed rats exposed rats suggesting that mercury may induce increased/ decreased production of NO depending on the dose, form and type of exposure (Fig.2). Increased MDA levels were accompanied with increase in serum NO levels in all the mercury
treated rats suggesting that mercury causes oxidative stress and increased production of NO.

A delicate balance exists between free radicals and NO released by endothelial cells on mercury exposure. When there is an increase in NO release, enhanced endothelial function is observed. When the balance is tipped in favor of oxidative stress, endothelial dysfunction is observed. The form of mercury, route, dose and period of exposure, play an important role in determining the harmful effect of mercury on the vascular endothelium. Therefore NO signaling mechanism and oxidative stress play an important function in the mercury-induced cardiovascular diseases in the populations exposed to mercury.

*P < 0.05

**Fig 1** Change in response of ACh after SOD + Catalase treatment

**Fig 2** Change in response of ACh after L-NAME treatment


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**PC140**

Evidence of a role for TRPV2 channels in retinal arteriolar myogenic signalling

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The TRP channels, TRPC6 and TRPM4, are known to play a key role in the myogenic response of cerebral arteries. Presently, little is known about the contribution of TRP channels in mediating such responses at the level of the microcirculation. Here we show that TRPV2 is the primary TRP channel that contributes to myogenic signalling in rat retinal arterioles. Sprague-Dawley rats were killed by schedule 1 methods and retinal arterioles isolated for RT-PCR, Fura-2 Ca^2+ microfluorimetry, patch-clamp electrophysiology and pressure myography studies as previously described. In some experiments, immunohistochemistry was performed on retinal wholemount preparations. Data were tested for normality using the D’Agostino-Pearson normality test and analysed using paired t-tests. mRNA transcripts for the mechanosensitive TRP channels, TRPC1, M7, P2 (PKD2), V1, V2 and V4, but not TRPC6 or M4, were detected in isolated retinal arterioles. Immunolabelling studies revealed cytosolic and membrane expression of TRPC1, M7, V1, V2 and P2 in retinal arteriolar myocytes, while TRPV4 appeared largely restricted to the nuclei of these cells. Hypo-osmotic stretch-induced Ca^2+ influx in retinal arteriolar myocytes was reversed by the TRPV2 inhibitor tranilast (100 μM; n=5; 100±33% vs -12.1±10.2%, P<0.05) and the non-selective TRPP2/V2 antagonist amlodipine (100μM; n=8; 100±17.7% vs 63.6±12.2%, P<0.01). Inhibitors of TRPC1, M7, V1 and V4 had no effect. Hypo-osmotic stretch activated wholecell currents were similar in Na^+ and Cs^+ containing extracellular solutions (33.6±8.7pA/pF vs -34.0±9.8 pA/pF respectively at -80mV; P>0.05) suggesting no contribution by TRPP2 channels. Application of 35.46±2.16 mmHg of negative pressure in 14 cell-attached patches resulted in an increase in integrated current from 0.38±0.10pC/s to 2.10±4.30pC/s (P<0.001). Pressure-induced stretch failed to significantly activate current in the presence of TNL (0.42±0.24pC/s to 0.53±0.23pC/s; n=10, P>0.05). Application of TNL inhibited delta-9-tetrahydrocannabinol (10μM) activated TRPV2 currents (4.0±1.0pA/pF vs -0.03±0.5pA/pF at -80mV; n=6, P<0.01), but had no effect on L-Type Ca^2+ channels (KCl-induced Ca^2+ entry; 61.7±19.3nM vs 59.7±18.6nM in the absence and presence of TNL, respectively; n=6, P>0.05) or ryanodine-sensitive store release (caffeine-induced Ca^2+ release; 183.6±26.7nM vs 172.2±28.7nM; n=7, P>0.05). Addition of TNL to isolated pressurised (40mmHg) retinal arterioles under conditions of myogenic tone resulted in significant dilation (98.5±0.5% vs 101.9±0.7% of initial diameter upon pressurisation; n=9, P<0.001). Our results suggest that rat retinal arteriolar myocytes express a range of mechanosensitive TRP channels, but only TRPV2 appears to contribute to myogenic signalling in this vascular bed.


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Translation of anti-fibrotic microRNA strategies into a mouse model of chronic allograft dysfunction

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Introduction: Chronic allograft dysfunction (CAD) is the most limiting factor of long term graft survival and characterized by fibrotic remodeling, renal injury and chronic inflammation. Recent studies identified several microRNAs (miRs), small non-coding RNAs involved in gene regulation, that are enriched in kidneys in response to injury and have pro-fibrotic potential [1]. Thus, miR antagonists (antagomiRs) could serve as a promising treatment strategy since they have been shown to be a powerful tool to reduce or even prevent fibrotic remodeling. The aim of this study is to investigate the inhibition of pro-fibrotic miR-21 in an evaluated murine kidney transplantation model.

Methods and Results: Allogenic kidney transplantation (KTx) was performed at day 0 from male C57BL/6 mice into female Balb/c mice (ca 25g, n=6) under isoflurane anaesthesia (3-4% induction, 2-3% maintenance, in 100% O₂ and analgesia (buprenorphin, 1mg/kg BW, s.c.) [2]. Recipient mice were treated at day -1 and day 7 either with LNA-SCR (control) or LNA-21 (inhibitor of miR-21) (20mg/kg BW, i.p.). Kidneys were harvested and analyzed six weeks after KTx.

Following data are compared by ANOVA. Using RT-PCR, we determined increased expression levels of markers for fibrosis (e.g. collagen 3 (Col3), fibroblast secretory protein-1 (FSP-1); p=0.002 and p=0.01 respectively) and inflammation (e.g. interleukin-6 (IL-6), macrophage inflammatory protein-1 (MIP-1); p=0.02 and p=0.006 respectively) in transplanted kidneys which were rescued by miR-21 inhibition. Moreover, allografts of LNA-21 treated mice showed significantly less fibrosis development (Sirius Red, p=0.001) and had a lower BANFF chronic rejection score (p=0.02).

The miR-21 promoter region harbors a putative binding site of transcription factor STAT3, which is activated by IL-6. We identified upregulated IL-6 expression in inflammatory cells (primary peritoneal macrophages and cell line RAW264.7 (p<0.0001 respectively) upon activation with lipopolysaccharide (10ng/mL, 24h) and hypothesized, that infiltrating immune cells produce and secrete cytokines that might affect resident renal cells causing fibrosis and injury development. Co-culture assays confirmed a crosstalk between RAW264.7 and renal fibroblasts NRK49F with increased expression levels of IL-6 (p=0.005), connective tissue growth factor (CTGF, p=0.02) and miR-21 (p=0.003) in NRK49F. Similar results were observed due to IL-6 treatment (10ng/mL, 12h) of NRK49F.

Conclusion: In our murine model of CAD allograft rejection is preserved by inhibition of miR-21 due to less inflammation and fibrosis. IL-6 was identified as a crucial signal mediator that increases miR-21 expression level in renal fibroblasts. Thus, our study suggests an essentially needed new treatment strategy based on inhibition of pro-fibrotic miR-21 in transplantation medicine.

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Acute differential changes in L-type Ca current density in surface sarcolemma and t-tubules of normal and failing rat cardiomyocytes

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Cardiac L-type Ca current (I_{Ca}) is the primary trigger for Ca release from sarcoplasmic reticulum (SR) and the major influx pathway maintaining cell Ca load and contractility. In normal ventricular myocytes, I_{Ca} is localised predominantly to t-tubules (TT) but its relocation to the surface sarcolemma (SS) may contribute to systolic dysfunction in heart failure. The aim of this study was to investigate the effect of this relocation on Ca loading.

Animal procedures were performed in accordance with UK legislation and approved by local ethics committee. Coronary artery ligation (CAL) and sham operations (Sham) were performed on adult male Wistar rats under surgical anaesthesia (ketamine 75 mg/kg, medetomidine 0.5 mg/kg ip) with appropriate analgesia (buprenorphine 0.05 mg/kg sc). Hearts were excised under pentobarbitone anaesthesia (140 mg/kg ip) 18 weeks after surgery and left ventricular myocytes isolated by enzymatic digestion. Cells were voltage-clamped to -80 mV using whole-cell patch-clamp. Following a ramp to -40 mV I_{Ca} was recorded at 0 mV. The pipette contained 100 mM fluo-4 for simultaneous measurement of Ca_i. Cells were stimulated at 1 Hz for 20 s followed by a 10 s gap before 10 mM caffeine was applied for 10 s to release SR Ca. 1 Hz stimulation was resumed 10 s after removal of caffeine. Recordings were made at room temperature from intact cells (Sham n=12, CAL n=8) and cells detubulated by osmotic shock (DT, Sham n=12, CAL n=7) to compare SS and TT I_{Ca} densities. Data are presented as mean±SEM and analysed using 2-way ANOVA with Bonferroni post-test; the limit of statistical confidence was P<0.05.

In all cells, Ca_i transient amplitude on the 1st stimulus after caffeine was smaller than steady-state and increased with repeated stimulation. However, there was no significant difference in the rate of recovery in Sham and CAL cells, and DT had no significant effect on recovery rate in either group. SS I_{Ca} density during the 1st beat after caffeine was not significantly different in Sham and CAL cells (Sham -2.98±0.26, CAL -3.31±0.31 pA/pF), and decreased during recovery from caffeine (P<0.01) to steady-state values that were not significantly different (Sham -2.42±0.21 pA/pF; CAL -2.50±0.26 pA/pF), consistent with accumulation of inactivation of SS I_{Ca}. In contrast, TT I_{Ca} density was markedly smaller in CAL cells during the 1st beat after caffeine (Sham -5.92±0.32, CAL -9.1±0.37 pA/pF, P<0.001), and increased by a similar amount in both groups of cells during recovery (Sham -6.85±0.27 pA/pF P<0.001, CAL -1.84±0.33 pA/pF P<0.001), indicating facilitation of TT I_{Ca}. These data provide evidence for differential acute regulation of I_{Ca} at SS and TT, and suggest that SS plays a key role in Ca loading.

This work was supported by the British Heart Foundation.
Characterisation of a novel interaction between the cardiac myosin binding protein-C and the ryanodine receptor/calcium release channel

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The type 2 ryanodine receptor (RyR2) mediates sarcoplasmic reticulum (SR) calcium release which is critical for cardiac excitation-contraction coupling. RyR2 is composed of ~5000 amino acids with C-terminal transmembrane domains forming the channel pore while the bulk of the protein faces the cytoplasm. Cardiac myosin binding protein-C (cMyBP-C) is a modular protein associated with the sarcomere thick filament through its C-terminus interactions with myosin and titin. cMyBP-C is thought to regulate myocardial contractility by modulating the actin-myosin association primarily through its N-terminal region. We have isolated cMyBP-C as a putative RyR2-binding partner by yeast two-hybrid screening of a human cardiac cDNA library. The RyR2 interaction with cMyBP-C was also verified by co-immunoprecipitation assays following co-expression of both recombinant human polypeptides in mammalian HEK293 cells (n = 5), and importantly between the native proteins from pig cardiac muscle homogenates (n = 5). Further mapping studies were carried out using co-immunoprecipitation assays on a series of RyR2 and cMyBP-C truncated fragments co-expressed in HEK293 cells in order to identify the minimal interacting regions. Cumulative data (n ≥ 5) following densitometry analysis indicated robust binding between the RyR2 N-terminal (amino acids 161-759) and cMyBP-C C-terminal (amino acids 820-972) domains. Additional experiments demonstrated that the corresponding RyR1 and RyR3 N-terminal fragments are also capable of interaction with the C-terminus of cMyBP-C (n ≥ 6 for both constructs), suggesting that the RyR:MyBP-C association could be conserved in both skeletal and cardiac muscle. The above results suggest that the RyR2 interaction with cMyBP-C is likely to be physiologically relevant. This novel, potential modulator of RyR2-mediated SR Ca2+ release by a sarcomere component could provide a retrograde regulation mechanism for cardiac excitation-contraction coupling.

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

Alterations to total and phosphorylated Cx43 and Cx40 levels in atrial fibrillation: role of Calcinuerin Aα, protein phosphatase 2A and CaMKII

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1School of Biosciences and Medicine, University of Surrey, Guildford, UK and 2School of Physiology and Pharmacology, University of Bristol, Bristol, UK

Atrial fibrillation (AF) is the most common cardiac arrhythmia and a major cause of stroke. Abnormal AP propagation is a central cause of AF associated with abnormal gap junction (GJ) conductance [1]. The latter is regulated by alterations to the phosphorylation state of GJ connexin (Cx) proteins, in particular Cx43 and Cx40. We have shown that the serine-threonine protein phosphatases (S-T PPs), calcineurin Aα (CnAα) and PP2A dephosphorylate Cx43-Ser365 and exposes Cx43-Ser368 for PKC phosphorylation, thus reducing GJ conductance. However, the role of S-T PPs and protein kinases (PKs) in AF remains unknown. This study aimed to: 1) investigate changes to Cx43 and Cx40 protein expression and phosphorylation state in tissue from sinus rhythm (SR) and AF patients; 2) measure protein levels of S-T PPs and PKs that may mediate these changes. Human left atrial (LA) appendage biopsies from patients with SR (n = 13) or AF (n = 13) were snap-frozen in liquid N₂ and homogenised. Samples were matched by age, gender and hypertension. Western blots measured the expression of total Cx43 (T-Cx43), Cx43 phosphorylated at Ser368 (Cx43-pSer368), T-Cx40, CnAα, PP2A, PKCα, Inhibitor 1 (I-1), I-1pThr35, PP1 and CaMKII. Values are integrated band-densities normalised to GAPDH (mean±SEM). Differences were tested by ANOVA, with post hoc parametric tests; significance was at p<0.05.

The ages of SR and AF groups were (SR 70±2.1 vs AF 72±1.9 yrs, p=0.05). T-Cx43 protein expression significantly decreased in AF (SR 1.73±0.26 vs. AF 1.02±0.07; p<0.001). This was associated with a two-fold increase of Cx43-pSer368 levels. LA T-Cx40 protein expression exhibited two bands (P₀ and P₁). T-Cx40 was significantly higher in AF (SR 0.90±0.06 vs. AF 1.05±0.05; p<0.001) with a significant increase of the P₀ band (SR 0.57±0.07 vs. AF 0.82±0.06; p<0.01). The profile of PPs and PKs were assessed. CnAα and PP2A protein expression were significantly increased in AF, whereas PP1 was unchanged. This was associated with an increase in I-1pThr35, suggesting that the I-1-P1 pathway is not involved in alteration in Cx phosphorylation associated with AF. PKCα protein expression decreased in AF with a ratio similar to that observed for Cx43 (T-Cx43 0.67±0.08 vs PKCα 0.88±0.16). CaMKII protein expression was significantly raised in AF. Thus, in AF there was an increase of T-Cx40 (especially P₁) compared with a decrease of T-Cx43 protein levels. The increase of CnAα, PP2A and Cx43-pSer368 supports previous data for their role in Cx43-pSer365 dephosphorylation leading to Cx43-Ser368 PKC phosphorylation. Finally, the increased T-Cx40 P₀ band may be mediated by enhanced levels of CaMKII. In conclusion, this is the first study to link changes to phosphatase and kinase profiles with alterations to Cx43 and Cx40 phosphorylation state in AF.


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Shear stress induces longitudinal Ca\(^{2+}\) wave via autocrine activation of P2Y\(_{1}\) purinergic signaling in atrial myocytes

J. Kim and S. Woo

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Atrial myocytes are exposed to shear stress during cardiac cycle and hemodynamic disturbance. It is known that shear stress elicits longitudinal global Ca\(^{2+}\) wave (“L wave”) in atrial myocytes (1). Here, we investigated cellular mechanisms for shear-mediated Ca\(^{2+}\) response in atrial myocytes using confocal Ca\(^{2+}\) imaging (30 Hz). We applied shear stress to single myocytes using pressurized micro flow system. Atrial myocytes were enzymatically isolated from male Sprague-Dawley rats (230–300 g) and from wild-type (WT) and type 2 inositol 1,4,5-trisphosphate receptor (IP\(_{3}\)R) knockout (KO) mice (C57/B6, 24-28 g) (anesthesia: pentobarbital sodium, 150 mg kg\(^{-1}\), i.p.). Shear stress of \(\sim 16\) dyn cm\(^{-2}\) aperiodically induced L wave (79±7.7 \(\mu\) m s\(^{-1}\)) 1.2±0.26 times for 8 s-long exposure (n=39), with a delay of 0.2-3 s. Shear-induced L wave was restored after 3-4 min resting period after the first occurrence. Values are means ± S.E.M. of wave events occurring for 8 s-long shear, compared by student t test. Blockade of ryanodine receptor (RyR) (zero for 1 mM tetracaine vs. 1.0±0.0 for control, n=5, \(p<0.0001\)) or IP\(_{3}\)R abolished the L wave occurrence under shear (zero for 3 \(\mu\)M Z-APB vs. 1.4±0.8 for control, n=8, \(p<0.001\)). In type 2 IP\(_{3}\)R KO cells, shear stress failed to induce L wave (WT, 1.8±0.34, n=13 vs. KO, zero, n=17, \(p<0.0001\)). Consistent with these results, inhibition of phospholipase C (PLC) using U73122 (5 \(\mu\)M) removed shear-induced L wave (0.13±0.13 vs. 1.1±0.26 for control, n=8, \(p<0.01\)), although its inactive analogue U73343 (5 \(\mu\)M) did not affect it (1.25±0.25 vs. 1.0±0.0 for control, n=4, \(p>0.05\)). These observations indicate that PLC-IP\(_{3}\)R signaling and Ca\(^{2+}\)-induced Ca\(^{2+}\)\(_{\text{release}}\) release via RyRs play a role in the generation of L wave under shear. Pre-treating atrial cells with the blockers for stretch-activated channel, TRP4 or NADPH oxidase did not alter the occurrence of L wave under shear. Suramin (10 \(\mu\)M), the inhibitor of purinergic receptor, suppressed the L wave occurrence under shear stress (zero vs. 1.2±0.50 for control, n=4, \(p<0.05\)). Antagonist of P2Y\(_{1}\) receptor MRS2179, but not P2X receptor antagonist (iso-PPADS), eliminated the L wave generation under shear (control, 1.0±0 vs. 200 nM MRS2179, 0.25±0.25, n=4, \(p<0.05\); control, 1.0±0 vs. 10 \(\mu\)M iso-PPADS, 0.83±0.17, n=6, \(p=0.05\)). Suppression of connexon that releases ATP using carbenoxolone (50 \(\mu\)M; zero vs. 1.2±0.17 for control, n=6, \(p<0.001\)), or extracellular application of apyrase (2 \(\mu\)M\(^{-1}\)) that metabolizes ATP inhibited the occurrence of L wave under shear (zero vs. 1.8±0.58 for control, n=5, \(p<0.05\)). Our data suggest that longitudinal Ca\(^{2+}\) wave is triggered by type 2 IP\(_{3}\)R-mediated Ca\(^{2+}\) release that is activated by connexon-mediated ATP release and subsequent activation of P2Y\(_{1}\) receptor-PLC signaling in atrial myocytes under shear stress.


We thank Dr. Ju Chen for type 2 IP\(_{3}\)R knockout mice.

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

PC146

Shear stress induces longitudinal Ca\(^{2+}\) wave via autocrine activation of P2Y\(_{1}\) purinergic signaling in atrial myocytes

J. Kim and S. Woo

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Atrial myocytes are exposed to shear stress during cardiac cycle and hemodynamic disturbance. It is known that shear stress elicits longitudinal global Ca\(^{2+}\) wave (“L wave”) in atrial myocytes (1). Here, we investigated cellular mechanisms for shear-mediated Ca\(^{2+}\) response in atrial myocytes using confocal Ca\(^{2+}\) imaging (30 Hz). We applied shear stress to single myocytes using pressurized micro flow system. Atrial myocytes were enzymatically isolated from male Sprague-Dawley rats (230–300 g) and from wild-type (WT) and type 2 inositol 1,4,5-trisphosphate receptor (IP\(_{3}\)R) knockout (KO) mice (C57/B6, 24-28 g) (anesthesia: pentobarbital sodium, 150 mg kg\(^{-1}\), i.p.). Shear stress of \(\sim 16\) dyn cm\(^{-2}\) aperiodically induced L wave (79±7.7 \(\mu\) m s\(^{-1}\)) 1.2±0.26 times for 8 s-long exposure (n=39), with a delay of 0.2-3 s. Shear-induced L wave was restored after 3-4 min resting period after the first occurrence. Values are means ± S.E.M. of wave events occurring for 8 s-long shear, compared by student t test. Blockade of ryanodine receptor (RyR) (zero for 1 mM tetracaine vs. 1.0±0.0 for control, n=5, \(p<0.0001\)) or IP\(_{3}\)R abolished the L wave occurrence under shear (zero for 3 \(\mu\)M Z-APB vs. 1.4±0.8 for control, n=8, \(p<0.001\)). In type 2 IP\(_{3}\)R KO cells, shear stress failed to induce L wave (WT, 1.8±0.34, n=13 vs. KO, zero, n=17, \(p<0.0001\)). Consistent with these results, inhibition of phospholipase C (PLC) using U73122 (5 \(\mu\)M) removed shear-induced L wave (0.13±0.13 vs. 1.1±0.26 for control, n=8, \(p<0.01\)), although its inactive analogue U73343 (5 \(\mu\)M) did not affect it (1.25±0.25 vs. 1.0±0.0 for control, n=4, \(p>0.05\)). These observations indicate that PLC-IP\(_{3}\)R signaling and Ca\(^{2+}\)-induced Ca\(^{2+}\)\(_{\text{release}}\) release via RyRs play a role in the generation of L wave under shear. Pre-treating atrial cells with the blockers for stretch-activated channel, TRP4 or NADPH oxidase did not alter the occurrence of L wave under shear. Suramin (10 \(\mu\)M), the inhibitor of purinergic receptor, suppressed the L wave occurrence under shear stress (zero vs. 1.2±0.50 for control, n=4, \(p<0.05\)). Antagonist of P2Y\(_{1}\) receptor MRS2179, but not P2X receptor antagonist (iso-PPADS), eliminated the L wave generation under shear (control, 1.0±0 vs. 200 nM MRS2179, 0.25±0.25, n=4, \(p<0.05\); control, 1.0±0 vs. 10 \(\mu\)M iso-PPADS, 0.83±0.17, n=6, \(p=0.05\)). Suppression of connexon that releases ATP using carbenoxolone (50 \(\mu\)M; zero vs. 1.2±0.17 for control, n=6, \(p<0.001\)), or extracellular application of apyrase (2 \(\mu\)M\(^{-1}\)) that metabolizes ATP inhibited the occurrence of L wave under shear (zero vs. 1.8±0.58 for control, n=5, \(p<0.05\)). Our data suggest that longitudinal Ca\(^{2+}\) wave is triggered by type 2 IP\(_{3}\)R-mediated Ca\(^{2+}\) release that is activated by connexon-mediated ATP release and subsequent activation of P2Y\(_{1}\) receptor-PLC signaling in atrial myocytes under shear stress.


We thank Dr. Ju Chen for type 2 IP\(_{3}\)R knockout mice.

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

PC147

Study on peak expiratory flow rate and its relationship with anthropometric characteristics in medical students

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The values of pulmonary function tests are influenced by age, sex, height, weight, body surface area (BSA), posture and race. This study was aimed to assess Peak Expiratory Flow Rate (PEFR) and its relationship with anthropometric characteris-
tics of medical students in relation to gender variation. 120 students having the age range of 17 to 23 years were selected at random from Manipal College of Medical Sciences, Nepal. The subjects were divided into male (n=60) and female (n=60) groups. Height, weight and Chest circumference were measured from the subjects. PEFR was measured in a standing position using a mini Wright’s peak flow meter. Three satisfactory readings were taken. The highest of the three values (L/min) was used in analysis. Values are mean±SD, compared by t test. Data was analyzed using Statistical Package for Social Sciences (SPSS, version 16.0). Results showed PEFR [male, 495.17±63.60; female, 362.67±48.43, L/min, p=0.001], values significantly higher in male. The height [male, 171.85 ± 7.1 Vs female 157.0 ± 6.3, cm, p=0.001], weight [male, 67.00±10.6 Vs female 53.7±9.50, kg, p=0.001], chest circumference [male, 86.1±5.5 Vs female 78.5±6.6, cm, p=0.001], BSA [male, 1.79±0.15 Vs female, 1.52±0.14, m², p=0.001] were found lower in female. The correlation of height with PEFR [male, r=0.375, p=0.003; female, r=0.338, p=0.008] was found significant in both gender. The chest circumference and BMI had significant positive correlation with PEFR in the study group. It was concluded that anthropometric characteristics had an influence on PEFR.

**Anthropometric parameters of male (n=60) and female (n=60) students.**

<table>
<thead>
<tr>
<th>Anthropometric parameters</th>
<th>Mean ± SD (Male)</th>
<th>Mean ± SD (Female)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>171.8±7.1</td>
<td>157.0±6.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.0±10.6</td>
<td>53.7±9.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.6±2.9</td>
<td>21.75±1.7</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.79±0.15</td>
<td>1.52±0.14</td>
</tr>
<tr>
<td>Chest circumference (cm)</td>
<td>86.1±6.5</td>
<td>78.5±6.6</td>
</tr>
</tbody>
</table>

Correlation coefficient between PEFR and anthropometric characteristics in medical students.

<table>
<thead>
<tr>
<th>Anthropometric Characteristics</th>
<th>PEFR (Male)</th>
<th>PEFR (Female)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>0.375**</td>
<td>0.338**</td>
</tr>
<tr>
<td>Weight</td>
<td>0.375**</td>
<td>0.395**</td>
</tr>
<tr>
<td>BMI</td>
<td>0.267**</td>
<td>0.340**</td>
</tr>
<tr>
<td>BSA</td>
<td>0.417**</td>
<td>0.363**</td>
</tr>
<tr>
<td>Chest Circumference</td>
<td>0.360**</td>
<td>0.303</td>
</tr>
</tbody>
</table>

* Significant at p<0.05, ** Significant at p<0.01


We would like to express our special thanks to volunteers who participated as subjects in this study and Dr. Brijesh Sathian for his help in statistical analysis.

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

**Influence of stretch on transmural gradient in mechanical properties of single ventricular cardiomyocytes**

A. Khokhlova 1, 2, G. Iribe 3, O. Solovyova 1, 2, K. Naruse 3 and V. Markhasin 1, 2

1 Ural Federal University, Yekaterinburg, Russian Federation, 2 Institute immunology and physiology, Yekaterinburg, Russian Federation and 3 Okayama University, Okayama, Japan

It has been shown previously that subendocardial (ENDO) and subepicardial (EPI) cells of ventricular wall differ in their mechanical properties. The subendocardial myocyte displayed a longer time to peak contraction and delayed relaxation in different animal species (1, 2). Although cardiomyocytes are exposed to mechanical load in ‘in situ’ beating heart, these studies were performed using mechanically unloaded cells. The aim of our study was to investigate the differences in the responses to stretch (mechanical preload) between EPI and ENDO cells from mouse left ventricle (LV) during auxotonic contractions.

All studies were conducted according to UK legislation. EPI and ENDO ventricular myocytes were enzymatically isolated from hearts excised from C57BL/6 mice (aged 9-11 weeks) and stored in normal Tyrode solution. Each cell end was hold by a pair of carbon fibers to apply 3-5 % axial stretch to the cells (3). Cells were stimulated at 1 Hz at room temperature. All values are presented as means ± SEM. Student’s unpaired t-test and two-way ANOVA were used for statistical analysis.

To predict electromechanical mechanisms responsible for the differences, we utilized our mathematical EPI and ENDO cell models that describe transmural gradient between the cells in some ionic currents and myofilament contractile mechanisms (4).

Although there were found significant differences neither in stiffness (slope of end-diastolic force-length relationship, EDSFLR) nor in contractility (slope of end-systolic force-length relationship, ESFLR) between ENDO and EPI cells, ENDO cells tended to show steeper slope in ESFLR (ENDO: 0.43±0.08 nN/ mm², n = 28, vs EPI: 0.40±0.07, n = 22).

ENDO cells showed significantly longer time to peak contraction (Tmax) compared to EPI cells during auxotonic contractions at non-stretched state (ENDO: 125.3±5.0 ms, n = 26, vs EPI: 110.6±3.5, n = 20, p<0.05). We did not find significant differences in the time constant of relaxation (τ) [ENDO: 39.46±1.81 ms, n = 26, vs EPI: 39.55±2.32, n = 20] between the groups.

Stretch delayed Tmax in both groups while EPI cells showed significantly greater delay in the Tmax compared with ENDO cells, resulting in a smaller difference in Tmax at stretched state (ENDO: 129.8±5.6 ms vs EPI: 118.8±3.9).

Modeling results suggest that differences in the kinetics of cross bridges and calcium-tropinin C complexes assumed in ENDO and EPI models may essentially contribute to the differences in the load-dependency between the cells. The present results demonstrate that transmural gradient in the characteristics of cellular contractile profile decreases under stretch that may lead to synchronization of contractions of LV in the intact heart.


Supported by The Russian Science Foundation (#14-35-00005) and JSPS KAKENHI 2628212.

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

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First measurement of cardiac heat-stress relation at 37 °C achieved using a novel muscle calorimeter

C.M. Johnston1, J. Han1, B.P. Ruddy1,2, D.S. Loiselle1,3, P.M. Nielsen1,2 and A.J. Taberner1,2

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When cardiac muscle contracts and generates stress, heat is liberated. The heat-stress relation reveals two key indices relating to muscle performance. The intercept reflects the metabolic energy for removal of calcium from the cytosol and restoration of membrane potential, and hence is termed ‘activation heat’. The inverse of the slope of the heat-stress relation represents the energetic cost associated with each unit of stress development, and hence is termed ‘economy’. Despite over three decades of investigation, the heat-stress relation has never previously been reported at body temperature. Previous experiments have been performed below body temperature (typically 27 °C). In consequence, the heat-stress relation at 37 °C is still unknown. We aim to determine, for the first time, the heat-stress relation at 37 °C, and to compare it to that at 27 °C.

We designed and constructed a calorimeter (Johnston 2014), the principle of which is based on the differential temperature of solution superfusing isolated cardiac tissue. We used thermoelectric sensors (Johnston et al. 2014) to achieve a 5-fold increase in thermal resolution. To measure the heat-stress relation, threemonth old male Wistar rats were deeply anaesthetised with isofluorane (5 % in oxygen, administered via inhalation according to protocol approved by the University of Auckland Animal Ethics Committee). Trabeculae were dissected from the right ventricle following cardiectomy, mounted in the calorimeter and stimulated at 3 Hz. Once muscle stress reached steady state, the entire calorimeter system was enclosed in a light-proof, temperature-controlled, enclosure. Active muscle stress development was varied by changing stimulus frequency and muscle length. Heat was simultaneously recorded.

Heat was linearly correlated with stress for each of the six trabeculae studied. The heatstress regression lines were averaged, and the effect of temperature was examined, using the ‘random coefficient’ model (the regression coefficients of which are derived from ANCOVA using the statistical software package SAS). Values are means ± SEM, compared by ANCOVA. The heat-stress relation shifted down at the higher temperature. The change in slope was not significant (0.54 ± 0.07 to 0.60 ± 0.07), indicating no difference in the ‘economy’, but the ‘activation heat’ per twitch decreased from 3.5 kJ.m⁻³ ± 0.3 kJ.m⁻³ to 2.3 kJ.m⁻³ ± 0.3 kJ.m⁻³. These results reveal that the energetics of Ca²⁺ cycling is temperature-dependent, but that of crossbridge cycling in producing a unit of stress is not. In summary, we have made the first determination of the heat-stress relation of cardiac muscle at body temperature. The ability of our novel calorimeter to resolve tiny differences of temperature change at 37 °C promies better understanding of the energetics of cardiac muscle in health and disease.


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

PC150

Characterisation of apelin receptor agonists in clinically relevant human tissue

C. Read1, C. Fitzpatrick2, A. Gandhi1, R. Kuc1, P. Yang1, J. Maguire1, R. Glen3, R. Foster2 and A. Davenport1

1Clinical Pharmacology, University of Cambridge, Cambridge, Cambridgeshire, UK, 2Chemical Biology and Medicinal Chemistry, University of Leeds, Leeds, UK and 3Centre for Molecular Informatics, University of Cambridge, Cambridge, UK

Apelin is emerging as a key transmitter in the human cardiovascular system while infusing apelin causes beneficial vasodilation and cardiac inotropy in patients with heart failure. Pulmonary arterial hypertension (PAH) is a devastating disease with a poor prognosis. It is associated with constriction of blood vessels, right ventricular hypertrophy and ultimately heart failure. Whilst current therapies reduce vasoconstriction, they have no effect on the heart and more efficacious treatments are required. Loss of apelin signalling is observed in PAH and infusion of apelin is beneficial in animal models. Despite the efficacy and specificity of peptides, they are limited therapeutically by their half-life and bioavailability. Additionally, rapid receptor internalisation and short-lived responses are observed. Biased ligands that are protective through G-protein signalling but are not internalised by β-arrestin recruitment would be advantageous. We hypothesise that small molecule apelin agonists could replace the missing endogenous peptide to produce beneficial vasodilatation and an improvement in cardiac remodelling (hypertrophy) of the right ventricle. As many drugs fail in the clinic due to lack of efficacy at the native human receptor, we propose to test compounds at an early stage in clinically relevant human tissue.

The aim of the study was to characterise four small molecule compounds in radioligand binding assays using human heart and to measure agonist potency in cell based assays. Radioligand competition binding used [Glp scaffold]apelin-13 in homogenised human left ventricle obtained with ethical approval and informed consent. Experiments were performed in triplicate, over the concentration range 100nM-50pM, and the mean ± SEM percentage specific binding plotted against log concentration to obtain a pKᵢ, Cell-based β-arrestin recruitment, receptor internalisation and cAMP reduction assays (DiscoveRx) were also performed in triplicate. Data were analysed to obtain values of pD₂ (logEC₅₀) as a measure of potency.

The rank order of potency for all four compounds was identical in binding and β-arrestin recruitment, with internalisation following a similar order. In the cAMP assay, however, CMF-019 possessed approximately ten fold higher affinity than [Pyr] Apelin-13.

Some of these molecules, in particular CMF-019, show bias towards G-protein signalling over β-arrestin and hence would be advantageous in therapy. These results will, therefore, be evaluated.

181P
used to inform future chemical modifications to obtain better drug molecules.

<table>
<thead>
<tr>
<th>Compound</th>
<th>µM</th>
<th>µM-0.05%</th>
<th>µM-0.1%</th>
<th>µM-0.2%</th>
<th>µM-0.5%</th>
<th>µM-1%</th>
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</thead>
<tbody>
<tr>
<td>PC151</td>
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</table>

Table 1: Binding and functional data of small molecule apelin agonists

The British Heart Foundation

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PC151

Characterisation of atrial volume receptors in the rat

E.F. Lucking¹, F.C. Shenton², S. Pyner² and J.F. Jones¹

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Excess plasma volume is a significant contributor to the hypertensive state. Atrial volume receptors (AVR) are an integral part of the neural circuitry that maintains plasma volume homeostasis. Abnormal mechano-sensing or transduction altered inadvertently by drug therapy may contribute to cardiovascular disease such as hypertension and heart failure. However, the molecular basis for mechano transduction in AVRs has not been studied.

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However, the molecular basis for mechanotransduction in AVRs has not been studied. This study aims to characterize cardiac vagal AVRs by first developing a reliable preparation using an isolated innervated rat atrium. Single cardiac vagal sensory recordings were obtained whilst recording atrial contractile state. A neuroanatomical and functional study has commenced to determine which ion channels are expressed and required for action potential generation during physiological stimulation of these receptors.

Wistar rats (145±28g; n=3) were euthanised with a stunning blow to the head followed swiftly by cervical dislocation. The intact right atrium was immediately harvested and superfused with oxygenated (95% O₂, 5% CO₂) Krebs solution at room temperature. All branches of the right vagus were dissected away with the exception of the cardiac branch. Right atrial contractile force was recorded via a force transducer. Using a suction electrode, the thoracic vagus was probed for action potentials generated as a result of AVR mechanotransduction, as determined by probing the atrial wall with Von Frey filaments (1.6MN).

Proteins expressed in AVRs could provide novel drug targets for the regulation of cardiovascular homeostasis (1). Early experiments showed that introduction of the TRPV4 channel antagonist Ruthenium Red (50µM) inhibited action potential generation in response to mechanical probing via Von Frey filaments. Knowledge of the mechanism whereby the mechanical perturbation is transduced into a neural input to the brain will lead to a better understanding of how they contribute to normal cardiovascular control. It is anticipated that this novel preparation will contribute to understanding the physiology of AVRs.

All procedures described conform with the Health Products Regulatory Authority of Ireland and Physiological Society ethical requirements.


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PC152

The anti-arrhythmic role of β3 adrenoceptors

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We have previously shown that the negative inotropic response of the β3-adrenergic receptor (β3-ADR) specific agonist BRL37344 was associated with a reduced systolic [Ca²⁺]i and SR Ca²⁺-content ([Ca²⁺]SR) which may have resulted from the shortening of action potential duration (APD). This negative inotropic action of β3-ADR has been linked by other groups to Nitric Oxide (NO) signalling [1]. As β3-ADR signalling is considered to be cardio-protective against Ca²⁺-overload injury [2] we set out to investigate a possible anti-arrhythmic effect of β3-ADR stimulation.

Ventricular myocytes were isolated by enzymatic digestion of male Wistar rat hearts. Arrhythmic activity was determined from video images of 20-30 myocytes electrically stimulated at 1Hz with high concentrations of β1-ADR agonist. [Ca²⁺]i was measured in myocytes loaded using Fura-2 and APD using whole-cell patch-clamp recording. Data shown as number of hearts (number of experiments), mean ± S.E.M., analysed via one-way ANOVA, with a Tukey’s post hoc test. To determine the anti-arrhythmic effect of β3-ADR stimulation, arrhythmic activity was induced by the β1-ADR specific agonist dobutamine (1µM for 5 minutes). Pre-treatment of cells with BRL37344 (200µM for 5 minutes) resulted in a significant reduction in the percentage of cells displaying arrhythmic activity in dobutamine from 34.5 ± 2.7% vs 5.5 ± 2.6% (n=4 (8), p<0.0001).

To determine whether the reduction in arrhythmic activity by BRL37344 was due to a modulation of the increase in APD by dobutamine, APD at 30% (APD30), 50% (APD50) and 90% (APD90) were recorded. Dobutamine resulted in a significant lengthening of the APD30 from 13.97 ± 1.53ms to 23.99 ± 1.94ms (n=4-6 (15-16), p<0.01) and APD50 6.29ms in pre-treated cells (n=3-4 (14-22), p<0.05), indicative of an additional increase in SERCA-2A activity beyond the dobutamine-induced increase in activity.

2 The anti-arrhythmic role of β3 adrenoceptors

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Our data suggests that β3-ADR pre-treatment causes an additional increase in the activity of SERCA-2A beyond the level of β1-ADR stimulation alone. As a result calcium is more rapidly sequestered to the SR during relaxation, removing the burden of Ca extraction from the Sodium-Calcium exchanger (NCX), potentially reducing the inward sodium current during cardiomyocyte relaxation.


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Loss of Na1.5 expression contributes to ventricular arrhythmogenicity in RyR2-P2328S hearts

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Introduction: Mutations in the ryanodine receptor 2 (RyR2) calcium release channel are commonly associated with abnormal diastolic Ca2+ release and catecholaminergic polymorphic ventricular tachycardia (CVPT)1. The RyR2-P2328S mutation, first identified in a Finnish family, has additionally been linked to atrial arrhythmogenicity. A reduced conduction velocity and Na1.5 in association with reduced Na1.5 expression has been demonstrated to contribute to the arrhythmic phenotype in murine RyR2-P2328S (RyR2S/S) atria2. However, whether this reduced Na1.5 expression is also observed in the RyR2S/S ventricle has not been established. This study assessed whether a reduction in Na1.5 is also observed in the ventricle, particularly within the membrane fraction, and correlated this with measures of arrhythmogenicity, action potential restitution and conduction.

Methods: WT and RyR2S/S mice were killed by cervical dislocation (Schedule 1, ASPA 1986), their hearts rapidly removed, snap frozen in liquid N2, homogenised in resuspension and lysis buffers in the presence of protease inhibitors, separated by centrifugation to obtain whole tissue and membrane fractions, and run against suitable markers. Programmed electrical stimulation (PES), standard S1S2 and dynamic restitution protocols, applied to Langendorff-perfused hearts, provided measures of arrhythmic incidence, action potential duration at 90% recovery (APD90) and conduction latency. Data are means ± SEM or % expression relative to WT and were compared by unpaired students t-test.

Results: Na1.5 expression was significantly reduced in the ventricle of RyR2S/S hearts compared to WT by 20% within the whole tissue fraction and more notably by 40% within the membrane fraction (Figure 1). RyR2S/S hearts were more arrhythmic than WT hearts as previously shown3, ventricular effective refractory period was similar between RyR2S/S (33.50 ± 4.55 ms) and WT (39.38 ± 4.16 hearts), and the maximum slope of the APD90 restitution curve was increased in the RyR2S/S. While conduction latency was similar between RyR2S/S (24.36 ± 2.08 ms) and WT hearts (19.06 ± 1.12 ms, P > 0.05) at 8 Hz pacing, RyR2S/S hearts had a much higher conduction latency compared to WT at the fastest pacing frequencies attained just prior to refactoriness or arrhythmia (36.44 ± 1.76 and 26.52 ± 1.19 ms respectively, P < 0.05).

Discussion: Reduced Na1.5 expression in RyR2S/S ventricles contributes to the slowed conduction, abnormal repolarisation and increased arrhythmogenicity. This is in accordance with previous findings within atrial myocardium4.

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Dual block-face imaging of wax-embedded whole heart on a motorized rotary microtome for improved serial two-dimensional histology stack acquisition

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Introduction: Histo-anatomical structure of the heart is a key determinant of electrophysiological and mechanical cardiac function, and it affects their mutual interactions. Quantitative study of these interactions requires accurate knowledge of cardiac 3D structure across multiple scales, from sub-cellular levels to whole organ. Histological staining of sectioned tissue is the method of choice to provide high-resolution identification of cells and sub-cellular structures. However, 2D sections cannot easily be projected to 3D volumes, as they do not represent an inherently co-registered stack and show significant sectioning-induced deformations. Therefore, re-integration in 3D needs to be guided by additional data, such as magnetic resonance imaging (MRI) [1,2]. However, processing prior to cutting sections involves dehydration and embedding, which already cause significant changes in volume and shape of the wax-embedded sample, compared to the MRI images. This makes combining the data obtained with both methods challenging, so we introduced dual block-face imaging as an intermediate step in the pipeline for tissue reconstruction.

Method: Briefly, rat hearts were excised after Schedule 1 killing, according to the UK Home Office guidance on the Operation of Animals (Scientific Procedures) Act 1986, and swiftly perfused using Tyrode solution (in [mM]: NaCl 140; KCl 5.4; MgCl2 1; HEPES 5; Glucose 10; CaCl2 1.8; pH 7.4). Hearts were then arrested using high-potassium Tyrode, and fixed with fast-acting Karnovsky’s fixative. Hearts were embedded in wax, mounted on a Leica RM2255 microtome and sectioned at 10µm. Two images of the wax block surface were taken prior to each cut with Matrix Vision USB 3.0 cameras (mBluexFOX3): image 1 - surface-perpendicular projection, image 2 - Brewster angle projection via a linear polarization filter to obtain an image dominated by light reflected from surface areas that consisted of wax, not tissue. While the latter identified the precise pre-sectioning location of tissue contained in the next histological cut, the former corrects angular distortion to obtain true shape and location of the tissue. The mutual information guided subsequent 3D integration of digitally-imaged stained 2D stacks.

Discussion: 3D histological reconstruction of extended tissue volumes at sub-cellular resolution with cell-type identification requires combined imaging methods to enrich in vivo reference (MRI), via identification of pre-cutting sample shape (dual block-face imaging), and cell type identification (stained 2D histology sections). Our improved system using a motorised microtome allows higher throughput efficiency of the collection of dual block-face data sets, required for effective 3D reconstruction of 2D histology image stacks.


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Poster Communications

PC154

Relationship between Blood Pressure and Blood Glucose Among Rural Adults in Niger Delta Region, Nigeria

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The incidence of hypertension, diabetes and overweight-obese are emerging increasingly as health problems globally. Oil spills and gas flares pollutants/toxins, though perceived to have resulted in ever increasing polluted air, also contaminated water and soil (1-2), the impact on the physiological functions of the body are poorly understood. The objective of the study was to evaluate the prevalence and the possible relationship between hypertension, diabetes and overweight-obese and the tendency of developing prehypertension and pre-diabetes in rural adults in the Niger Delta region. A cross-sectional, population-based descriptive design was used. A total sample of 250 subjects from Erema / Obagi in Rivers State, aged 20 years and older, exposed in excess of 10 years, were recruited. Blood pressure and fasting blood glucose were recorded by standard methods, body mass index was calculated. Overall, mean value (±SD) of blood pressure (mm Hg), blood glucose level (mmol/L) and body mass index (kg/m²) for the participants were 122.2±18.1/75.1±11.8, 6.6±3.6 and 26.3±4.6 respectively. Pre-diabetes and diabetes prevalence respectively, were 9.8% and 12.0%. About 30.8% of the participants are pre-hypertensive and 36.4% are hypertensive The mean (±SD)body mass index for males 26.2±4.0 and females 26.4±4.8 fall in the range defined as overweight (p<0.05) . Distribution of blood glucose and blood pressure (%) revealed that 13% pre-hypertensive coexisted with pre-diabetes, whereas 2.4% pre-hypertensive and 18.2% hypertensive had diabetes. Correlation of blood glucose and body mass index showed that 19.6% pre-diabetes and 7.2% diabetes are overweight, while 16.8% diabetes are obese .The correlation between body mass index and blood pressure depicts that 15.4% pre-hypertensive are overweight, 1.4% hypertensive are overweight, 16.8% are both hypertensive and obese . Overall, the prevalence (%) of obesity 16.4, diabetes 24, hypertension18.2, coexisting prehypertension and pre-diabetes 35.0, pre-non-communicable disease 21.7 and main non-communicable disease 23.7 respectively. ANOVA analyses show that diastolic and systolic blood pressure was positively associated with blood glucose and body mass index (P<0.001). Correlation ratio analysis showed that systolic blood pressure and diastolic blood pressure are associated with blood glucose by 11 to 18 – fold. High blood pressure and elevated blood glucose was by 1 to 2- fold. Body mass index and blood glucose was 1-4 fold. High body weight was directly proportional with elevated blood glucose by 1:1 - fold. These complex interactions possibly provided the underlying complex mechanisms about the pathophysiology of hypertension-diabetes in humans and its complications. In conclusion, this study provides popula-
Quantitative characterization of cardiac tissue using confocal microscopy: New methods for sample mounting, image acquisition and image analyses

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Myocardial infarction (MI) is associated with apoptosis of cardiomyocytes (CM) and differentiation of cardiac fibroblasts (CF) into myofibroblasts (MF). The latter leads to an increased production of extracellular matrix (ECM) proteins and to scar formation [1]. In order to characterize cardiac tissues and their remodelling, we introduced new methods for mounting of samples as well as for image acquisition and 3D analysis of tissue microstructure at submicrometer scale. Our new approach for tissue mounting has two advantages: (1) avoiding morphology changes induced by ample mounting and (2) an increased penetration depth during imaging.

We obtained left ventricular tissue biopsies from an Institutional Animal Care and Use Committee approved rabbit MI model. Animals were anesthetized prior to surgery by IM injection of ketamine (50mg/kg) and xylazine (10mg/kg). Narcosis was maintained by isoflurane. Infarction was induced by ligation of the circumflex coronary artery as described in [3]. Additionally, fentanyl patches (25µg/h) were applied to all animals 24 h before until 48 h after the surgery. Animals were euthanized by IV injection of sodium pentobarbital (100mg/kg) before hearts were excised and tissue biopsies taken. After fixation and cryosectioning, we fluorescently labelled ECM, cell nuclei, α-smooth muscle actin (αsCMA), and vimentin (Vim). Specificity of these labels for identification of CF and MF is limited [4]. Thus, αsCMA antibodies label not only MF, but also cells in the wall of arterioles. Vim labels CF but also other cell types located adjacent to capillaries. We applied a new approach for embedding samples in a mounting medium without compression. In contrast to our prior approaches, probes were mounted on glass slides and sealed without additional cover glass slides. Scattering in the sample was largely removed by controlled desiccation in a humidity chamber before sealing. We acquired 3D images with a linear increase in laser power to compensate for depth-dependent attenuation. An algorithm for attenuation correction under conditions of linear power increase was developed and applied by fitting histogram based segmentation (mode + 2 standard deviations) was performed, followed by semi-automatic segmentation of CM and blood vessels using the water shedding algorithm [5]. Vessel lumens were dilated by 1 µm to reconstruct vessel walls. Positively stained for Vim, these were excluded from segmented CF and MF.

Our approach resulted in extended imaging depths and improved tissue morphology preservation, compared to conventional methods [5,6] (Fig.1B versus A). The prior approach led to compression of samples, which made it difficult to identify vessels and flattened CMs. With the new approach capillary lumens and laminae of connective tissue were identifiable and more realistic CM shapes were maintained. We conclude that our new approach allows for improved quantitative characterisation of cardiac tissue morphology.
Ursodeoxycholic acid decreases the activity of ENaC in normal and cystic fibrosis airway epithelia

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Cystic fibrosis (CF) is a disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) that result in reduced Cl− secretion and increased Na+ absorption, airway surface liquid (ASL) dehydration, decreased mucociliary clearance, infection and inflammation leading to lung injury. Cystic fibrosis patients often present bile acids in the lower airways, however the effects of bile acids on ASL and ion transport in CF airways are not known. Secondary bile acids, such as ursodeoxycholic acid (UDCA), have been shown to modulate immune responses (1,2) and epithelial ion transport (3). Here we investigated the effects of UDCA in normal and CF airway epithelial cell models.

NuLi-1 (normal genotype) and CuFi-1 (CF genotype, Δ508/Δ508) primary immortalized airway epithelial cells were grown under an air-liquid interface. Electrogenic transepithelial ion transport was measured as short-circuit current (∠) and epithelial ion transport (3). We here investigated the effects of UDCA in normal and CF airway epithelial cell models.

UDCA treatment reduced the amiloride-sensitive current (∠) across cell monolayers mounted in Ussing chambers. Data are presented as mean ± SEM and were statistically analysed by ANOVA or Student’s t-test, one way-ANOVA or two way ANOVA as appropriate. Differences were considered significant if P<0.05. After 6–7 weeks, heart/body weight ratio increased by 139% & fractional shortening fell by 50%. Internal ventricular systolic & diastolic dimensions increased by 58% & 22%, respectively. Recordings from conscious mice showed a 6% decrease in heart rate & 20% prolongation of QRS duration in the HF group. In anaesthetised mice, the PR interval was increased by 9% & QRS duration by 17% in the HF group. In isolated sinus node (SAN) preparations, cycle length was prolonged by 22% in the HF group. Superfusion of this preparation with 2 mM Cs (to block funny current, ∠) increased cycle length by 25% in the HF & 34% in the control mice. These data demonstrate dysfunction of the CCS consistent with changes in mRNA measured by qPCR, which showed significant downregulation of HCN4 (responsible for ∠) & Tbx18 (transcription factor). Expression of 384 miRs in the SAN was measured by microarray & compared to that in the left (LA) & right (RA) atrium. In the control group, 100 miRs showed significant differences in expression between the SAN & LA (47 were more abundant in SAN & 53 in LA), & 15 miRs showed significant differences in expression between the SAN & RA (11 were more abundant in SAN & 4 in RA). In HF there were 43 significant changes in miRs in the SAN. For example, miR-370-3p (using Ingenuity IPA software, predicted to affect HCN4) was upregulated by 81% in the SAN & 57% in the LA. To validate the effect of this microRNA on the SAN, miR-370-3p mimic was injected into SAN tissue preparations & maintained in culture medium for ~48 hours. miR-370-3p resulted in significant bradycardia 5 hours after injection (heart rate was 289 ± 5 bpm in SAN preparation injected with scrambled miR & 231 ± 24 bpm injected with miR-370-3p) & this effect continued & after 20 hours the heart rate was 284 ± 20 bpm in the control SAN preparations & 193 ± 18 bpm in the SAN preparations injected with miR-370-3p mimic. These data reveal that increased expression of endogenous miR-370-3p contributes to bradycardia associated with HF & suggest that it might be targeted therapeutically to restore function.

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Fundamental gating defects of sudden cardiac death-linked mutant cardiac ryanodine receptors determine Ca\(^{2+}\) release dynamics in cells

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Normal rhythmic contraction of the myocardium depends on the controlled release of Ca\(^{2+}\) from intracellular stores via cardiac ryanodine receptors (RyR2). Mutations in RyR2 are linked with arrhythmia and give rise to catecholaminergic polymorphic ventricular tachycardia (CPVT)\(^1\). A consensus view on the mechanisms of RyR2 dysfunction in CPVT proposes that mutant channels exhibit gain-of-function activity due, at least in part, to abnormal interactions with, or enhanced sensitivities to modulatory ligands\(^2\). We have previously described the gating of wild type (WT) human RyR2 channel in response to cytosolic Ca\(^{2+}\)\(^3\), resulting in a gating scheme, which includes a number of gating phenomena never before described for RyR2 and serves as a platform for modelling the effects of disease-linked mutations on the channel. In the present study, we used identical minimal experimental conditions (i.e. with \([K^+]\) at 210mM as the permeant ion, using purified recombinant human channels devoid of regulatory co-proteins) in order to characterize molecular defects in exemplar CPVT-linked mutant channels. Data are given as mean±SEM and compared using t-tests or ANOVA where appropriate. In contrast to WT channels and a central domain mutation (S2246L), the N4104K mutation exhibited a higher frequency of gating events in the absence of Ca\(^{2+}\). These data suggest that the N4104K mutation introduces intrinsic instability in the channel structure. This concept was explored further using Hidden Markov Modelling of N4104K gating data using QuB\(^4\) which revealed that this propensity for unliganded gating was likely due to an increased susceptibility of N4104K channels to enter a conformational state that favours subsequent opening. This was quantified as a decrease in the ratio of isomerisation between responsive and non-responsive closed states found in our model, \(E_{\text{m}}\). WT = 0.42±0.14 vs N4104K = 0.07±0.06 (n=2-6). Confocal imaging of spontaneous Ca\(^{2+}\) release events in fluo-3 loaded HEK293 cells expressing WT or mutant RyR2s demonstrated that both CPVT-linked mutations resulted in a gain-of-function with both S2246L and N4104K showing an increased duration of Ca\(^{2+}\) release event compared to WT (WT 7.97±0.25 s, vs SL 9.01±0.41 s, NK 10.52±0.61 s, p<0.05, n = 17-46). However, cells expressing these CPVT mutants exhibited markedly different inter-transient durations (decreased for NK 3.82±0.76 s, increased for SL 8.47±0.73 s vs WT 6.46±0.50 s, p<0.05, n = 17-46) compared to WT. The data suggest that a fundamental conformational defect gives rise to unliganded gating in N4104K channels which leads to an increased propensity for Ca\(^{2+}\) release in cellular systems. These data point to the likelihood that the generic assignment of CPVT-linked RyR2 mutations as ‘gain-of-function’ masks different mutant modes of RyR2 channel dysfunction.


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Reducing drug-induced cardiac arrhythmia risk through impairment of drug-ion channel interactions: An attenuating effect of macrolides?

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hERG channels mediating the rapid delayed rectifier K\(^+\) current (I\(_{\text{Kr}}\)) are important for normal ventricular repolarization (1). Various cardiac and non-cardiac drugs inhibit hERG channel function and thereby prolong the QT interval of the electrocardiogram; this carries a risk of potentially fatal torsades de pointes (TdP) arrhythmia (1, 2). Macrolide antibiotics such as erythromycin have been associated with cases of TdP, but this is usually against a background of other risk factors (3, 4). By contrast, very recent in vitro data suggest that pre-treatment with low concentrations of erythromycin (that produce little pharmacological blockade of hERG) may significantly antagonise - and therefore potentially protect against - the inhibitory effects of normally potent hERG blocking drugs (5). To date the therapeutic potential and the mechanism underlying this concept have not yet been established. Here we investigated the effect of a low concentration of erythromycin on the pharmacological sensitivity of hERG current (I\(_{\text{hERG}}\)) and native I\(_{\text{Kr}}\) to terfenadine, a potent inhibitor of the hERG channel. Whole cell patch clamp recordings were made of hERG/I\(_{\text{Kr}}\) tail currents elicited by a repolarising step to -40 mV following an activating step to +20mV either from HEK-293 stably expressing WT hERG or from rabbit ventricular myocytes isolated from the hearts of New Zealand White rabbits killed in accordance with UK Home Office legislation. Data are presented as mean ± SEM; statistical comparisons were made using an unpaired t-test. I\(_{\text{hERG}}\) and I\(_{\text{Kr}}\) tail were inhibited by 3 \(\mu\)M of erythromycin, a concentration previously reported to cause a substantial reduction in hERG sensitivity to terfenadine (5), by 15.3 ± 6.0 % (n=5 cells) and 28.2 ± 3.8 % (n=6 cells) respectively. Terfenadine sensitivity was then assessed in the absence and presence of 3\(\mu\)M erythromycin. 100 nM of terfenadine, a concentration close to the half-maximal inhibitory (I\(_{\text{50}}\)) value for I\(_{\text{hERG}}\) in our hands (95.7 ± 0.13 nM; n=4 to 6 for each of four concentrations), produced an inhibition of ventricular I\(_{\text{Kr}}\) of only 17.6 ± 3.5 % (n=7 cells; p<0.01 versus hERG). Finally, the effect of 100 nM terfenadine was tested following a preincubation for 15 min of the cells with 3 \(\mu\)M erythromycin. In the maintained presence of 3 \(\mu\)M erythromycin, I\(_{\text{hERG}}\) was inhibited by 54.3 ± 12.4 % (n=7) [vs 47.6 ± 6.1 % in absence of erythromycin, n=10, NS] and I\(_{\text{Kr}}\) by 10.9 ± 4.3 % (n=5) [vs 17.6 ± 3.5 %; n=7, NS]. These data indicate that, under the conditions of this study, exposure to a low concentration of erythromycin did not significantly alter the extent of inhibition of either I\(_{\text{hERG}}\) or I\(_{\text{Kr}}\) by 100 nM terfenadine.


Reducing drug-induced cardiac arrhythmia risk through impairment of drug-ion channel interactions: An attenuating effect of macrolides?


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

PC161

The use of colour-coded three dimensional printed models of a congenital heart defect to assist surgical planning for heart transplantation

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Purpose - We created a 3D printed colour-coded model of a complex CHD to aid visualisation of the aberrant anatomy to enhance surgical planning for transplantation for end stage heart failure. The anatomy was of a 41-year old patient with right atrial isomerism, dextrocardia, left inferior and superior vena cava to a single atrium, single ventricle with right ventricular morphology, double outlet right ventricle with transposition of the great vessels and total anomalous pulmonary venous drainage to the superior vena cava.

Introduction - In the past decade, 3D printing has demonstrated a role in medical research and pre-operative planning. Studies have shown that patient-specific models of CHDs are a valuable aid to surgical planning (Ejaz et al., 2014). Due to the wide variation and complexity associated with CHDs, 3D visualisation of the aberrant structures based on the 2D images can prove challenging.

Methods - Imaging: The data were obtained using a Somaton Definition AS+ 128 slice scanner. A cardiac gated contrast enhanced Commuted Tomography study was performed at 120kV with automatic mAs modulation. Images were acquired in 0.6mm slices and reconstructed using a Siemens Syngo system. The image matrix size was 512 x 512. All data were anonymised in keeping with local ethical guidance.

Segmentation: A medical expert proficient in segmentation in conjunction with a Consultant cardiac Radiologist performed image analysis. Using open source 3D Slicer software, the structures of interest were segmented. The segmented images represented the bounding lumen of the heart and Great vessels. The generated 3D model was then exported as a stereolithographic (STL) file and further model optimisation was performed using Meshlab.

Processing: Z Edit Pro was used for mesh editing and to attribute colours to the structures. The design process took 8 hours with 1 hour for attributing colours. This was then printed using a Z Print 250 binder jetting printer in 6 hours and 5 minutes. Post processing involved oven curing to improve model strength before an epoxy infusion system was applied to allow intensive model manipulation without fear of breakage.

Results - We produced a colour-coded patient-specific model which represented the lumen of the heart and great vessels. This model was qualitatively verified in conjunction with the CT images by the Consultant Radiologist. The model was utilised immediately preoperatively and was considered very helpful. The surgical team felt that the model was of use, particularly in the setting of dextrocardia. The colour-coding provided additional benefit.

Conclusion - We suggest that colour-coded models of CHDs should be utilised as an adjunct to anatomic study and surgical planning. Surgeons operate in a 3D world relying on 2D imaging for preoperative planning; 3D models may aid understanding of complex anatomy.
Methods: Marrow was extracted from adult Wistar rat hindlimbs (n=4) immediately following sacrifice by cervical dislocation. BMCs were isolated by centrifugation then suspended at 107 cells/ml in 2x50μl gadolinium (Gd) doped alginate for injection. Myocardial infarction was induced by ligation of the left anterior descending artery, for surgery and imaging rats were anaesthetised by inhalation of isoflurane and oxygen (2% / 2%). BMCs were either directly injected into the ischemic region during surgery (n=3) or injected closed chest using ultrasound guidance at 7d after infarction (n=3) (fig.1). MRI was performed 3d after injection. A cine-stack covering the left ventricle (LV) (0.4x0.4x1.5mm;TE/TR 1.2/5ms) was acquired to measure LV properties. A pre-Gd inversion recovery (IR) sequence located Gd-doped hydrogel (0.27x0.27x1.5mm; TE/TR 1.6/3.9). After intraperitoneal injection of 0.5mmol/kg Gd-DTPA a 2nd post-Gd IR sequence was acquired to measure infarct size. A tag-cine sequence measured regional contraction (0.2x0.2x1.5mm).

Results: Gd-doped bioscaffold was visible pre-Gd, demarcating the region of gel administration (fig.2a). Post-Gd enhanced the ischemic myocardium demonstrating good co-localisation of the therapy and the damaged tissue (fig.2b). Combining this with tag-cine MRI permits the presence of grafted cells to be correlated with changes in regional strain, determining the local effect of therapy on myocardial contractility (fig.2c). Although this study is not currently powered to identify changes in function, the data in table 1 suggest improvement in treated hearts.

Conclusion: This work establishes a method for assessment of BMCs as a regenerative cardiac therapy using MRI. This imaging strategy has applications in evaluation of other cell transplantation regeneration therapies, where the impact of bioscaffolds on their local myocardial environment is not well known.

Global cardiac function:

<table>
<thead>
<tr>
<th></th>
<th>Infarct to treatment</th>
<th>Infarct + BMC’s</th>
<th>Chronic infarct + BMC’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejection Fraction (%)</td>
<td>59.3</td>
<td>73.1</td>
<td>67.24</td>
</tr>
<tr>
<td>LV mass (mg)</td>
<td>608±22</td>
<td>799±17</td>
<td>716±23</td>
</tr>
<tr>
<td>Stroke Volume (μl)</td>
<td>2852±76</td>
<td>3990±92</td>
<td>4122±45</td>
</tr>
<tr>
<td>Infarct mass (mg)</td>
<td>1056.30</td>
<td>61325</td>
<td>77310</td>
</tr>
<tr>
<td>Infarct volume/LV</td>
<td>15.72±5.0</td>
<td>8.53±3.3</td>
<td>10.98±1.2</td>
</tr>
<tr>
<td>HUdoped volume/(%Infarct)</td>
<td>29±14</td>
<td>29±14</td>
<td>21±17</td>
</tr>
</tbody>
</table>

Image 1 - Snapshot from ultrasound guided injection. Red line parallel to needle and green arrows define the epi/endocardial border.

Images 2–4 - Representative images from chronic infarct + treated rat. The pre contrast image shows the location of Gd-doped and cell enriched alginate (a) while the post Gd image defines the infarcted myocardium (b) strain analysis shows positive strain (tissue stretching) at infarct (c).

Funded by MRC

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In silico study of the effects of hERG-linked short QT syndrome on the electrical and mechanical activities of human atrial cells

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Atrial fibrillation (AF) is a common clinical presentation of the short QT syndrome (SQTS). Despite its prevalence, the link between gene mutations underlying the SQTS and increased susceptibility to AF remains unclear. This study aimed to investigate the functional impact of the SQT1-related N588K gain-of-function mutation to human Ether-à-go-go-Related Gene (hERG) potassium channels on the electrical and mechanical activities of human atrial cells.

Multiple contemporary human atrial action potential model (e.g. Colman et al. 2013) were coupled to the Rice et al. (2008). A Markov chain formula of the rapid delayed rectifier current, Ikr, (the α subunit of which is encoded by hERG) was implemented in both wild type (WT) and N588K conditions, based on whole-cell patch clamp recordings from CHO cells performed at 37°C (McPate et al., 2005). The effect of the mutation on action potential duration (APD), peak current density, intracellular calcium transient, and contractile force was evaluated both with and without inclusion of a stretch-activated current.

Inclusion of the new formulation of Ikr, was validated by a complete block of this channel-current, reproducing the proportional APD prolongation observed under experimental Ikr blocker (E-4031) conditions (Wettwer et al., 2004). The SQT1-related N588K mutation was found to increase peak Ikr current density by ~2-fold, in agreement with experimental findings (McPate et al., 2009), which served to significantly accelerate atrial repolarisation, reduce the APD and stabilise re-entrant circuits in tissue. Secondary effects of the mutation resulted in a decreased calcium transient amplitude, consequently reducing the contractile force. This effect was reduced when stretch-activated channels were included in the model. Both the significant acceleration in atrial action potential repolarisation and the modest impairment in contractile function could have important implications for atrial electro-mechanical function and provide insight into the mechanisms underlying the relationship between SQT1 and AF.


This project was funded by the British Heart Foundation (FS/14/5/30533)

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Reproducibility of normalised five minute heart rate variability recordings in healthy subjects

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Heart rate variability (HRV) is increasingly being used to assess the autonomic regulation of the heart. Altered HRV is a risk factor for adverse cardiovascular events, including sudden cardiac death [1]. One of our concerns was investigators using one five-minute ECG recording to measure baseline HRV and then comparing it with an intervention [2, 3]. We have previously shown a significant decrease in heart rate over three occasions [4]. This finding has gained importance in light of recent literature showing that heart rate can affect HRV; its influence can be removed through normalisation [5]. Our aim was to investigate whether there was any effect of repetitive testing on normalised HRV in healthy subjects.

65 healthy subjects volunteered to undergo three consecutive five-minute HRV measurements. Subjects were all staff and students of the Arabian Gulf University (AGU). The study was given ethical approval by the Research and Ethics committee, College of Medicine & Medical Sciences, AGU. All participating subjects gave informed and signed consent. Subjects were supine and each test was separated by a three minute time period. An ECG lead was attached to each limb. LabChart software and a PowerLab were used for data acquisition. The results were analysed by repeated measures ANOVA. P < 0.017 was considered as significant.

The subjects’ mean (± S.D.) age was 25.9 ± 11.1 years. Over the three tests, mean heart rate showed a significant decrease for the 1st vs. 2nd (p=0.00004) and 1st vs. 3rd test (p=0.00003). There was no difference between the 2nd vs. 3rd test (72.88 ± 10.1 beats min⁻¹ vs. 72.37 ± 10.0 beats min⁻¹; p=0.35).

With respect to non-normalised data, two parameters were significantly different. These were an increase in the low frequency power in normalised units over all occasions (LFnu; p=0.0017) and an increase in the non-linear parameter, standard deviation along the line of identity from the Poincare plot (SD2; p=0.009). After normalisation of the complete data re-analysis, only one frequency domain, high frequency power in normalised units over all occasions (HFnu; p=0.0037) showed a decrease over all occasions.

The most important finding from this study is that normalisation of our data radically alters the study results. The only factor for adverse cardiovascular events, including sudden cardiac death [1]. One of our concerns was investigators using one five-minute ECG recording to measure baseline HRV and then comparing it with an intervention [2, 3]. We have previously shown a significant decrease in heart rate over three occasions [4]. This finding has gained importance in light of recent literature showing that heart rate can affect HRV; its influence can be removed through normalisation [5]. Our aim was to investigate whether there was any effect of repetitive testing on normalised HRV in healthy subjects. After normalisation, only HFnu showed a fall, reflecting a decrease in parasympathetic stimulation. We suggest that HRV investigators may need to make sure they have a stable baseline before giving subjects any interventions.


Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Sympathetic nerve activity is associated with decreased grey matter volume in men with essential hypertension

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Background and aim: Hypertension is the leading modifiable risk factor for the development of cardiovascular-related cerebral events (including ischaemic stroke and cerebral small-vessel disease)[i]. Hypertensive humans have lower cerebral blood flow compared with normotensives, which may contribute to the pathophysiology of the disease, particularly grey matter loss [ii][iii]. Lower cerebral blood flow may trigger reflex activation of sympathetic nerve activity (SNA); a hallmark of essential hypertension [iv]. The level of SNA may therefore reflect the likelihood and severity of cerebral events occurring in hypertensive individuals. Here we sought to investigate the association between cerebral tissue volumes (grey and/or white matter; GM, WM) and the level of SNA in hypertensive vs. normotensive humans.

Methodology: Muscle SNA (MSNA) was measured using peroneal microneurography in 16 healthy, normotensive individuals, and in 25 otherwise healthy, patients with hypertension (treated and untreated mixture) [(mean age: 42±4 and 54±2 years respectively, p<0.001) (mean BMI: 23.9±2 and 29±1 respectively, p<0.001)]. Tissue volumes were measured using T1-weighted 3T-Magnetic Resonance (FSPGR) Imaging of the brain. Total GM and WM volumes were established using automated segmentation software (FIRST, FSL) and normalised as a % of total intracranial volume. Comparisons of age, BMI and blood pressures between the normotensive and hypertensive cohorts were analysed using an unpaired student’s T-test. Associations between MSNA and cerebral tissue volumes were analysed using non-linear multivariate regression, controlling for age and BMI. Values are Mean ± S.E.M.

Results: Office systolic blood pressure (SBP) and mean arterial pressure (MAP) were higher in hypertensives (134±10 mmHg vs. 122±9 mmHg respectively) vs. normotensives (122±8 vs. 92±9 mmHg respectively) (p<0.01). In hypertensive, but not normotensive men, MSNA incidence (bursts/100 heart beats) and frequency (bursts/minute) were both negatively correlated with the ratio of GM to WM (p=0.002, r=0.001 and p=0.009, r=0.002 respectively). In normotensive and hypertensive women, no significant relationship between MSNA and cerebral tissue volume could be established (p>0.05).

Conclusions and interpretations: In hypertensive men, MSNA levels negatively correlate with the ratio of GM to WM volume.

This is not observed in normotensive men. Interestingly, this relationship appears to be sex-specific. We suggest that lower cerebral blood flow in hypertensive men may cause GM loss and subsequent reflex elevations in MSNA in order to aid perfusion. It is therefore possible that the level of MSNA reflects the severity of disease progression, and target organ damage in males with essential hypertension. Those with higher MSNA may therefore have a cerebral-vascular component driving their hypertension.


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MEFs (25.69±2.47 hrs; n=4). In HL-1-6 cells, Per2 and Bmal1 mRNA exhibited anti-phasic expression similar to MEFs. Per2 mRNA levels and rhythmicity were maintained in HL-1-6 cells in control and ATXII. However, ATX II caused a 12 hrs phase shift in the ΔCT Per2 peak gene expression when compared with control. At ZT 18 Per2 gene expression was significantly higher in ATXII treated vs control cells (Control ΔCT 7.89±0.16 vs ATX ΔCT 9.13±0.26; n=3; p<0.01). Similarly, at ZT 12 Bmal1 gene expression was significantly higher in ATXII treated versus control cells (Control ΔCT 7.57±0.21 vs ATX ΔCT 8.70±0.34; n=3; p<0.01).

This study showed that in cultured atrial myocytes, Per2 and Bmal1 genes are endogenously expressed and exhibit 24 hrs circadian rhythm under control conditions. However, with ATXII, a significant increase in Per2 and Bmal1 gene expression levels were observed at a given ZT. Also, ATXII caused a 12 hrs phase shift in Per2 gene expression. In conclusion, this study provides a novel correlation between ATXII induced atrial arrhythmias and Per2 and Bmal1 gene rhythmicity patterns, indicating a role for clock genes in atrial arrhythmogenesis.


We would like to thank Dr E Dupot for providing us with the HL-1-6 clone.

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**PC168**

**Lung function in children with sickle cell disease receiving either chronic transfusion or hydroxyurea**

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Children with sickle cell disease (SCD) can develop pulmonary complications, such as Acute Chest Syndrome, which are associated with high mortality rates. In addition, their lung function deteriorates with increasing age, with an increasingly restrictive picture developing. Certain SCD patients receive either chronic transfusion or hydroxyurea in an attempt to reduce SCD complications, but their effects on lung function are not known. A cross sectional observational study was undertaken. Thirty-three control children not receiving treatment, 22 children receiving chronic transfusion and 11 children receiving hydroxyurea therapy were recruited. Spirometry was carried out to measure forced vital capacity (FVC), Forced Expiratory Volume in 1 Second (FEV\(_1\)), FEV\(_1\)/FVC ratio and Forced Expiratory Flow between 25 and 75 percent of the forced vital capacity (FEF25-75). Whole body plethysmography was carried out to assess Total Lung Capacity (TLC) and Residual Volume (RV). Single breath gas transfer was used to measure the Diffusion Lung carbon monoxide (DLCO) and the transfer coefficient carbon monoxide (KCO) and impulse oscillometry was used to measure resistance. The results were expressed as the percentage predicted for age and height using specific reference ranges.

Chronically transfused patients had a higher median FVC (94.0, (72.9-142.1)%predicted) compared to the controls (89.0, (58.2-135.6)%predicted), (p=0.014) and a higher median TLC, (95.6 (71.6-135.2)%predicted), compared to the controls (86.4 (63.1-113.8)%predicted), (p=0.022). The DLCO was higher in the hydroxyurea treated patients, 102.7 (79.2-138.3)%predicted), compared to untreated controls, 89.1 (64.9-122.0)%predicted), which only reached statistical significant on multivariate analysis (p=0.0083).

In conclusion, these results suggest that chronic transfusion might at least slow the decline in lung function in SCD children. The greater DLCO in the hydroxyurea treated patients may be due to the increase in fetal haemoglobin and higher oxygen carrying capacity compared to the SCD controls.


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**PC169**

**Changes in expression of inward rectifier potassium channels and microRNAs in cardiac hypertrophy**

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Cardiac hypertrophy carries a risk of heart failure and sudden cardiac death, and is a growing health problem because of the increasing prevalence of hypertension in the aging population. MicroRNAs show great promise as therapeutic agents and targets in disease treatment. Maximising their therapeuetic potential for heart disease requires an understanding of the effects of microRNAs on target genes that influence cardiac function. Important among these are inward rectifier potassium channels that are downregulated in cardiac hypertrophy, increasing the risk of sudden death from cardiac arrhythmia. Reduction of inward rectifier current enhances delayed afterdepolarisations, thereby increasing the risk of extrasystoles and consequent fatal ventricular arrhythmias. Several cardiac microRNAs are known or predicted to target cardiac inward rectifier genes ( KCNJ2 and KCNJ12), and therefore may be useful therapeutic targets, especially at early stages of the disease. Using next-generation sequencing, we aimed to determine the changes in these cardiac microRNAs in spontaneously hypertensive, heart failure prone (SHHF) rats at 8 months of age, when cardiac hypertrophy is at an early stage and inward rectifier current is reduced. Small RNA was isolated from the left ventricles of SHHF and Wis-tar-Furth (WF, control) rats. cDNA libraries were prepared with separate barcodes, combined, and sequenced on an Illu-
The effects of upper airway obstruction on sternohyoid muscle PO$_2$ in the anaesthetised rat

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Intermittent hypoxia is a hallmark feature of the debilitating sleep-related breathing disorder - obstructive sleep apnoea (OSA). OSA patients experience intermittent cycles of hypoxia and reoxygenation due to recurrent apnoea throughout sleep. Upper airway muscle dysfunction is implicated in the pathophysiology of OSA. Indeed, recurrent exposure to hypoxia, often employed in animal models of OSA, has been shown to drive aberrant muscle remodeling, with redox remodelling postulated as the underlying mechanism. The effects of intermittent occlusions with concomitant hypoxia on tissue oxygenation within upper airway muscles has not been explored to date.

In the present study, we sought to determine sternohyoid muscle (upper airway dilator) partial pressure of oxygen (PO$_2$) in normoxia and to characterise the dynamic tissue response to arterial oxygen desaturations associated with upper airway occlusions in anaesthetised rats.

Adult male Sprague-Dawley rats (n=7) were anaesthetised (urethane 1.5g/kg; 20% w/v; i.p.) and exposed to intermittent tracheal airway occlusion trials. Respiratory airflow and arterial blood pressure were measured. Arterial oxygen saturation (SaO$_2$, %) was measured via a pulse oximetry (sensor STARR Life™) placed on the hind paw, whilst the sternohyoid PO$_2$ was...
measured using an oxygen sensor probe (Oxford Optronix™) inserted directly into the sternohyoid muscle. Sternohyoid muscle PO₂ under normoxic conditions was 41 ± 3 mmHg (mean ± SEM, n=7). SaO₂ and PO₂ were significantly positively correlated; Pearson correlation r=0.4350, p<0.0001, n=7. Upper airway occlusion led to significant decreases in sternohyoid muscle PO₂ at all target SaO₂ desaturations: 60%, 70% and 80%, 20±3 mmHg, 25±2 mmHg and 30±2 mmHg, respectively; r=0.0001; one-way ANOVA. There were differences in the temporal relationship comparing muscle PO₂ and SaO₂ responses with significantly slower latency to nadir and recovery consistently observed in muscle PO₂ compared with SaO₂. This is the first report of sternohyoid muscle PO₂ during normoxia and hypoxia. We have demonstrated and quantified sternohyoid muscle hypoxia in response to arteriovenous desaturations that are characteristic of animal models of intermittent hypoxia modelling human OSA. We reason that tissue hypoxia characterised in our study underpins functional plasticity reported in previous studies exploring the effects of intermittent hypoxia on upper airway muscle physiology. Extending our study to a chronic model of upper airway occlusion/intermittent hypoxia to characterise and quantify respiratory muscle PO₂ may help inform studies exploring the mechanisms driving intermittent hypoxia-induced muscle remodelling.

Physiology Department UCC

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The interaction between prenatal smoke exposure, infection and environmental temperature on neonatal rat breathing

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Background: Sudden Infant Death Syndrome (SIDS) is one of the most common causes of post-natal infant mortality in the developed world. Identified risk factors for SIDS include prenatal cigarette smoke exposure, hyperthermia and infection, but the mechanisms underlying SIDS remain elusive. Objective: To examine if prenatal cigarette smoke (CS) exposure, lipopolysaccharide (LPS)-induced infection and environmental temperature interact to alter cardio-respiratory outcomes of neonatal rats before, during or after hypoxia. Methods: During the gestational period dams were treated daily with CS. Sham treatments were performed in parallel. Offspring were studied at postnatal day 6-8 (11-20g). These neonates were examined under both thermoneutral (33°C) and hyperthermic (38°C) conditions. Within each group, rats were allocated to control, saline or LPS (200µg/kg I.P.) treatments. Cardio-Respiratory pattern was examined using head-out plethysmography and ECG before, during and after hypoxic stress (10% O₂). Body surface temperature was monitored throughout the experiments

Results: Two hours post LPS administration, body temperature was not different compared to sham, in rats studied at 33°C (P=0.9995; One-way ANOVA) and 38°C (P=0.8272; One-way ANOVA); n=10-13.

Heart rate (HR) was analysed using 3-way ANOVA (smoke x LPS x x LPS temperature; n=10-13), during last minute of baseline, hypoxia and post hypoxic periods. Prenatal CS did not significantly alter HR by itself (P=0.18) nor did it interact with infection (P=0.88) or high temperature (P=0.61). Hyperthermia increases HR during normoxia (P<0.0001) and even further during hypoxia (P=0.0001). Mild infection in a high temperature environment increases HR during normoxia (P=0.043) and blunts normal hypoxic response (P=0.033). In the post hypoxic period, neonatal rats at 33°C had significantly lower HR than their initial baseline rate, compared to those rats at 38°C (P<0.001).

Under thermoneutral, but not hyperthermic conditions, there was an increased number of apnoea’s in prenatal CS exposed rats compared to sham in the post hypoxic period (P=0.0015; two way ANOVA smoke x LPS; n=10-13), LPS had no effect on apnoea count in thermoneural (P=0.8219) or hyperthermic conditions (P=0.6559).

Under high temperature conditions minute ventilation in LPS-treated neonates was significantly higher than saline treated rats post hypoxia (P=0.0214; two-way ANOVA smoke x LPS; n=6).

Conclusion: Multiple risk factors may interact in a hyper additive manner (eg. increased heart rate during high temperature in animals with mild LPS-induced infection). It is important we understand these interactions in the context of SIDS. However, it is clear that environmental risk factors predominately increase work rate of cardio-respiratory system in the neonatal rat. This may lead to overburden and instability of the system.

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Serum levels of interleukin-8 and intercellular adhesion molecule-1 as biomarkers for hidden fibrotic processes in moderate chronic obstructive pulmonary disease with comorbid cardiovascular pathology

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Chronic obstructive lungs disease has been describing as a disease, which has plenty of comorbidities, including cardiovascular diseases. It was established, that development of cardiovascular disease in COPD is supported with system inflammation, underlining the role of non-specific proinflammatory cytokines and assessing of chronic inflammatory reaction associated with activation of macrophage and endothelial cells in the lung [1]. Interleukin -8 is an important activator and chemoattractant for neutrophils. The accumulation of inflammatory leukocytes in the lung is a hallmark of pulmonary inflammation. After stimulation by inflammatory mediators, endothelial cells are able to express leukocyte adhesion molecules such as intercellular adhesion molecule -1 [2]. We investigated intercellular adhesion molecule-1 and interleukin-8 among patients with COPD and comorbid cardiovascular pathology. The Ethics Committee of the Institute approved the design of the present research. In addition, informed consent was signed prior to patient enrollment. 34 patients with ischemic heart disease and chronic obstructive lung disease
Hydrocortisone acutely reduces cardiovagal baroreflex sensitivity and heart rate variability

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Acute stress is associated with increased cardiovascular risk (1). The autonomic nervous system and hypothalamic-pituitary-adrenal axis are activated during stressful events and result in an acute surge in cortisol. In rats, glucocorticoid administration acutely reduces cardiovagal baroreflex sensitivity (2). It is not presently known whether this occurs in humans, but this is important to ascertain as it may contribute to the acute stress-induced increase in cardiovascular risk. To determine the effects of hydrocortisone on cardiovagal baroreflex sensitivity in healthy humans, 10 healthy males (mean±SD 29±5 yrs, 23±3 kg/m²) received a single intravenous bolus of hydrocortisone (200 mg) and placebo (saline) one week apart according to a randomized single-blinded cross over design. Three hours after hydrocortisone administration heart rate (HR) and blood pressure (BP) were recorded at rest and during sequential sodium nitroprusside and phenylephrine infusion (modified Oxford technique, MOT). Cardiovagal baroreflex sensitivity was determined from the slope of the systolic BP versus R-R interval during the phenylephrine induced rise in BP. Heart rate variability was assessed using the square root of the mean of the sum of the differences between successive R-R intervals (rMSSD) to provide an index of cardiac parasympathetic activity. Statistical differences were determined using a one way ANOVA (serum cortisol) and paired t test (HR, BP, cardiovagal baroreflex sensitivity, rMSSD). Non-parametric data was transformed. A P value of less than 0.05 was considered statistically significant.

Serum cortisol increased 3 hours after hydrocortisone administration (mean difference±SD 1649±623 nmol/L, p<0.05; hydrocortisone vs. placebo). Compared to placebo, resting HR (+7±4 beats/min; p<0.001) and systolic BP (+5±2 mmHg; p<0.05) were increased, whilst cardiovagal baroreflex sensitivity (-10±2 ms/mmHg; p<0.05) and rMSSD were decreased (-25±21 ms, p<0.05) 3 hours following hydrocortisone infusion.

Our data show that cortisol administration acutely reduces cardiovagal baroreflex sensitivity and cardiac parasympathetic activity, possibly via a central mechanism. These findings suggest that the acute surge in cortisol accompanying stress may result in adjustments of cardiac autonomic control that could contribute to an increased cardiovascular risk.

This study was supported by a grant from Arthritis Research UK (grant number 196633). We would like to acknowledge Jacqueline Smith for biochemical analysis.

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

Hydrocortisone acutely reduces cardiovagal baroreflex sensitivity and heart rate variability (PC174)

Genetic basis of arrhythmogenesis in hypertrophic cardiomyopathy patients

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Aims: Hypertrophic cardiomyopathy (HCM) mainly results from autosomal-dominant inherited single heterozygous mutations in cardiac sarcomere genes. Arrhythmia such as ventricular tachycardia and fibrillation is one of the main causes of sudden death especially in young HCM patients. Genetic background contributions to arrhythmogenesis in HCM patients were investigated.

Methods: One hundred and thirteen HCM probands with both clinical diagnosis and positive gene screening results were investigated. Ninety-six 96 cardiac relative genes exon and boading intron were analysis using second-generation sequencing. The identified mutations were confirmed by bi-directional Sanger sequencing and 300 healthy controls.
Results: The results showed the incidence of male was higher than female (1.69:1). Ten patients were less than 19 years old, 8.08%. The 40-49 years old group took 35.4%. Twenty five patients (22.1%) carried single mutation, 88 patients (77.9%) carried two or more mutations. In 96 gene tested, mutations were identified in 69 genes. The detection rate was >5% in 9 genes, including TTN (61.95%), MYH7 (24.78%), OBSCN (23.89%), MYBPC3 (23.01%), ANK2 (8.85%), RYR2 (7.08%), SCN5A (7.08%), AKAP9 (5.31%), DMD (5.31%) and DG2 (5.31%). Fifty nine (52.21%) patients were found carried cardiac arrhythmic relative gene mutations. Thus, 20 patients (17.70%) carried long QT syndrome related genes. Others included Brugada syndrome 17 (15.04%), short QT syndrome 7 (6.19%), atrial fibrillation 5 (4.42%), sick sinus syndrome 10 (8.85%), catecholaminergic polymorphic ventricular tachycardias 9 (7.96%), and arrhythmogenic right ventricular cardiomyopathy 19 patients (16.81%) respectively. Then a three generation family with four missense mutations was intensively investigated. These were two novel MYH7-H1717Q and MYLK2-K324E mutations accompanied by the KCNQ1-R190W and TMEM70-I147T mutations. The proband carried all four mutations with dual HCM and LQT1 phenotypes. Five subjects carried two mutations. II-1 only carried TMEM70-I147T. Left ventricle mass indexes in MYH7-H1717Q carriers were significantly higher than in non-H1717Q carriers (90.05±7.33g/m², 63.20±4.33 g/m² respectively; P<0.01). Four KCNQ1-R190W carriers showed QTc intervals that were significantly more prolonged than those in non-R190W carriers (472.25±16.18 ms and 408.50±7.66 ms respectively, P=0.05). All MYLK2-K324E carriers showed inverted ECG T waves. The subject with only a TMEM70-I147T mutation showed normal ECG and echocardiographs suggest that this had less pathological effects at least in this family.

Conclusion: Multi gene mutations contribute HCM phenotypes. Arrhythmic associated gene mutation might be the genetic background for rhythmogenesis in HCM patients.

Poster Communications

PC176

Epigenetic regulation of development of cardiac conduction system (sinoatrial node)

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microRNAs (miRNAs) are post-transcriptional regulators of gene expression that have been shown to play an important role in the establishment of cellular programs. The heart develops from two small patches of cells in the mesoderm, the heart fields, which originate the different cardiac cell types, including cardiomyocytes, vascular smooth muscle and endothelial cells. These progenitors proliferate and differentiate to establish a highly connected three-dimensional structure, involving a robust succession of gene expression programs strongly influenced by miRNAs. To test the role of miRNA in the development of cardiac conduction system, sinoatrial node (SAN) from embryonic day E 12.5 and E 18.5, and from 1-day-old and 10-day-old mice were flash frozen in cold isopentane and stored at −80°C. The study was conducted in accordance with the Guide for the Care and Use for Laboratory Animals. Tissues collected from 2 hearts were combined to increase the yield of total RNA from each region (n=18 mice). Single-stranded cDNA was synthesised from 100 ng total RNA. Using qPCR, I investigated the changes in ion channels and transcription factors at 4 different time points: at E12.5 and E18.5 and at 1-day and 10-days postnatal. Data are presented as mean±SEM and statistical differences assessed by Student’s t test, one-way ANOVA or as appropriate. Differences were considered significant if P<0.05.

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

PC177

Respiratory muscle remodelling following acute sustained hypoxic stress in the mouse

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Hypoxia is a common feature of respiratory-related diseases. There remain, however, a general paucity of information concerning the effects of sustained hypoxia (SH) on respiratory muscle performance. We assessed the effects of acute exposure to SH on ventilation, sternohyoid (upper airway dilator) and diaphragm muscle contractile function and gene expression. Adult male C57BL/6J mice (n=8 per group) were exposed to 1, 4 or 8 hours of SH (F O2 = 0.10) or normoxia (F O2 = 0.21). Whole-body plethysmography was used to record breathing during gas exposures. Respiratory muscles were excised post-mortem. Muscle isotonic contractile and endurance performance was assessed ex-vivo following 8 hours of SH. qRT-PCR was used to examine changes in respiratory muscle gene expression at 1, 4 and 8 hours of SH and normoxia. Respiratory rate and minute ventilation were increased (p<0.001) and p<0.01 respectively, two-way ANOVA & Bon-
ferroni post hoc test) after 10mins of SH compared with control, returning to levels equivalent to normoxia by 30mins and remaining similar to normoxia for the remainder of the 8 hour SH exposure. For the sternohyoid, SH decreased tetanic force (12.80 ± 1.152 vs. 9.717 ± 1.054 N/cm², mean ± SEM, p=0.0683, unpaired t-test) and it depressed work-load (p<0.0001) and power-load (p=0.0009) relationships. For the diaphragm, SH decreased tetanic force (29.53 ± 3.151 vs. 20.75 ± 1.983 N/cm², p=0.0334) and power-load (p=0.0011) relationship. Isotonic fatigue tolerance of both muscles was improved (p<0.0001) following SH exposure. Differential changes in PGC1α, NfxB1 and selenoprotein N1 mRNA levels were observed in the diaphragm while decreased mRNA levels for NRF1, NRFB1, junctophilin 1 & 2, ryanodine receptor 1, calsequestrin 1, dihydropyridine receptor, and selenoprotein N1 were seen in the sternohyoid after 1, 4 or 8 hours of SH (p<0.05, one-way ANOVA & Tukey’s post hoc test).

Force generation and resistance to fatigue are important functional parameters in muscle. Respiratory muscle weakness is reported in COPD and animal models of chronic SH. Here we show that acute SH is sufficient to cause diaphragm and sternohyoid muscle weakness with resultant decreased mechanical work and power outputs. Interestingly, both muscles demonstrate apparent increased fatigue tolerance following acute SH. Acute SH appears to influence the regulation of metabolism and atrophy, and SR Ca²⁺ handling. Of note, following the acute hypoxic ventilator response at 10mins, ventilation in SH remains at normoxic levels thereafter (most likely due to a decreased metabolic O₂ demand during SH exposure), suggesting that the gene expression and functional changes observed are not due to enhanced respiratory muscle activity but relate to hypoxic stress per se. We aim to explore hypoxic signalling in respiratory muscle in this animal model to elucidate the mechanism of functional plasticity.

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

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**PC178**

**Role of Interleukin 6 and TNF-α in mediating the inflammatory effect in petrol exposed Wistar rats**

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Exposure to petrol has been associated with reduced immunity in previous work. This work is aimed at assessing the role played by IL-6 and TNFα in the suspected inflammatory reaction produced by exposure to petroleum product.

Methodology: 25 adult male rats were grouped to 5 with 5 rats in each group. All rats were made to inhale petrol for 5 minutes every day for 2 weeks using compressor nebulizer except the control group. Group 1 (control) had normal feed and water ad-libitum. Groups 2, 3 and 4 were given moringa oleifera in their feed (ratio 1:4), candesartan (AT1 receptor blocker) pre-treatment, captopril (Angiotensin converting enzyme inhibitor) pre-treatment before inhaling petrol while group 4 had petrol only every day. At the end of the exposure, rats were anaesthetized with chloroform and blood collected into EDTA bottles from the heart. Rat ELISA kit IL6 and TNFα were used by analyzing in duplicate wells for each sample. Result: Significantly increased release of pro-inflammatory cytokins IL6 was observed in the group exposed to petrol only compared with the value in the group pretreated with captopril and moringa oleifera. There was increase in the value of IL-6 in the group exposed to petrol only (89.47±46) compared with the pretreated with captopril (42.05±24) while the group given alovera had the least value (14.88±22).

Significantly increased serum level of TNF-α (453.6±319 pg/mL) found in the group exposed to petrol inhalation only when compared with group 2 (moringa oleifera 129.56±28 pg/mL) [candesartan (191.83±46 pg/mL)] and group three [captopril 92.62±23 pg/mL] was. Synthesis and secretion IL-6 interleukin 6, a cytokine is induced by TNF-α. Moringa oleifera, candesartan and captopril ameliorated the inflammation resulting from exposure to the petroleum hydrocarbon and IL6 as well as tumor necrosis factors increase that followed. TNF-α and IL6 were suspected to play a significant role in manifesting effect of petrol in the body of the Wister rats.

**Key words:** Interleukin-6, Tumor necrotic factor –α (TNF-α), petrol, captopril, moringa oleifera


We acknowledge the central research laboratory Staff for their assistance in carrying out the analysis where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

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**PC179**

**Effect of B-type natriuretic peptide and phosphodiesterase 2A in neurotransmitter release from pre-hypertensive rats**

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Introduction: B-type natriuretic peptide (BNP) is regarded as an early compensatory response to cardiac myocyte hypertrophy. However, the role of the recombiant BNP (Nesiritide) in clinical trials proved disappointing in the treatment of hypertension and heart failure, with some suggesting it actually enhances cardiac sympathetic activity. Our previous work showed that BNP decreases cardiac sympathetic neurotransmission by attenuating activation of neuronal calcium channels and the intracellular calcium transient via a cGMP-protein kinase G (PKG)-phosphodiesterase 2 (PDE2A) coupled pathway. Emerging evidence suggests that myocardial PDE2A expression and activity is up-regulated in heart failure. Therefore we tested whether PDE2A was directly involved in modulating cardiac neurotransmitter release from pre-hypertensive spontaneously hypertensive rats (SHRs) that show an enhanced Ca²⁺ phenotype.

Methods & Results: Four week old pre-hypertensive SHR and the age matched normotensive Wistar-Kyoto (WKY) rats were humanely killed with schedule 1 method in accordance with the Home Office Animals (Scientific Procedures) Act 1986 (UK). PDE2A activity in the SHR (n=8) was higher (~60%) than in WKY stellate ganglia tissue (n=8). [³H]labelled noradrenaline (NA) release was measured from isolated spontaneously beating atrial in response to 5Hz field stimulation for 1 minute at the 16th (S1, control) and 40th (S2, with 250nM BNP) minutes. All data are expressed as mean±SEM. Comparison within groups are performed using the paired t-test. We
Vascular resistance is preferentially increased by a respiratory-frequency bursting pattern rather than tonic sympathetic nerve stimulation

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Understanding the cause and maintenance of hypertension is critical for generating effective clinical treatments. Vascular tone is controlled by sympathetic nerve activity causing peripheral vasoconstriction. Respiratory-sympathetic coupling is the phenomenon whereby bursts of sympathetic nerve activity are modulated by respiration. In an animal model of essential hypertension the amplitude of sympathetic activity is elevated during the post-inspiratory period, contributing to hypertension (Simms et al. 2009). We do not yet understand the significance of this respiratory-sympathetic bursting pattern and phase vascular tone generation. Thus, we aimed to assess the impact of respiratory modulated bursts of sympathetic activity on vascular resistance (VR). Adult male Wistar rats (n=8) were anaesthetised with 1.2-1.5g/kg urethane and 60mg/kg alpha-chloralose i.p. The left carotid artery was cannulated to record arterial pressure and a flow probe applied to the left femoral artery to record femoral artery flow. Femoral VR was calculated as mean arterial pressure divided by mean vascular flow. The left lumbar sympathetic chain was located and the L4 sympathetic ganglia isolated and a cuff electrode applied for electrical stimulation. The working stimulus voltage was determined as that producing a half-maximal effect in VR (0-2.5V, 2ms pulse width, 40Hz). The nerve was stimulated with two respiratory bursting patterns to model tonic and 1Hz respiratory rates. The burst duration matched previous reports of the respiratory-sympathetic burst duration (250ms). Each pattern was compared to tonic stimulation at of the same average spike frequency (2Hz, 4Hz, 8Hz and 10Hz). Values are the maximum change in VR relative to base-

Poster Communications

PC180

How does fat cause heart diseases? Effects of epicardial adipocytes on cardiomyocyte signalling and contractility

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The heart is surrounded by a layer of fat known as epicardial adipose tissue (EAT). The adipocytes within EAT are critical to the function of the heart; they are an essential source of free fatty acids, which cardiomyocytes utilise for energy. EAT also provides thermal insulation and physical protection for the heart. However, the beneficial functions of EAT can be negated by the release of signals that evoke pathological changes in cardiomyocytes. EAT releases molecules, such as adipokines and cytokines, and due to the close proximity of EAT to cardiomyocytes these adipose-derived factors can affect the heart in a paracrine manner. The thickness of EAT is a predictor for several types of cardiac diseases. It is not possible to co-culture adipocytes and cardiomyocytes in a 2 dimensional co-culture, because mature adipocytes do not attach to cell culture surfaces. As a result, most studies on the effects of adipocyte released factors on cardiomyocytes use the addition of adipocyte-conditioned medium to cardiomyocytes. However, this does not allow paracrine effects between both cell types to become established after their isolation, and does not allow the study of long-term interactions. Adipocytes can be trapped and maintained in a 3-dimensional collagen matrix. We are currently optimising such a 3-dimensional culture system to study the effects that adipocytes have on adjacent cardiomyocytes, and vice versa. The translucent nature of the collagen matrix allows studying cellular phenotypes, Ca\(^{2+}\) signalling and contractions within the cultures. We investigated whether signalling pathways are activated or inhibited by the addition of adipokines and
cytokines, and whether co-culture with adipocytes affects the contractility of cardiomyocytes. TNF-α (3 ng/ml), activin A (1 ng/ml) and adiponectin (10 μg/ml) were added to cardiomyocytes for a period of 6 hours, and the activity of signalling pathways was subsequently examined using a PathScan Signalling Array (Cell Signalling Technology). We found a significant up-regulation of cardiomyocyte pro-apoptotic signalling pathways by TNF-α and Activin A. Whereas, adiponectin reduced ERK and mTOR activity, in line with its proposed cardioprotective effects. Co-culture of cardiomyocytes with adipocytes for 3 days caused negative chronotropic and negative inotropic responses. The presence of adipocytes reduced the frequency of cardiomyocyte beating from 0.51 ± 0.0 to 0.32 ± 0.04 Hz, and reduced the amplitude of contraction from 6.9 ± 0.3 to 3.8 ± 0.5 μm (n= 10 regions, 3 wells each with or without adipocytes, mean ± SEM, unpaired t-test, P<0.05). These data illustrate that adipose-derived cytokines and adipokines cause an up-regulation of inflammatory and pro-apoptotic pathways in rat ventricular cardiomyocytes. In addition, the proximity of adipocytes to cardiomyocytes leads to changes in contractility.

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

PC182

The effect of non-obesogenic high fat diet on cardiac Epac expression: implications for excitation-contraction coupling

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Non-obesogenic high-fat diet can directly trigger cardiac changes without obesity, by altering cardiac metabolism and/or function (Littlejohns et al., 2014). These changes include an increase in cardiomyocyte percentage shortening and were associated with an increase in diastolic calcium during staircase response. Excitation-contraction coupling is regulated by sympathetic stimulation involving cAMP signalling. Recent evidence has shown that in addition to protein kinase A (PKA), cAMP/guanine nucleotide exchange factor directly activated by cAMP (Epac) is also important in mediating cAMP signalling (Okumura et al., 2014). The aim of this study was to determine whether a non-obesogenic high-fat diet alters the relative protein expression of Epac. Consequently, we measured the expression of Epac in hearts from normal and high fat diet. Male C57BL 6 mice aged 6 weeks fed a high-fat diet (45% calories from fat and 0.15% cholesterol) for approximately 20 weeks had an increase in blood cholesterol but no evidence of cardiac hypertrophy or insulin sensitivity with little increase in body weight (Littlejohns et al., 2014). Extracted ventricular tissue from normal diet (control) and high-fat diet groups were used to detect the relative protein expression of the two Epac isoforms (Epac1 and Epac2) using western blotting. RIPA buffer was used to extract proteins and the expression was normalised to GAPDH. For western blots, monoclonal mouse antibodies for Epac1& 2 (dilution 1:1000) and rabbit GAPDH (1:5000) were purchased from Cell Signaling Technology. Data are presented as mean±SEM (n= 4 hearts/group) and were analysed using unpaired t-test.

High fat diet was associated with a significant (p<0.05) increase in the relative level of Epac1 protein expression compared to normal diet (1.03 ± 0.01 vs. 0.4 ± 0.08). However, high-fat diet did not change Epac2 expression compared to normal diet (0.58 ± 0.08 vs. 0.6 ± 0.04). These data show that a high-fat diet increases the expression of Epac1 which can be involved in altering excitation-contraction coupling in cardiomyocytes. Littlejohns B, Pasdois P, Duggan S, Bond AR, Heesom K, Jackson CL, Angelini GD, Halestrap AP & Suleiman MS. (2014). Hearts from mice fed a non-obesogenic high-fat diet exhibit changes in their oxidative state, calcium and mitochondria in parallel with increased susceptibility to reperfusion injury. PLoS one 9, e100579.


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PC183

Ageing-induced ultrastructural changes of mitochondria of pulmonary vein sleeve cells

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Atrial fibrillation (AF) is the most common form of sustained cardiac arrhythmia, with age being a significant risk factor. Inside the pulmonary veins a sheath of cardiomyocytes called pulmonary vein sleeve cells (PVCs) can be found. PVCs can cause atrial fibrillation by generating spontaneous increases in the intracellular Ca2+ concentration and ectopic electrical activity. Ultrastructural changes during atrial fibrillation have been described in atrial cardiomyocytes. Besides an increase in the number of glycogen granules, the reported changes include a greater variability in the number and size of mitochondria. However, the published data are inconsistent; some studies reported an increase of mitochondrial number and size in AF models, whereas other studies reported a decrease. So far, no studies have been performed to investigate changes in the mitochondrial structure occurring in PVCs. Since age is a significant risk factor for the development of AF we expected that the ultrastructure and/or number of mitochondria in PVCs would change with age. Here, we describe the results of a comparative ultrastructural study of pulmonary vein sleeve cells, atrial and ventricular cardiomyocytes from 3 and 24 month-old mice. PVCs were isolated from mouse lung slices after agarose-inflation of the lungs. Atrial and ventricular cardiomyocytes were prepared from wedge-shaped sections of mouse hearts. We counted the number, and measured the size, of mitochondria in 25 μm² sections of three to five EM images in four animals per age group. The quantification showed an increased number of mitochondria in PVCs from the older animals (14.2 ± 0.9 vs. 19.6 ± 1.2 mitochondria / 25 μm², 3 vs. 24 month respectively, P < 0.05, unpaired t-test, n = 4 animals per group). Additionally, mitochondria were significantly enlarged (0.7 ± 0.02 vs. 1.0 ± 0.1 μm², 3 vs. 24 month respectively, P < 0.0001, unpaired t-test, n = 4 animals per group). Values are means ± S.E.M. In contrast, we did not see any significant differences
in mitochondrial number or size in atrial and ventricular cardiomyocytes. Besides the changes in mitochondria, we found the presence of lipofuscin in PVCs, atrial and ventricular myocytes from the older animals. Lipofuscin is a known marker for ageing, and contains larger amounts of oxidized unsaturated fatty acids and non-degraded non-functional mitochondria. These results show age associated structural changes in pulmonary vein sleeve cells. Since mitochondria are an important Ca\(^{2+}\) buffer in cardiomyocytes, an increase in mitochondrial number and size could contribute to the pro-arrhythmic signalling described for pulmonary vein sleeve cells.

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**PC184**

Respiratory system dysfunction in the mdx mouse model of Duchenne muscular dystrophy

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The authors wish to submit this work as title only.

Muscular Dystrophy Ireland & Department of Physiology, University College Cork.

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**PC185**

Providing students with opportunities to test their understanding of key physiological principles: the challenge of curriculum congestion and large cohorts

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Curriculum congestion, which has arisen from an increasing knowledge base, forces many students to take a pragmatic approach to their learning; rote learning from didactic lectures. This approach may allow students to pass examinations but limits their ability to integrate and apply their knowledge. Moreover, large cohorts impact on our ability to provide sufficient opportunities for students to consolidate lecture content and test their understanding of and ability to apply core concepts, eg small groups tutorials. We sought to identify ways in which we could address these challenges for a cohort of 350 year 2 MBChB students studying cardiovascular science at the University of Birmingham. From performance in small group teaching sessions and summative assessments over a number of years we identified that students were able to recall basic details of the cardiac cycle but were unable to apply their knowledge to simple clinical cases. Thus we reduced didactic lecture content and introduced an interactive session in which 90 students were given a Wiggers diagram and were asked to accurately plot left ventricular pressure against volume for one cardiac cycle; they had not previously seen a P-V loop. To test their understanding of the physiological principles underlying pressure generation, valve opening and blood movement we asked them to mark on their loop the points at which the aortic and mitral valves open and close, to indicate end diastolic and end systolic volume and to calculate stroke volume and ejection fraction. With only one lecturer facilitating the session, we used peer instruction (Lasry et al, 2008) to enable students to complete the task. The activity was extended by asking the students to discuss and illustrate what would happen to the P-V loop with changes in pre-load, inotropy and after-load, giving them the opportunity to explore this before subsequent teaching on regulation of cardiac output.

Evidence from performance in summative assessments suggests that this activity increased the students’ understanding of the cardiac cycle and their ability to apply the physiological principles underlying it. Student feedback on the session was very positive, interestingly however, a significant minority of students expressed a wish to have more facilitators in the sessions, suggesting that there are a number of students who are not confident in their ability to learn when they are not being lectured didactically. This could be ameliorated by explaining to students the rationale for and the benefits of this type of learning.

This approach could be applied in any area in which curriculum pressure has forced students to rely largely on rote learning and in which large cohort size limits how resources can be used to provide students with opportunities to test their own learning.


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**PC186**

An online procedure for anonymous peer marking of physiology laboratory reports

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Well-designed peer marking procedures can give students rapid and accurate feedback on their work. Harris (2011) described and evaluated such a procedure for hand-marking ‘pro-forma’ physiology laboratory reports in a large (ca. 200) first year undergraduate class. Staff and student feedback on the approach was positive but its format made it difficult for the reports to include free text and many students disliked the lack of anonymity in peer marking. We have now adapted the procedure so that anonymous peer marking of reports that include free text can be carried out online within eBiolabs, a web-based system developed to support and assess laboratory-based work (Hughes et al, 2012). Students upload physiological data, including numerical analysis and graphs, obtained in the lab plus free text answers to related questions. All work must be submitted within one week of the practical class. Each student’s work is assigned randomly to two peers who allocate marks with reference to marking criteria and specimen answers that are released online, and explained verbally, immediately after the submission deadline but before the peer marking opens. Peer marking (anonymous to both the writer and marker) must then be completed within one week. Staff are able to review all uploaded work, the identity of student markers and the marks awarded. Anomalous marking is checked and is moderated if the two peer marks differ by more than 15%. Finalised marks
are made available to students within one week of the marking deadline and contribute 2% to the final unit mark. The system has now been used for 5 years to peer-assess a first year renal physiology practical report submitted by ca. 200 BSc students. In the first 3 years a range of technical and student compliance issues were encountered but these have now been largely resolved. In 2013-14 and 2014-15, 68% and 74% respectively of the submitted reports required no staff moderation and students were awarded the average of the two peer marks. Furthermore, 86% (13-14) and 91% (14-15) of the student cohort were totally compliant with the process. Examples of student non-compliance included failure to upload their own report and/or to peer-mark the reports that were allocated to them.

Feedback from ca. 75 students in 13-14 showed that 80% agreed that they received a fair mark for their work whilst 64% agreed that evaluating other students’ work was helpful for their own learning.

We conclude that online peer assessment of laboratory reports can give students rapid, anonymous and fair feedback on work that includes free text, and that around two-thirds of students find the process helpful for their learning. Although generally efficient in staff time, some intervention is required to resolve issues of peer marker non-compliance or inaccuracies in student marking.


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Essay writing skills workshop for first year physiology students
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First year undergraduates, on a systems physiology unit, write a summative timed essay. One aim of this is to train students in scientific writing as many will have had limited prior experience. A marking grid, developed at Bristol, is used to assess writing skills for which 50% of the marks are allocated and 50% is for scientific content. Prior to the assessment students write two formative essays receiving feedback from staff. Following the first practice essay students anxiously requested more essay writing practice and feedback. In response an optional workshop was run which aimed to: give all students an opportunity to practice essay writing; improve students’ confidence in planning and writing essays; provide good quality feedback, with minimum staff input. All 186 students taking the unit were invited to an essay writing workshop. Interested students were given a title prior to the workshop and asked to bring an essay and an attempt at a scientific content mark scheme, 20 attended. In the session groups students were allocated a section of a skeleton mark scheme to discuss. Their suggestions were fed back to the whole group and a master mark scheme composed. Students were given 15 minutes to marked an essay and write feedback comments. They were then encouraged to give verbal feedback to each other.

Students completed pre and post session questionnaires which included a self-assessment of confidence in planning and writing essays on a 1-5 scale, 5 being high. Permission was gained from all students to collect their marks for their other essays.

Attendees marks for two practice essays (essay 1 pre & essay 2 post workshop) and the summative essay were compared (mean ±SEM, unpaired T test) with those of students who did not attend. Students reported attending the workshop to improve essay writing skills (74%) and to improve scientific essay content (16%). 89% of students agreed that the session had fulfilled their goals. The questionnaire showed a reported increase in attendees’ confidence for essay planning from 2.8 ±0.2 to 3.5 ±0.2 and for writing timed essays from 2.0 ±0.2 to 2.7 ±0.2. The mean mark for essay 1 was similar for both attendees (72.8% ±2.8) and the rest of the cohort (73.8% ±0.9). Attendees did better in the essay 2 than non-attendees (mean mark 65.0% ±2.3 vs 60.3% ±1.1) and in the final summative essay (mean 57.3% ±1.9 vs 53.3% ±1.1). However this was not statistically significant: for essay 1, p=0.7, for essay 2 and summative essay, p=0.08.

This workshop provided students with an extra opportunity to practice essay writing, and improved students’ confidence in this type of assessment. Those students who attended the session achieved a higher mean mark in the summative exam. The peer review aspect of the workshop also gave students the opportunity to experience other students’ writing styles and set a framework for them to follow in their independent study.

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

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Academic motivation, strength of motivation and need of cognition of Romanian first-year medical and dental students involved in a Physiology course
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Objective: The purpose of this study was to examine the determinants of academic study motivation, strength of motivation for medical/dental school students involved in a Physiology course. Methods: The participants of this descriptive, cross-sectional study were 221 first year medical and dental students at the University of Medicine and Pharmacy “Carol Davila” Bucharest that completed a questionnaire assessing demographic variables, Academic Motivation Scale (AMS)(Val-lerand et al., 1992, 1993), The Strength of Motivation (SMMS)(Nieuwhof et al., 1992, 1993), The Strength of Motivation (SMMS)(Nieuwhof et al., 2004) questionnaire and Need for Cognition scale (NCS)(Caccioppo et al., 1984). Results: Reliability analy-ses indicated that all scales and their subscales have adequate internal reliability, as measured by Cronbach’s alpha. Results revealed that females were more intrinsically (knowledge, accomplishment) and extrinsically (identification) motivated and less amotivated toward academic activities than males students. Female students also scored higher than males on the Relative Autonomy Index (RAI)(132.29 ± 40.42 vs. 105.41 ± 52.84; P<0.0001), SMMS and NCS. Age negatively correlated with AMS subscale: Extrinsic Motivation-External Regulation (r = -0.15; P<0.05) and with Relative Autonomy Index (RAI) (r = 0.14; P<0.05), while incomes were significantly correlated
Factors affecting performance in physiology examination among dental and medical students at the University of Ibadan. A pilot study

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Motivation/problem statement: Medical students are among the brightest of students high and students are admitted selectively based on past academic performance. However with the failure rate in medical school, factors other than intelligence may affect academic performance. Contrary to the traditional belief that stressful experiences are necessary for future medical practice, research suggests that stress and anxiety are major causes of cognitive dysfunction (Vitaliano, 1984). Dyrbye et al.,(2006) in a systematic review reported that there are limited data available regarding the causes of student distress and its impact on academic performance, dropout rates, and professional development. The aim of this study was to determine factors that affect performance in Physiology Part 1 examination among Medical and Dental students at the University of Ibadan, Nigeria. Methods: Semi structured self administered questionnaire was used. The questionnaire contained questions on sociodemographic data. Perceived Stress Scale developed by Cohen and Mermeistein in 1983 was used. Subjects were grouped as stressed or not stressed. Hospital anxiety and depression scale was used to collect data on psychological distress. Scores in Physiology examination was obtained from Departmental records.

Results: Of the 130 questionnaires that were filled, examination results were obtained for 100 students, who provided student identification numbers.

Factors affecting performance in Physiology - There was no association between gender and success in physiology (p=0.42). The mean age of those who passed (20.44±2.58 years) was lower than the mean age of those who failed (22.55±5.13 years). There was statically significant association between age and performance (p=0.028). A higher proportion of medical students (93.7%) passed compared with dental students (71.4%) (p=0.004). There was statistically significant association between Cumulative GPA groups in first year and success in Physiology examination, (p=0.003).

Association between stress and psychological distress and performance in Physiology (Figure 3) Among those who failed, 72.7% had perceived stress compared with 27.3% who did not perceive stress (p=0.001). There was a statistically significant association between anxiety and performance in physiology. A higher proportion of those who failed were anxious (60%) compared to those who were not anxious (40%) (p=0.042). There was no association statistically significant association between depression and success in Physiology (p=0.173).

Conclusion. Anxiety and perceived stress were associated with poorer performance, however depression did not affect performance among the students. Further research needs to be done to determine source of stress and anxiety. Preventive measures need to be put in place to enhance performance.

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

TRPV4 receptor expression and function in the aging bladder

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Urine incontinence and bladder dysfunctions are highly prevalent in the aging population with a huge financial cost(1). However, the underlying mechanisms are poorly understood. Recent evidence suggests that transient receptor potential vanilloid 4 (TRPV4) channels present in the urothelium, which facilitate Ca2+ influx, may serve as key sensory molecules in response to noxious stimuli and bladder stretch. We previously demonstrated that this receptor is functional in native bladder tissue, triggering release of ATP from the urothelium. This study tested the hypothesis that the expression of TRPV4 receptors is altered in aging bladders. Bladder tissues were obtained from young (2-5 months) and aging (24-36 months) guinea-pigs (GP) (male Dunkin-Hartley, schedule-1 procedure) in compliance with UK and EU regulations. Immunohistochemistry and Western blotting were employed to detect TRPV4 expression. Fixed full-bladder wall slices (14 microns) were probed for TRPV4 and examined by confocal microscopy. The presence of TRPV4 in mucosal and denuded smooth muscle lysates was evaluated using a standard Western blotting protocol and the relative expression analysed. Mucosal
and intact smooth muscle strips were isolated from GP bladders and superfused with a Tyrode’s solution. The superfuse adjacent to the tissue was sampled and measured for ATP release using luciferin-luciferase assay(2). Positive staining for TRPV4 was observed in both young and aging GP bladders. Quantification of relative band intensity from Western blots by densitometry revealed a significantly greater mean TRPV4 expression (7 fold) in aging mucosa than young (arbitrary units normalised to loading control - aging: 65.0±11.6, young: 9.2±1.2; mean ± SEM, n=5, p<0.05, Mann-Whitney) and (6 fold) in smooth muscle (aging: 23.5±1.9, young: 3.8±2.7; n=5, p<0.05). Additionally, TRPV4 expression in the aging mucosa was significantly greater compared to smooth muscle (Mann-Whitey, n=5, P<0.05), which was not seen in young tissue. TRPV4 activator GSK1016790A (GSK, 1μM) triggered significant increase in ATP release in tissue preparations from both GP groups (young GP - mucosa: 281±60% of control; full thick strip: 346±40% of control; aging GP - mucosa: 232±47% of control; full thick strip: 269±40% of control; mean ± SEM, n=9, p<0.05, Wilcoxon’s). No significant difference in receptor-activated ATP release was observed between GP age-groups. These results provide the first evidence that TRPV4 expression is altered in bladders with increasing age. The up-regulated expression of TRPV4 receptors and their abundance in the urothelium indicate that these receptors may contribute to the urothelium-mediated sensory dysfunction during aging. This highly increased TRPV4 expression may influence bladder function through altered Ca^{2+} entry or other signalling pathways independent from ATP release.


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Pathological role of extracellular vesicle cell-to-cell signalling in nephrotoxic renal injury

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Drug toxicity is one of the most common causes of kidney injury in hospital. There is neither an effective therapy nor an effective diagnostic test. Extracellular vesicles (ECVs) are released into human urine from all regions of the nephron, so downstream kidney tubular cells are exposed to ECVs originating proximally. ECV signalling could be contributing to pathological cell-to-cell signalling, raising the question of their role in renal injury and their potential as therapeutic targets. Collecting duct (CD) immortalised cells were injured with lithium chloride (LiCl; vehicle, 52mM and 300mM) for 24 hours. ECVs were harvested from the supernatant using ultracentrifugation. These ECVs were co-cultured with healthy ‘recipient’ CD cells for 24 hours. Inhibition of dynamin-dependant endocytosis, prior to inoculation with ECVs, nullifies this response (figure b). Our work has raised the exciting possibility that ECVs derived from severely injured cells increase apoptosis in healthy cells but may, at an earlier stage of nephrotoxic injury, confer protection to downstream cells. This is in line with previous observations implicating ECV signalling in apoptosis, a signal which may be mediated by mRNA and miRNA exchange²⁻⁵, raising the interesting physiological and pathophysiological role of ECV signalling in the nephron.

Figure a) ECVs isolated from CCD cells treated with 300mM LiCl trigger apoptosis in healthy CCD cells b) Inhibition of dynamin-dependent clathrin-mediated endocytosis blocks ECV–induced apoptosis. Boxplot diagram of caspase activity of CCD cells. Results normalised to control (cells, vehicle ± dynasore as necessary). Whiskers represent min and max data, n=6, *p<0.05.

Pisitkun T, Shen RF, Knepper MA. *PNAS*. 2004; 101(36): 13368-73


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

PC192

The immunoglobulin loop of the voltage-gated Na⁺ channel β1 subunit regulates adhesion and Na⁺ current in metastatic breast cancer cells

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Voltage-gated Na⁺ channels (VGSCs) are composed of a pore-forming α subunit and one or more auxiliary β subunits. The β subunits regulate channel gating and are members of the immunoglobulin (Ig) superfamily of cell adhesion mole-
cules. Although classically expressed in excitable cells, VGSC α and β subunits are also present in cancer cells, including breast cancer (BCa). In the metastatic MDA-MB-231 BCa cell line, Na⁺ influx through Naᵥ1.5 increases invasion and migration. β1 expression is up-regulated in BCa specimens relative to control tissue. β1 over-expression also increases tumour growth, invasion and metastasis in an orthotopic tumour model. In addition, β1 promotes neurite-like process outgrowth from BCa cells, and this is dependent on the presence of the Ig loop (1). Therefore, targeting and inhibiting the adhesive function of β1 may provide a novel therapeutic method for inhibiting metastasis. The purpose of this study was to test the hypothesis that the Ig loop is required for β1 to regulate adhesion and Na⁺ current in BCa cells. We studied cell-cell adhesion using an aggregation assay. Briefly, tumour cells were maintained in suspension for up to 2 h with gentle shaking, aliquots were taken at 30 min intervals, placed on glass slides and aggregation was monitored under a microscope. The number of particles per field of view was counted. A particle was defined as a single cell or cluster of cells of any size. Increased adhesion was therefore reflected in a decrease in particle number. After 30 minutes, the number particles per field of view was 52.5±2.4 (mean±SEM) in β1 over-expressing cells, compared to 79.4±1.4 in control MDA-MB-231 cells, in agreement with previous observations (2) (t-test, n=4, P<0.001). In contrast, over-expression of a truncated β1 mutant lacking the Ig loop (β1Δ40-124) did not increase adhesion compared to control cells (t-test, n=3, P=0.26). Therefore, the Ig loop is necessary for the increase in adhesion of MDA-MB-231 cells. We next studied Na⁺ currents by whole-cell patch clamp recording in voltage clamp mode. The peak Na⁺ current density increased from -6.7±0.3 pA/pF in control MDA-MB-231 cells to -15.3±2.4 pA/pF in β1 over-expressing cells, in agreement with previous observations (P<0.05) (2). However, the peak Na⁺ current density was not significantly altered in MDA-MB-231 cells overexpressing the β1Δ40-124 truncation mutant, compared to control cells (control, n=14; MDA-MB-231-β1, n=15; MDA-MB-231-β1Δ40-124, n=13; Kruskal-Wallis with post-hoc Dunn’s tests). These data suggest that the Ig loop of β1 is required to modulate both adhesion and Na⁺ current in BCa cells. Thus, the Ig loop of β1 may enhance invasion and metastasis through dual roles, and may therefore represent a useful novel therapeutic target.


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**PC193**

**Pathological NO and Ca²⁺ signaling in pancreatic stellate cells**

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Pancreatic stellate cells (PSCs) are the resident cells of the exocrine pancreas. In healthy pancreatic tissue they play an important role in regulation of extracellular matrix proteins turnover, and store retinoids. In response to pancreatic tissue injury PSCs undergo a phenotypic transformation and contribute to tissue fibrosis (1). Despite a growing body of evidence of the role PSCs might play in e.g. chronic pancreatitis and pancreatic cancer, relatively little is known about the exact mechanism of PSC activation and pathological transformation (2). Currently, the role of nitric oxide (NO) signaling, and its potential link with Ca²⁺ signaling, in these cells remains unknown and should be elucidated.

In the present study NO and Ca²⁺ responses were examined in murine pancreatic lobules which contained both the acinar (PACs) and the stellate cells, as well as in the cultured human PSC line. C57BL/6 mice were humanely killed in accordance with the UK Schedule 1 of the Animals (Scientific Procedures) Act, 1986. Ca²⁺ signals were recorded in a ratiometric manner using Fura-2, and NO was measured with the specific fluorescent dyes: DAF-2 or DAF-FM.

The simultaneous increases in intracellular Ca²⁺ and NO were observed in the lobular (murine) stellate cells co-loaded with DAF-2 and Fura-2 probes, after treatment with bradykinin (BK; 20 nM). Interestingly, the BK-evoked changes in the cellular levels of Ca²⁺ and NO showed markedly different profiles: BK caused a rapid increase in cellular Ca²⁺, with an initial peak and subsequent plateau, and a small, sustained increase in cellular NO. Responses were detected in the lobular PACs after BK stimulation. Hydrogen peroxide (H₂O₂), an oxidative pathophysiological stimulus, caused a dose dependent (0.125–0.5 mM) increase in the cellular level of NO in the lobular PSCs.

A sharp increase and sustained plateau was observed after treatment with H₂O₂ in the higher (0.3–0.5 mM) concentration range, and a more modest increase and sustained plateau after treatment with H₂O₂ in the lower concentration range (0.125–0.25 mM). The development of H₂O₂-evoked responses was diminished substantially (p < 0.005) after the application of a NO synthase inhibitor L-NAME (0.6 mM). Other pathological stimuli, such as bile acid sodium salts: chololate (0.1–5 mM) and taurocholate (5 mM) caused elevations in cytosolic Ca²⁺, detected in the lobular (murine) and in the human PSCs, and related increases in cellular NO. We conclude that NO signaling, together with Ca²⁺ signaling, play an important role in regulation of physiological and pathological processes in the pancreatic stellate cells.

Apte, MV, et al., (1999); Gut 44, 534–541

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Identification of tryptophan metabolite transporters in human astrocytes and their regulation by uric acid

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The tryptophan metabolite kynurenic acid (KYNA) plays a vital role in brain health as it provides neuroprotection (Schwarz et al., 2012). Once synthesized in astrocytes from L-kynurenine (L-KYN) KYNA is released into the synaptic cleft to modulate release and effect of glutamate. However, transporters for L-KYN and KYNA in astrocytes have not been identified yet. Uric acid is a purine metabolite that is neuroprotective and connected to the tryptophan metabolism. Our aim was to identify the transporters for L-KYN and KYNA in human astrocytes and to investigate their regulation by uric acid.

U373MG and primary human astrocytes (NHA) were used to examine expression of candidates for the two unidentified transporters such as BOCT1, many urate or known renal KYNA transporters such as OAT1 (Bahn et al., 2005), OAT3, URAT1, OAT4, OAT2, LAT1, GLUT9 or MRPS by q-PCR and western blot analyses. We performed siRNA knock-down, uptake and efflux studies over 5 minutes at 37°C with [3H]-KYNA or [3H]-L-KYN and suitable inhibitors such as L-leucin or MK-571 to identify the transporters for L-KYN and KYNA. The influence of urate (500μM) and L-KYN (1.5mM) on expression of all candidate transporters as well as KATII and EAAT1 were tested.

Uptake of 25nM [3H]-KYNA into U373MG and NHA cells was inhibited by 1mM of L-leucin down to 34% and 9%, respectively, indicating involvement of L-amino acid transporter 1 (LAT1). This was further examined by siRNA knock-down of LAT1 in U373MG and NHA cells, leading to a reduction of [3H]-KYNA uptake by 47% and 27%. qPCR and western blot analyses confirmed LAT1 as the L-KYN uptake transporter in human astrocytes. To identify the efflux transporter for KYNA we screened U373MG and NHA cells by qPCR for possible candidates, revealing BOCT1, MRPS and OAT1, of which only BOCT1 and MRPS could be confirmed by western blot. Efflux of KYNA could be inhibited by MK-571 down to 50%, indicating that MRPS may be the KYNA efflux transporter. Further siRNA studies are underway to confirm this finding. To evaluate the impact of urate on the kynurenicine pathway, we incubated U373MG and NHA cells with urate (500μM) or L-kynurenine (1.5mM) and performed qPCR analysis. Interestingly, none of the tested proteins (LAT1, BOCT1, MRPS, VGLUT1, KATII or GLUT9) were regulated by urate.

In summary, we have identified LAT1 as L-KYN uptake transporter in human astrocytes. Moreover, we have characterised possible KYNA efflux transporters and postulate that MRPS is the KYNA efflux mechanism. The impact of urate on the kynurenicine pathway is still open. Identification of the L-KYN and KYNA transporter paves the way for further understanding of diseases such as schizophrenia, Huntington or Alzheimer’s disease, in which KYNA levels in the brain are disturbed.


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Investigation of MCT1 transporter localization in the human gastrointestinal tract

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Short chain fatty acids (SCFAs), such as butyrate, are products of the fermentation of dietary fibres and undigested carbohydrates by anaerobic intestinal microbiota in the human colon. These SCFAs are vital in maintaining the symbiotic relationship that exists between humans and these colonic microbial populations. For example, butyrate induces cell differentiation and regulates growth and proliferation of colonic mucosa (1).

A key step in this process is the transport of butyrate into colonic epithelial cells via MCT1 transporters (2). However, the exact cellular localisation of MCT1 protein along the human gastrointestinal tract remains controversial (3-4). Our previous studies have shown MCT1 protein abundance is higher in ascending compared to sigmoid colon (5). The aim of this present study was to investigate the precise MCT1 cellular localization in various human gastrointestinal tissues.

Using 10μm sections of paraffin-embedded tissue and an anti-MCT1 antibody, immunolocalization studies confirmed MCT1 protein was strongly detected in the human colon, but not the ileum or stomach. This colonic MCT1 staining was only detected in surface epithelial cells. Further experiments showed strong MCT1 staining in the ascending colon, particularly in the basolateral region of the surface epithelial cells. In contrast, only weaker MCT1 staining was detected in the sigmoid colon and had a more general intracellular distribution within these cells. Finally, strong MCT1 staining was surprisingly also detected in the surface epithelial layers of the foetal colon.

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Inhibition of store-operated calcium entry as a potential therapy for acute pancreatitis

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Introduction: Store-operated calcium entry (SOCE) is implicated in many pathologies including acute pancreatitis (AP)¹. Calcium-release activated calcium (CRAC) channels, composed of the endoplasmic reticulum (ER) Ca²⁺ sensor STIM1 and the pore-forming subunit ORAI1 in the plasma membrane, are expressed in pancreatic acinar cells (PAC)². During pathological stimulation by necrotising agents, such as fatty acid ethyl esters (FAEE) or bile acids, CRAC channels are activated by the depletion of ER Ca²⁺. Subsequent SOCE maintains sustained increase in [Ca²⁺]i – thought to be the trigger for premature, intracellular activation of digestive enzymes and the autodigestion of the pancreas, hallmarks of AP.

Methods: C57BL/6 mice were killed humanely in accordance with the UK Schedule 1 of the Animals (Scientific Procedures) Act, 1986. [Ca²⁺]i measurements were made in PAC loaded with the Ca²⁺-sensitive dye, Fura-2.

Results: In order to activate CRAC channels it was necessary to deplete ER store. Stores were depleted by the application of SERCA inhibitors thapsigargin or CPA in nominally Ca²⁺ free extracellular solution. Extracellular application of CRAC channel antagonists such as GSK7975-A (10µM)¹, dramatically reduced [Ca²⁺]i, when applied before the re-admission of CAO²⁺. When applied on top of a sustained increase in [Ca²⁺]i, due to the re-admission of CAO²⁺, acute application of GSK7975-A also reduces [Ca²⁺]i. Concurrently a reduction in cellular necrosis was also seen. Increases in [Ca²⁺]i, usually induced by FAEE were markedly reduced too.

2-APB is known for its biphasic actions on SOCE⁴ – potently inhibitory at 100µM, whilst potentiating SOCE at 1-5µM. Analogues of 2-APB were generated: DBP162 and DBP163, and are more potent than 2-APB at inhibiting SOCE. Both analogues markedly inhibited SOCE in PAC (3µM), when applied before the re-admission of CAO²⁺. In addition to CRAC channels, PAC express TRPC3 – a Ca²⁺-permeable cation channel. Pyr10, a selective TRPC3 inhibitor, blocked SOCE when applied to PAC (10µM), however, GSK7975-A was more effective.

ORAI1 is reported to have a calmodulin (CaM) binding domain, thought to regulate its channel function⁵. CaM agonist, calcium-like peptide-3 (CALP-3), markedly inhibited SOCE when applied to PAC and reduced [Ca²⁺]i. Furthermore, when CaM itself was applied to cells there was a significant reduction in SOCE.

Conclusion: Inhibition of SOCE, via entry channels such as CRAC and TRPC3 is an important target for reducing [Ca²⁺]i. Using a direct pharmacological approach or a more indirect method by modulating CaM has been effective in reducing SOCE and as such reducing [Ca²⁺]i. A combination of both methods could prove an efficient mechanism in acutely targeting cytosolic Ca²⁺ overload, reducing the intracellular activation of digestive enzyme and ameliorating AP.


Stromal-interaction molecule 1 contributes to the effect of the new sp²-castanospermine α-glucosidase II inhibitor in breast cancer cells

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Breast cancer is the most common cancer in women in both industrialized and developing countries. Currently, breast cancer ranks as the second leading cause of death in women. Several studies have highlighted the role of calcium channels and their implications on the regulation of cell proliferation, apoptosis, migration and invasion in breast cancer. The two major regulators of SOC channels, STIM1 (stromal-interaction molecule 1) and ORAI1 are responsible for Store Operated Calcium Entry (SOCE) activation. ORAI1 molecule, an essential pore-forming component of the SOC channel, translocates to the same STIM1-containing structures during store depletion and opens to mediate Ca²⁺ entry.

Cellular glycosylation is a fundamental post-translational event that has been shown to orchestrate key biological processes including cell–cell communication, signal transduction, protein folding and stability. In mammalian cells, oncogenic transformation is often accompanied by altered glycosylation pattern of proteins and lipids and these appear to have functional implications in tumors development. Furthermore, the events involved in the glycosylation process are modulated by glycosidases and glycosyltransferases, the levels of which have been correlated with tumor metastasis. Moreover, tumor cell-surface oligosaccharides have been shown to be remarkably distinct from those of normal cells such as increase in branching of N-linked glycan. Inosiminos is competitive inhibitors of glycosylation, known to have several pharmacological effects in medicine, among them their anti-cancer effects, which make them potential drug candidates to fight cancer.

Previous results of our laboratory have shown anti-proliferative and apoptotic effects of the new generation of sp²-Castanospermine inosiminos α glucosidases II inhibitors (CO-OCS) [1] on MCF-7 breast cancer cell line, without affecting normal mammary cells MCF-10A[2]. In this study, we found that CO-OCS decreased cell proliferation, increased cell mortality, impaired STIM1 expression levels and SOCE among MCF-7 cells. Silencing of STIM1 reduced cell proliferation without affecting cell mortality. Furthermore, in MCF-7 cells treated with CO-OCS, silencing of STIM1 has no additive effect on cell proliferation, but decreased cell mortality induced by CO-OCS. Finally, CO-OCS has no effect on the STIM1 expression, SOCE, cell proliferation and mortality in normal mammary cells MCF-10A. Therefore, we suggest that STIM1 and may be SOCE could be used as attractive targets for CO-OCS.

A pharmacological characterization of ovine CFTR Cl\(^{-}\) channels expressed in mammalian cells

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Cross-species comparative studies have highlighted differences in the response of cystic fibrosis transmembrane conductance regulator (CFTR) homologues to small-molecule modulators (1, 2). Compared to human CFTR, ovine CFTR shows a high degree of sequence conservation (91% identity and 95% similarity at the amino acid level) (3), but exhibits enhanced conductance and ATP-dependent channel gating. Therefore, we were keen to characterise the pharmacology of ovine CFTR. In this study, we investigated the effects of the CFTR potentiator phloxine B (4) and the inhibitor glibenclamide (5) on the single-channel activity of ovine CFTR using excised inside-out membrane patches from transiently transfected Chinese hamster ovary cell (CHO-K1) cells at 37 °C (1).

In contrast to its potentiation of human CFTR, low micromolar concentrations of phloxine B (0.1 – 5 μM) were without effect on inhibited weakly ovine CFTR Cl\(^{-}\) currents (e.g. phloxine B (1 μM), human, \(I_{\text{drug}}/I_{\text{control}} = 134 \pm 11\%\), \(n = 9\), \(P < 0.05\); ovine, \(I_{\text{drug}}/I_{\text{control}} = 98 \pm 4\%\), \(n = 11\); \(P > 0.05\); means = SEM (n observations); Student’s t-test). However, elevated concentrations of phloxine B (≥ 10 μM) inhibited strongly both human and ovine CFTR (n = 4 – 8). Single-channel studies indicated that phloxine B (1 μM) potentiated human CFTR by increasing open probability \((P_{o})\) (control, \(P_{o} = 0.31 \pm 0.03\); phloxine B, \(P_{o} = 0.54 \pm 0.03\); n = 15; \(P < 0.05\)). However, phloxine B had little impact on the \(P_{o}\) of ovine CFTR (control, \(P_{o} = 0.46 \pm 0.04\); phloxine B, \(P_{o} = 0.46 \pm 0.05\); \(n = 8\); \(P > 0.05\)). The open-channel blocker, glibenclamide (50 μM) inhibited ovine CFTR Cl\(^{-}\) currents, albeit with reduced efficacy compared with human CFTR (human, \(I_{\text{drug}}/I_{\text{control}} = 31 \pm 3\%\), \(n = 8\); ovine, \(I_{\text{drug}}/I_{\text{control}} = 44 \pm 2\%\), \(n = 9\); \(P < 0.05\)). To investigate the mechanism of glibenclamide inhibition of ovine CFTR, we examined the voltage-dependence of channel block. Although glibenclamide inhibition of ovine CFTR was weaker than that of human CFTR (human, \(K_{i}(0 \text{mV}) = 16 \pm 2 \mu M\); ovine CFTR, \(K_{i}(0 \text{mV}) = 42 \pm 12 \mu M\), \(n = 5\); \(P < 0.05\)), there was no difference in the electrical distance across the membrane sensed by glibenclamide (human CFTR, \(z' = 0.25 \pm 0.05\), \(n = 4\); ovine CFTR, \(z' = 0.31 \pm 0.05\), \(n = 5\); \(P = 0.44\)).

We conclude that the pharmacological profile of ovine CFTR shows similarities to, but also differences from that of human CFTR. Variations in the pharmacology of ovine CFTR might result from subtle differences in the three-dimensional structure of CFTR and the local environment (e.g. hydrophobicity and charge) in the vicinity of drug-binding sites.

Supported by the CF Foundation, CF Trust, National Institutes of Health and the Strategic Scholarships Fellowships Frontier Research Networks, Office of the Higher Education Commission of Thailand.

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

Poster Communications

PC198

Aquaporins in pig urinary bladder

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Background: Bladder urothelium, considered a poorly permeable urine-blood-barrier, expresses transmembrane channels called aquaporins (AQPs). AQPs are important for transepithelial water and solute movement in various tissues and their discovery in bladder urothelium supports a more controversial hypothesis that urothelium is able to modify the composition and volume of urine in the lower urinary tract. Thus, AQPs may act as potential targets for treatment of debilitating conditions such as nocturia. The aim was to investigate the expression of AQPs in porcine bladder, a valuable alternative to human bladder for elucidating physiological principles.

Methods: 6-month old female pig bladders were obtained from the local abattoir. PCR was carried out on the c-DNA synthesized from total RNA isolated from the mucosa, and also its component urothelium. Primers were designed for the Sus scrofa AQPs 1-11. PCR products were separated by electrophoresis and sequenced. For immunohistochemical studies, sections of fresh intact bladder dome were fixed (4% formaldehyde), processed and embedded in paraffin. Tissue sections were sectioned at 3 μm and placed on charged slides. After blocking endogenous peroxidase activity and subsequent antigen retrieval, tissue sections were incubated with primary antibodies (dilutions made up in PBS+0.5% Triton and 1% horse serum) overnight at 4°C. Detection used an avidin biotin peroxidase system and a diaminobenzidine substrate. Sections were counterstained with haematoxylin and examined using a Nikon Eclipse 50i microscope.

Results: AQP 1, 3, 9 and 11 mRNA expression were found in the mucosa and in the urothelium itself. AQPs 2, 5, 6, 7, 8 and 10 expression were not detected at the mRNA level. Immunohistochemical analysis demonstrated the expression of AQP 1 in the endothelial layer of arterioles in the sub-urothelial layer. AQPs 3 and 11 were expressed throughout the urothe-
Bladder disorders are highly prevalent and significantly affect the quality of life. Specific treatment is not currently available due to poor understanding of the pathophysiology and there is an urgent need to identify novel therapeutic targets. Since the elucidation of the sensory role of the urothelium—the inner mucosal lining of the bladder, (2) there has been intense interest in identifying new physiological and pathological regulators in this structure. Oxidative stress has been shown to play a significant role in mediating many pathologies including: diabetes and cardiovascular disorders (3). Of many sources of reactive oxygen species (ROS), NADPH oxidase (Nox) is the only enzyme that produces ROS as its sole function and may serve as a promising specific therapeutic target. To date few studies have explored the role of ROS in bladder mucosa. The aim of this study was to identify the presence of Nox in the bladder and its functional significance. Guinea-pigs (male Dunkin-Hartley 450-550g) were euthanized with schedule-1 anesthesia and transmural blood flow was measured by the femoral artery approach before and after bilateral intravesical injection of 100 μM H2O2. ATP release was inhibited by 100 μM Apocynin in mucosa-attached muscle: (pmoles/g/min, median (25%-75% range); control: 13 (7-20) vs. 100 μM Apocynin: 9 (2-17); n=12, p<0.05, Wilcoxon signed rank test). These data present the first evidence for the presence of in situ ROS production and Nox2 and Nox4 in urothelium and bladder smooth muscle. Stimulation of ATP release by exogenous ROS and suppression of its release by Nox inhibitor suggest the functional relevance and the contribution from Nox enzymes.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
control from $96 \pm 22 \Omega \cdot \text{cm}^2$ to $107 \pm 18 \Omega \cdot \text{cm}^2$. While apical LXAh incubation TEER changed from $101 \pm 36 \Omega \cdot \text{cm}^2$ to $113 \pm 37 \Omega \cdot \text{cm}^2$ ($P<0.051; n=3$) was less effective and no change was found with basolateral treatment. This compares to previous research with $1 \text{mM Metformin}$ added basolaterally for 18 hours increased TEER by $36 \%$. Previous research with $1 \text{mM Metformin}$ added basolaterally for 18 hours increased TEER by $36 \%$ ($P<0.0001; n=15$) and reduced glucose flux. With LXAh an increase in TEER was inversely correlated with a decrease in glucose flux by $12 \%$ ($P<0.09; n=2$), no significant difference was observed with apical incubation with LXAh.

In conclusion we provide evidence that LXAh could potentially reduce glucose flux by reducing transepithelial permeability in airway epithelial cells. This highlights a new possible therapeutic benefit of LXAh in the prevention of lung infection.


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PC202

Analysis of the impact of icavafactor on the functional stability of VX-809-rescued F508del-CFTR

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The most common cystic fibrosis mutation, F508del, disrupts cystic fibrosis transmembrane conductance regulator (CFTR) function by impairing protein processing, plasma membrane stability and channel gating. Small molecules called correctors (e.g. lumacaftor [VX-809]) and potentiators (e.g. ivacaftor [VX-770]) have been developed to rescue F508del-CFTR. In a recent clinical trial [1], VX-770 + VX-809 combination therapy achieved milder than expected therapeutic benefit, possibly due to chronic VX-770 (cVX-770) destabilising VX-809-rescued F508del-CFTR [2,3]. Here, we use single-channel recording and Ussing chamber studies to investigate the effects of cVX-770 on VX-809-rescued F508del-CFTR.

Single-channel data from BHK cells expressing F508del-CFTR (see Ref 4) revealed that acute treatment of VX-809-rescued F508del-CFTR with VX-770 (10 $\mu$M) improved greatly open probability ($P_o$) and mildly stabilised F508del-CFTR activity. At the single-channel level, this stabilising effect was enhanced markedly by cVX-770 treatment (1 $\mu$M, 24 h). However, when F508del-CFTR $I_{sc}$ currents were studied in excised membrane patches treated with VX-809 (3 $\mu$M, 24 h) and cVX-770 (1 $\mu$M, 24 h) the majority of current declined rapidly to leave a small population of highly active channels, similar to those observed in single-channel recordings. We interpret this result to suggest that there are two populations of F508del-CFTR $I_{sc}$ channels one of which is stabilised by cVX-770 treatment.

To understand how cVX-770 impacts transepithelial $I_{sc}$ transport, we performed Ussing chamber studies on Fischer rat thyroid (FRT) epithelia expressing F508del-CFTR. After 24 h treatment with VX-809 (3 $\mu$M) and VX-770 (1 $\mu$M), we recorded the F508del-CFTR-mediated short-circuit current ($I_{sc}$) in the presence of a basolateral to apical $Cl^-$ gradient. Chronically administered VX-809 and VX-770 enhanced greatly F508del-CFTR $I_{sc}$ compared to untreated controls, reaching ~25% of wild-type levels. To determine the impact of VX-770 administration on F508del-CFTR functional stability, small molecules were washed from epithelia and $I_{sc}$ recordings made at time (t) = 0, 2, 4 and 6 h after washout. In epithelia treated with VX-809 alone, $I_{sc}$ rapidly declined to untreated levels by 2 h, suggesting that in the absence of VX-809 little newly synthesised CFTR was trafficked to the apical membrane. While VX-809 + cVX-770 did not improve $I_{sc}$ at t = 0 compared to VX-809 treatment alone, it did improve F508del-CFTR functional stability consistent with single-channel data.

We conclude that chronic co-administration of VX-809 and VX-770 appears to rescue a sub-population of F508del-CFTR $Cl^-$ channels in recombinant cells. Further work is required to identify and characterise these channels.


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PC203

Hyperosmolality regulates UT-A6 urea transporter expression in the Caco-2 cell line

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Urea transporters (UTs) encoded by the Slc14a1 (UT-B) and Slc14a2 (UT-A) genes facilitate the movement of urea across plasma membranes. Gastrointestinal urea transporters are thought to play a significant role in the urea nitrogen salvaging process that occurs between mammalian hosts and their gut bacteria (1). UT-A6 has previously been reported in human colon (2) and also in the Caco-2 intestinal cell line (3). Since UT-A gene expression has been shown to be regulated by osmolality (4), the aim of this study was to investigate the role of external osmolality on UT-A6 RNA expression in the human Caco-2 cell line.

Initial experiments using end point PCR (RT-PCR) showed human UT-A6 expression in the ascending colon but not the descending colon. Using this RT-PCR, an effect of varying external osmolality was observed when Caco-2 cells were treated for 24 hours with media of different osmolalities - 200, 300 and 600 mOsm. Further experiments using quantitative PCR (qPCR) then confirmed a stimulatory effect of hyperosmolality (i.e. 600 mOsm) on UT-A6 expression ($P<0.01$, $n = 4$, ANOVA) as detailed in Table 1.

These results confirm the expected regulation of the human UT-A6 urea transporter by external osmolality in Caco-2 cells. The cellular regulatory pathways involved in this process require further investigation. The possible physiological significance of this regulation of hUT-A6 could be related to the increased osmolality of fluid in the ascending colon compared to the sigmoid colon.
Table 1. qPCR data for Caco-2 cells treated with media of varying osmolarity. Primers for UT-A6 and Actin were used. Data are mean ± standard deviation. Fold difference and statistical analysis (ANOVA) is relative to untreated control (300mOsm).

<table>
<thead>
<tr>
<th>Media Osmolality (mOsm)</th>
<th>UT-A6 average CT</th>
<th>Actin average CT</th>
<th>UT-A6 - Actin ACT</th>
<th>Fold difference in UT-A6</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>32.08 ± 0.63</td>
<td>15.55 ± 1.15</td>
<td>16.53 ± 1.31</td>
<td>-3.7</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>300</td>
<td>30.38 ± 0.60</td>
<td>15.18 ± 1.15</td>
<td>15.60 ± 1.54</td>
<td>8.7</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>600</td>
<td>26.32 ± 0.61</td>
<td>17.00 ± 1.36</td>
<td>11.32 ± 1.49</td>
<td>8.7</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

PC204
P2X7 receptor antagonism improves renal blood flow and oxygenation in angiotensin-II infused rat
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Chronic activation of the renin angiotensin system leads to hypertension and ultimately target organ injury. In the kidney, typical early signs are microvascular dysfunction, hypoxia, inflammation and ultimately fibrosis/necrosis. We found previously that the F344 rat is particularly susceptible to renal injury following chronic angiotensin II (ANGII) infusion. We further identified p2rx7, encoding the P2X7 receptor (P2X7R), as a candidate gene for this differential susceptibility (Menzies et al. Front. Physiol. 4:305 2013). Here we have tested the hypothesis that P2X7R antagonism improves vascular function under high ANGII tone. Osmotic minipump containing ANGII (30ng/min) or saline vehicle were implanted into male F344 rats under isoflurane anesthesia. After 14 days, rats were again anaesthetised (Thiobutabarbital; 120mg/kg) and arcuate arteries were killed by cervical dislocation and the left kidney fixed minus PC205
Expression and localisation of the P2X7 receptor in mouse kidney
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P2X7 receptors (P2X7R) are cation channels activated by extracellular ATP. They are expressed in cells of the haematopoietic lineage and play a key role in the normal inflammatory response. Enhanced P2X7R activation is implicated in chronic disease. P2X7R expression is up-regulated in models of renal injury and P2X7R deficiency/antagonism is renoprotective, attributed to reduced inflammation. Despite this, P2X7R antagonists have not improved outcome for inflammatory diseases in clinical trial. The receptor is also expressed in non-immune cells under normal conditions and this may be important in the disease process. Here, we used immunofluorescence to define P2X7R distribution in the kidney of male C57BL6/J mice. We used a commercially available polyclonal P2X7R antibody raised against a C-terminal sequence in exon 13 (APR-004, Alomone, Israel). Since the encoding gene, p2rx7, is highly polymorphic we first compared the antibody’s target sequence against known C57BL6/J mouse variants. The NCBI database (accessed 30 March 2015) identified 5 protein coding splice variants for P2X7R. P2X7-001 was the canonical full-length protein; the remaining forms exhibited variation in either the C terminal sequence or exon number. Antibody APR-004 recognised the full length variant, P2X7-001, and the variant P2X7-005, which has sequence variation in the N-terminus. In Western analysis of whole-kidney protein extracts, APR004 identified products of the predicted molecular weight (n=5). The expression profile of this antibody was then established using immunofluorescence. Male C57BL6/J mice (n=3) were killed by cervical dislocation and the left kidney fixed by immersion fixation in 4% paraformaldehyde. Kidneys were then paraffin-embedded and sectioned at 6µm. Sections were blocked with 1% goat serum and incubated overnight with rabbit anti-P2X7 APR004 (1:1000). An HRP-conjugated secondary antibody was added, followed by a tyramide signal amplification step. APR004 consistently identified immune-positive in the endothelium of the segmental, interlobular, interlobar and arcuate arteries. Punctate staining in the smooth muscle of arteries and arterioles was also observed and there was weak and diffusely intracellular staining in proximal tubules. These findings indicate that the canonical, full-length P2X7R variant is expressed in epithelial, endothelial and vascular cells of the kidney under normal, non-inflammatory conditions. The physiological function of P2X7R is not yet established but may be important if the full therapeutic potential of P2X7R antagonists is to be realised.

Menzies RJ, Unwin RJ, Bailey MA. 2015. Renal P2 receptors and hypertension.
LDF oscillatory components described by the wavelet transform, the detrended fluctuation analysis (DFA) and the multiscale entropy analysis (MSE)

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Laser Doppler Flowmetry (LDF) is a powerful tool to explore, non-invasively, in vivo skin microcirculation. LDF produces a complex signal with oscillatory and fractal properties, sometimes difficult to evaluate. This study aims to characterize the oscillatory components of the LDF signal by mathematical modelling during a classic ‘oxygen challenge test’. A group of 35 healthy subjects (22.1±3.7 years old), both genders, were enrolled after giving their written informed consent. The measurement procedure included: baseline measurement, while breathing room atmosphere; provocation by breathing a 100% oxygen atmosphere; and recovery, while resuming normal atmosphere breathing. LDF signal (PF5010 system, Perimed, Sweden) was recorded on the inferior side of the second toe and then decomposed by: the wavelet transform, giving its major components’ activities (cardiac, respiratory, myogenic, sympathetic, and endothelial); detrended fluctuation analysis (DFA) which allowed the characterization of the self-similarity properties through its alpha (α) exponent; and multiscale entropy analysis (MSE), quantifying the signal complexity over multiple time scales. All statistical comparisons were done with the Wilcoxon signed-rank test (p<0.05). The hyperoxia led to a significant perfusion reduction (p=0.001) followed by full recovery. The endothelial and sympathetic components contributed the most to the LDF signal. During provocation, a significant increase in the respiratory activity (p=0.001) was noted. Cardiac and myogenic activities also increased, while sympathetic and endothelial activities decreased, all of which showing no statistical significance. Wavelet components showed α~0.5, meaning positive self-correlated signals. On baseline, cardiac and respiratory activity components have shown α~1.00, suggesting that these phenomena have a 1/f noise-like behavior. However, myogenic (α~1.44), sympathetic (α~1.54) and endothelial (α~1.47) activity components reflected other characteristics closer to the Brownian noise (α~1.50). α values decreased for all, except for the endothelial component. The sole α value significant change was noted for the cardiac component (p=0.02), which also exhibited the highest entropy level, suggesting a more random-like signal. The components’ entropy levels changed, although non significantly, during provocation - cardiac and endothelial levels decreased, while respiratory, myogenic and sympathetic levels increased. These results suggest that the combined use of the wavelet transform, DFA and MSE contribute to characterize the complex LDF signal and thus helps to better understand the different mechanisms underlying peripheral perfusion regulation.

Buard B et al. (2010). Med Phys 37(6), 2827-2836

Cutaneous microcirculatory reactiveness can be quantitatively described by topical drug application and Laser Doppler Flowmetry (LDF). Nicotinates are often used for this purpose as vasodilator drugs, and the influence of age, gender and skin location on this response has been addressed. This work focuses the effect of local perfusion conditions on that response. 14 young healthy volunteers (22.7±2.8 years old) participated in this study, after giving their written informed consent. Methyl nicotinate (MN) was applied on the dorsum of a randomly chosen foot on two different circulatory dynamics protocols: (a) on the seated position while breathing room atmosphere and then breathing a saturated oxygen atmosphere; (b) on the supine position with both feet at heart position and while performing a passive leg raising (PLR, 45°). The microcirculation response was evaluated with LDF (PF 5010 system, Perimed, Sweden) for 15 minutes after MN application. Several curve-dependent parameters, including full response mean perfusion value and area under the curve (AUC) were compared for each protocol. Furthermore, the signal was assessed at a 32 Hz sampling frequency and analyzed with: the wavelet transform, giving its major components’ activities (cardiac, respiratory, myogenic, sympathetic, and endothelial); and the detrended fluctuation analysis (DFA), which allowed the characterization of the self-similarity properties through its alpha (α) exponent. The first 5 minutes of each volunteer’s plateau phase were used for comparison. All statistical comparisons were done with the Wilcoxon signed-rank test (p<0.05). MN induced vasodilation, steadily increasing perfusion, which was maintained on a plateau until reduction by dermal absorption. The saturated oxygen breathing, a constrictive stimulus, did not significantly change the vasodilation parameters’ values. While breathing the saturated oxygen atmosphere, a significantly lower myogenic activity was noted (p=0.006). No changes for the cardiac, respiratory, sympathetic or endothelial activities were detected. Although no difference as found for the respiratory activity, this alpha exponent was found to be significantly higher while breathing a saturated oxygen atmosphere (p=0.038). The PLR technique reduced the magnitude of vasodilation significantly by reducing the mean perfusion level and AUC. Significant lower activity and alpha exponents were noted for the cardiac component (p=0.004 and 0.005, respectively) relative to the
Assessing the impact of the regular use of hand sanitizers on the epidermal barrier

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Hand washing is an important instrument to prevent disease transmission. Alcohol-based hand sanitizers are preferred for their germicide effectiveness. However, their high alcohol content is reported to cause skin dryness and sensitization upon repetitive use. Stratum corneum is the skin segment responsible for the skin ‘barrier’ against water loss, and is non-invasively assessed with Transepidermal Water Loss (TEWL). Dynamic tests such as plastic occlusion stress test (POST) are commonly used to increase this variable’s discriminative capacity. This study intended to evaluate the impact of the regular use of a commercially available alcohol-based gel on the epidermal ‘barrier’ function, using a bi-compartmental kinetic model that simulates the physiological distribution of water, where the data is analyzed as TEWL decay curves. 13 healthy females (21.6 ± 2.6 years old) were enrolled after informed written consent. Subjects washed the dorsum of one randomly chosen hand for 15 days with an 80% (v/v) alcohol gel. The contralateral hand served as control. TEWL obtained in the inflammatory response. The cutaneous inflammatory response produces a visible erythema that can be quantified with Laser Doppler Flowmetry (LDF). Our aim was to test the efficacy of our antioxidant-containing formulations to reduce the erythema response to a topically applied vasodilator. 12 subjects (26.6±6.4 years old) participated in this study after informed consent. Four 9 cm² areas were marked on the volar surface of both forearms. Three of these areas were topically treated with sunscreen formulations containing or not antioxidant nanoparticles (F1, F2, F3) twice-daily, for 7 days, with the fourth remaining non treated. The formulations’ composition is shown in Table 1. After this period, ethyl nicotinate (EN) was applied on each site to induce an inflammatory response and skin perfusion was measured for 20 minutes with LDF (PF 5010 system, Perimed, Sweden). Area under the curve (AUC) and the slope of the curve on the hyperemic phase were chosen as comparison parameters between sites’ responses. The LDF signal was decomposed with the wavelet transform into its main components (cardiac, respiratory, myogenic, sympathetic, endothelial). Wilcoxon signed-rank test was used as comparative statistics (p<0.05). Significant differences were found for the curve slope between the three formulations. Our results indicated that the presence of gelatin nanoparticles decreased the intensity of the erythema caused by EN when compared to F1. No significant differences were found for the components’ activities between control and treated sites or between the sites themselves. Our results suggest that topical formulations containing antioxidants show potential to inhibit the cutaneous erythema response.

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

PC208

Assessing the impact of the regular use of hand sanitizers on the epidermal barrier

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PC209

Assessing in vivo cutaneous antioxidant activity of sunscreen formulations with Laser Doppler Flowmetry

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Reactive oxygen species are known to play an important role in the inflammatory response. The cutaneous inflammatory response produces a visible erythema that can be quantified with Laser Doppler Flowmetry (LDF). Our aim was to test the efficacy of our antioxidant-containing formulations to reduce the erythema response to a topically applied vasodilator. 12 subjects (26.6±6.4 years old) participated in this study after informed consent. Four 9 cm² areas were marked on the volar surface of both forearms. Three of these areas were topically treated with sunscreen formulations containing or not antioxidant nanoparticles (F1, F2, F3) twice-daily, for 7 days, with the fourth remaining non treated. The formulations’ composition is shown in Table 1. After this period, ethyl nicotinate (EN) was applied on each site to induce an inflammatory response and skin perfusion was measured for 20 minutes with LDF (PF 5010 system, Perimed, Sweden). Area under the curve (AUC) and the slope of the curve on the hyperemic phase were chosen as comparison parameters between sites’ responses. The LDF signal was decomposed with the wavelet transform into its main components (cardiac, respiratory, myogenic, sympathetic, endothelial). Wilcoxon signed-rank test was used as comparative statistics (p<0.05). Significant differences were found for the curve slope between the three formulations. Our results indicated that the presence of gelatin nanoparticles decreased the intensity of the erythema caused by EN when compared to F1. No significant differences were found for the components’ activities between control and treated sites or between the sites themselves. Our results suggest that topical formulations containing antioxidants show potential to inhibit the cutaneous erythema response.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Table 1. Qualitative and quantitative (% w/w) composition of the sunscreens formulations

<table>
<thead>
<tr>
<th>Composition</th>
<th>Concentration (% w/w)</th>
<th>Formulation Code</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F1</td>
</tr>
<tr>
<td></td>
<td>Hydroxyethyl Acrylate</td>
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</tr>
<tr>
<td></td>
<td>/ Sodium Acryloyl</td>
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</tr>
<tr>
<td></td>
<td>Dimethacrylate Taurine</td>
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</tr>
<tr>
<td></td>
<td>Copolymer &amp; Isobornyl</td>
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</tr>
<tr>
<td></td>
<td>Isobornyl) Acrylate</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Copolymer &amp; Polyisobornyl 60</td>
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<tr>
<td></td>
<td>Glycine</td>
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<td></td>
<td>Disodium EDTA</td>
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<td>Cyclomethicone</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>(methylisobutyl) polyurethane</td>
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</tr>
<tr>
<td></td>
<td>Ethylhexyl methoxyisobutyl</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Butyl methoxyisobutyl</td>
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<tr>
<td></td>
<td>Ethylhexyl p-methoxybenzyl</td>
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<td></td>
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<td></td>
<td>PABA</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>B-NC</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>R-NC</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Age</td>
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</tr>
</tbody>
</table>

B-NC – blank gelatin nanoparticle; R-NC - rutin-loaded gelatin nanoparticles

* Qualitative composition was reported in accordance with INCI (International Nomenclature of Cosmetic Ingredient)

** Enough to complete 100%


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**PC210**

The impact of eccentric and concentric exercise on muscle function in young and older men

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Skeletal muscle repair is vital for the maintenance of muscle mass & function, both of which decline with age. Research suggests a greater susceptibility to muscle injury & prolonged recovery with advancing age (1), which may contribute to sarcopenia & dynapenia. We investigated the effects of ageing on functional responses to a single bout of eccentric (ECC) or concentric (CON) exercise. We hypothesized: 1) ECC would cause greater functional decline & slower recovery in old (O) vs. young (Y) men, & 2) CON would minimally impact function. We recruited 8 Y (22±1 y, mean±SEM) & 8 O (70±1 y) healthy, exercise naive volunteers. Participants performed a single bout of unilateral ECC & CON (legs randomized to ECC or CON): 7×10 repetitions at 80% of ECC/CON one-repetition maximum (1-RM). We assessed muscle soreness, sensitivity to pain, peak torque, power & lower body function at baseline (BL), immediately after (0 h) & 5, 24, 72 & 168 h following ECC & CON. Y had a higher ECC & CON 1-RM than O (ECC: Y: 265±18 vs. O: 190±13; CON: Y: 152±14 vs. O: 95±6 kg, t-test P<0.005). At BL, Y exhibited greater peak torque than O (Y: 253±21 vs. O: 166±15 Nm; two-way ANOVA P<0.05). ECC resulted in peak torque declines (compared to BL) in both Y & O at 0 h (Y: 171±15.3, P=0.0001; O: 128.5±11, P<0.05) which persisted up to 24 h post-ECC in the O (113±13, P<0.0005) & 72 h in the Y (201±24, P<0.0005). Peak torque had returned to BL in both groups by 168 h. CON produced peak torque declines in the Y at 0 h (207.5±20.3, P<0.05), which remained at 72 h (210±29, P<0.05) & returned to BL by 168 h. CON had no effect on peak torque in the O. No BL age-related differences were observed for power (Y: 228±32 vs. 179±26 W). Immediately after ECC, power declined in the Y at 0 h (169±16, P<0.05) & persisted until 24 h (150±24, P<0.0005). Power did not decline in the Y following CON or in the O following ECC or CON. No changes in lower body function, measured by a short physical performance battery test (SPPBt), were seen in the Y or O. Sensitivity to pain was heightened in both Y & O at 5 h (Y: 11±2; O: 7±1 lbs, P<0.05) post-ECC compared to BL (Y: 13±1; O: 10±1) & remained until 72 h (11±1, P<0.05) in the Y only. Sensitivity to pain did not change post CON. Following ECC, muscle soreness was elevated at 0 h (6±1 cm, one-way ANOVA P<0.005) & continued until 72 h (4.9±0.8, P<0.005) in the Y, & was elevated at 24 h in O (6±1, P<0.0001). Soreness was elevated at 24 h post CON in both Y & O (Y: 5±1; O: 4±1, P<0.005). We find no evidence to support an age-related increase in ECC injury susceptibility or delayed functional repair. These findings may be explained by differences in absolute loads lifted with O being exposed to markedly lower loads during the study, perhaps due to reduced cross sectional area. Furthermore, the data suggests ECC is safe to perform in the O. Overing, R.M. & Brooks, S. V (2013) Eccentric exercise in aging and diseased skeletal muscle: good or bad? Journal of applied physiology (Bethesda, Md. : 1985). Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23471953>

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**PC211**

Acute mental stress and its impact on systemic vascular endothelial function in obese adults

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Background and aims: Everyday life stressors such as acute mental stress can result in transient impairments in vascular endothelial function (Ghiadoni et al., 2000). Similarly, obesity is known to impair arterial smooth muscle function (Ayer et al., 2011). However, their combined effect remains unknown. To investigate this, we determined to what extent a battery of neuropsychometric tests can result in transient impairments in vascular endothelial function in obese adults related to non-obese controls.

Methods: Eight obese [26 (mean) ± 8 (SD) years old; BMI = 33 ± 4] and 8 non-obese [21 ± 4 years old; BMI = 25 ± 3] male participants were recruited. Brachial artery flow-mediated dilatation (FMD, Acuson P50, Siemens) was measured 1 hour before and immediately following a standardised battery of neuropsychometric tests. Following a 1 min baseline, the occluding cuff (distal) was inflated (>250mmHg) for 5 min and a subsequently released for 5 min into recovery. Brachial artery diameter and flow were recorded continuously throughout the test. Baseline data correspond to the 1-min average pre cuff inflation and peak diameter was measured as the average of the highest 3 values recorded. Data were analysed using automated edge-detection and wall-tracking.
software (Brachial Tools, Medical Imaging Application). Following confirmation of distribution normality (Shapiro-Wilk W tests), data were analysed using a 2-way (Time x Group) repeated measures ANOVA. Data are expressed as mean ± SD with significance set at \( P < 0.05 \).

**Results:** Psychometric stress was shown to impair FMD (Pre: 5.3 ± 1.4% vs. Post: 4.4 ± 1.4%, \( P < 0.05 \), Figure 1A). The obese group also displayed a lower FMD than their non-obese peers during both FMD tests (P<0.05). The obese further exhibited a decrease in baseline flow from 0.11 ± 0.02 m/sec before to 0.08 ± 0.02 m/sec after acute mental stress (P<0.05, Figure 1B).

**Conclusion:** The present results confirmed that acute mental stress impairs systemic vascular endothelial function. Contrary to our original hypothesis, this impairment was not further compounded by obesity.

**Figure 1.** Flow-mediated dilatation (FMD) and baseline flow in the non-obese (open bars) and obese (closed bars) groups before and following acute mental stress. Values are means ± SD. \(* P<0.05\) vs. Pre Mental Stress; \(\dagger P<0.05\) vs. non-obese

Ghiadoni et al. (2000). *Circulation* 102, 2473-2478

Ayer et al. (2011). *Obesity* 19, 54-60

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

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**PC212**

**Resistance exercise increases mTOR and Integrin β-3 association in human skeletal muscle**

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**Introduction:** The maintenance of skeletal muscle mass and function is critical for health and wellbeing, with deterioration in muscle size and strength associated with numerous chronic diseases. Skeletal muscle protein balance is highly dependent on the activity of the serine/threonine protein kinase, mammalian target of rapamycin complex 1 (mTOR), which when active can stimulate both protein synthesis and attenuate protein degradation. Mechanism(s) of mTOR activation in human skeletal muscle after contractile activity are still not fully understood but are thought to involve signal transduction events through mechanical sensing proteins located within or in close proximity to the cell membrane. The Integrin-β proteins play a role in cardiac muscle hypertrophy; however, little is known about the role of integrin β-3 in response to resistance exercise in human skeletal muscle.

**Methods:** Fourteen young, healthy male volunteers (24.7 ± 2.7 yrs, 177.4 ± 6.8 cm, BMI 26.1 ± 2.2 kg m\(^{-2}\)) performed an acute bout of resistance exercise (4 sets of leg press and knee extension at 8-10 repetition maximum) prior to ingesting a beverage providing 20/44/1g of protein/carbohydrate/fat (PRO: \( n=7 \)) to enhance post-exercise muscle anabolism or an energy-free control (CON: \( n=7 \)). Muscle biopsies were collected from the vastus lateralis pre and post-exercise and 1h and 3h after beverage ingestion. Skeletal muscle samples were immediately frozen in isopentane cooled by liquid nitrogen. Samples were serially sectioned for subsequent immunofluorescent analysis. Pearson’s correlation coefficient analysis was conducted to quantify protein-membrane and protein-protein interaction.

**Results:** Integrin β-3 was observed to translocate to the sarcolemmal membrane following resistance exercise. Quantification showed an enhanced association between Integrin β-3 and cell membrane 1 and 3h post exercise with no difference between groups (CON: 0.29±0.02 pre, 0.33±0.03 1h post, 0.31±0.03 3h post \( P<0.05 \); PRO: 0.31±0.02 pre, 0.37±0.02 1h, 0.36±0.01 3h post \( P<0.05 \)). In addition, we observed increased association between Integrin β-3 and mTOR on the cell periphery in both CON and PRO groups (CON: 0.40±0.02 pre, 0.44±0.03 1h, 0.45±0.03 3h post \( P<0.05 \) and PRO: 0.43±0.02 pre, 0.49±0.02 1h, 0.48±0.02 3h post exercise respectively \( P<0.05 \)).

**Conclusion:** We present novel data detailing Integrin β-3 redistribution to the cell membrane following a single bout of resistance exercise. Further, we report that Integrin β-3 and mTOR interact at the cell periphery following resistance exercise, suggesting that Integrin β-3 may be involved in mTOR activation in human skeletal muscle.

Funded by: China Scholarship Council Li Siguang Scholarship (ZS) and Natural Sciences and Engineering Research Council of Canada (DRM/LLS)
Predicting individual oxygen uptake responses to interval exercise in humans

M.J. Davies, G.K. Lyall, C.K. Berry, K.M. Birch, A.P. Benson and C. Ferguson

School of Biomedical Sciences, University of Leeds, Leeds, UK

Interval exercise (IE) interventions drive superior physiological adaptations compared to continuous exercise (Wisløff et al., 2007). However, identification of which IE protocol most effectively promotes physiological adaptations is unclear as the IE work rate (WR) and work:recovery durations interact effectively to influence the intensity domain in which an individual is working (Turner et al., 2006). Therefore, we assessed whether an individualised computational model along with measures from two standard exercise tests would allow prediction of the WRs that would place subjects in a desired exercise intensity domain for a variety of IE protocols.

Eight healthy males (mean±SD age 21±1 y, height 178±6 cm, mass 75±8 kg) performed a ramp incremental test to the limit of tolerance on a cycle ergometer. Lactate threshold (LT), an index of critical power (CP) and peak VO$_2$ (VO$_2$peak) were estimated from these data to obtain thresholds for the exercise intensity domains. On a separate visit, subjects performed a moderate-intensity step protocol to measure baseline VO$_2$ (VO$_2basal$), gain (dVO$_2$/dW) and phase 2 VO$_2$ kinetics (rVO$_2$). These data were used to individually parameterise a computational model of gas exchange and circulatory dynamics (Benson et al., 2013). The model was used to predict the WRs that would place each subject in the moderate, heavy and very heavy exercise intensity domains for work:recovery durations of 15:15, 30:30, 60:60 and 120:120 s (i.e. 12 WRs per subject), with these each performed for up to 22 min over 12 subsequent visits. Measured IE breath-by-breath VO$_2$ was then compared to model-predicted VO$_2$ outputs. Measured parameters were: VO$_2$basal: 0.7±0.07 l/min ($p$=0.61–0.8); rVO$_2$: 29.9±6.0 (20.7–39.3) s; LT: 1.82±0.22 (1.58–2.28) l/min$^{-1}$; CP index: 2.77±0.40 (2.35–4.05) l/min$^{-1}$, and VO$_2$peak: 3.58±0.42 (3.09–4.53) l/min$^{-1}$, highlighting the large inter-variability in model inputs and intensity domain thresholds (LT range 39–59 % VO$_2$peak; CP index range 56–90 % VO$_2$peak). Mean error between measured and model-predicted VO$_2$ responses ranged from 0.11±0.02 l/min for the very heavy intensity 15:15 s protocol to 0.32±0.11 l/min$^{-1}$ for the very heavy intensity 15:15 s protocol. However, peak metabolic disturbance during IE (i.e. the peak of the VO$_2$ oscillation in each work bout) did not exceed LT (for moderate-intensity IE protocols) and the CP index (for heavy intensity IE protocols), and in 59% of very heavy intensity IE protocols the limit of tolerance was reached.

Thus, a computational model and data from two standard exercise tests can predict individualised WRs that place subjects in the desired exercise intensity domain for a range of IE protocols. This therefore provides a methodology to control for exercise intensity when investigating how IE can be optimised to maximise physiological adaptations.


PC215

A comparison of the active range of motion of the cervical spine in elite sportmen

B.B. Zietsman, A. Heusch and P. McCarthy

Life Science and Education, University of South Wales, Pontypridd, mid-glamorgan, UK

Performance of sport at elite level creates enormous demands on the physiology. However, unless damage obviously impacts on performance, it can be ignored in preference to the resolution of the more noticeable problems. We have shown the impact of this in relation to changes in the functional capacity of the cervical spine in rugby (Lark and McCarthy; 2007, 2010). The study presented here initially arose from observations when sourcing suitable control groups for the rugby study. The protocol used in this study is the same as that described previously (Lark and McCarthy, 2007). Over the period spring 2012 to autumn 2014, 12 participants were included in a range of sports (see Table 1) were invited, via their coaches, to take part in this study. Exclusion criteria included: current neck pain or trauma, previous surgery, current or previous serious pathology/trauama to the cervical spine. A cervical range of motion device (CROM device; Performance Attainment Associates, Minneapolis, USA: Capuano-Pucci et al., 1991) was used to measure active cervical range of motion (ACROM) following a standardised warm up procedure. Full flexion and full extension were measured from the subject’s neutral point, in alternating order, to reduce potential order effects. Ethical approval was granted by the Faculty of Health Science and Sport’s Ethics Committee, University of Glamorgan, written informed consent was obtained from each participant. These findings confirm that playing elite contact sports such as rugby, both union and league and ice hockey can be associated with a decrease in ACROM. Swimmers appear to generally have the greater ACROM. In the case of helmet wearing sports such as American Football and Ice Hockey, these players appear to have an altered head neutral position.

Table 1: The data for each sport was normally distributed (Shapiro-Wilk), ANOVA with post-hoc tukey analysis. Data is presented as mean ± SD: * indicates p<0.05 and ** p<0.01 compared to rugby union.

Table: Human rehydration following exercise typically advises drinking 120-150% of the body mass lost (∆BM), depending on sodium ingestion. No allowance is made for the possibility that substantive mass loss in exercise may arise from glycogenolysis (substrate loss plus water release; [1]), which would presumably require rehydration at a rate more commensurate with glycogenosis [2]. Renal reabsorption of sodium and water might also benefit from less aggressive rehydration [3, 4]. We tested the hypothesis that BM recovers more slowly following hypohydration incurred by exercise than by heat, whereas plasma volume would expand more rapidly following exercise than by heat, whereas human rehydration more slowly but expand plasma volume rapidly when hypohydration is incurred by exercise than by heat per se.


Figure 1: Functional capacity of coagulating blood, d, Tc (A); Gel time of coagulating blood, TC (B); Plasma clotting time, aPTT (C).

PC216

Humans rehydrate more slowly but expand plasma volume rapidly when hypohydration is incurred by exercise than by heat per se

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1School of Physical Education, Sport and Exercise Sciences, University of Otago, Dunedin, Otago, New Zealand, 2Alma College, Alma, MI, USA, 3School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, UK, 4Department of Physiology, University of Otago, Dunedin, New Zealand and 5Université de Lorraine, Nancy, France

Guidelines for rehydration following exercise typically advise drinking 120-150% of the body mass lost (∆BM), depending on sodium ingestion. No allowance is made for the possibility that substantive mass loss in exercise may arise from glycogenolysis (substrate loss plus water release; [1]), which would presumably require rehydration at a rate more commensurate with glycogenosis [2]. Renal reabsorption of sodium and water might also benefit from less aggressive rehydration [3, 4]. We tested the hypothesis that BM recovers more slowly following hypohydration incurred by exercise than by heat, whereas plasma volume would expand more rapidly following exercise.

Eight physically-active volunteers (6 males, 2 females; BM 72.5 kg (9.3); mean (SD)) dehydrated to at least 3% BM passively in the heat (40.3 °C) to ingest 120% of their ∆BM as rapidly as comfortably possible; ¼ as a sports drink (5.6% carbohydrate, 24 mMol/L sodium) and ¼ as water. Fluid balance was recorded for 2 h, after which they left the lab and recorded ad libitum-drinking and micturition volumes until returning the next morning. The ∆BM averaged -3.6% (0.5) in EX-HYPO and -3.7% (0.8) in HEAT-HYPO (t-test: p= 59), while plasma osmolality was 293 (3) and 293 mOsmol/kg (5) (p = 83). Drinking duration was 66 min (38) in EX-HYPO and 85 min (37) in HEAT-HYPO (p= 0.9). Urine production during this initial 2 h was larger in EX-HYPO (496 mL (124)) than in HEAT-HYPO (336 mL (202); p=0.05; mean ±95%CI difference: 160 ±160 mL), and was also
less variable (range: 285 to 605 mL, vs 40 to 690 mL). Urine volume thereafter was similar between EX-HYPO (2093 mL (303)) and HEAT-HYPO (2118 mL (889); p= .93), as was the gain in BM from baseline (0.65 (0.40) vs 0.47 kg (0.62); p= .51). Plasma volume exceeded its resting baseline by 5.6% (5.9) at 1 h, and by 8.6% (2.0) at 2 h in EX-HYPO, but not in HEAT-HYPO (-1.7% (7.8) at 1 h and 0.9% (5.3) at 2 h; mean differences: 7.2 ±3.7% and 7.7 ±3.6%). These divergent responses were still evident the following morning (4.1 vs. -3.6%; difference: 8.8 ±6.5%). In conclusion, water balance is regained more slowly following exercise- than heat-induced hypohydration; presumably at least partly because some of BM deficit is attributable to glycogen metabolism and associated water release. Physiologically, this BM deficit may therefore not represent a metabolic deficit, as the gain in BM was identical for the first 60 min of exercise but increased on CHO vs. KE by 2 h (p<0.05). No differences in cortisol, blood glucose or insulin were observed between CHO and KE. Mean exercise RQ was significantly lower on KE vs. CHO (0.89±0.02 vs. 0.97±0.02, p<0.05). IMTAG and glycogen were not significantly different between conditions at baseline, however IMTAG fell by 24% on KE vs. 1% on CHO (p<0.01) after 2 h of exercise. There was a direct relationship between IMTAG oxidation and % of slow type muscle fibre content (r = 0.65, p<0.05) on KE. Conclusion: Nutritional ketosis is able to harness the innate metabolic response to starvation, increasing IMTAG oxidation 20-fold during exercise in the presence of normal muscle glycogen, co-ingested carbohydrate and elevated insulin. These findings may have important implications not just for endurance exercise performance, but for conditions of dysregulated fatty acid oxidation such as insulin resistance.

Poster Communications

PC217

Nutritional ketosis increases intramuscular fat oxidation during exercise


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Introduction: Human metabolic responses to energy crisis are hardwired to favour ketosis[1]. Ketone bodies act as substrates and signals to conserve glucose stores by altering substrate competition for respiration[2]. Endurance exercise performance may be constrained by the same metabolic considerations pertinent to starvation, albeit occurring on an accelerated scale[3]. We investigated whether acute nutritional ketosis could mimic starvation physiology and increase fat oxidation in muscle during exercise, without restricting carbohydrate intake.

Methods: After providing informed consent, and after an overnight fast, 7 highly trained male volunteers (age 29.7 y, VO2 max 4.8 L/min) drank 1.14 g/kg BW of ketone ester (KE) and dextrose, or isocaloric carbohydrates alone (CHO), in a randomised, blinded, crossover design. After 30 min, athletes performed 2 h of bicycle exercise at a fixed intensity of 70% Wmax. Blood samples were obtained via an intravenous catheter at regular intervals during exercise, and assayed for β-hydroxybutyrate (BHB), lactate, glucose and fats (FFA). Indirect calorimetry (Cortex, Metalyser) was performed at identical times to quantify respiratory quotient (RQ) and VO2. Muscle biopsies of the vastus lateralis were obtained before and after exercise. Muscle samples were cryosectioned, stained for intramuscular triglyceride (IMTAG), glycogen, and fibre type, and quantified using confocal microscopy. A 2-way repeated measures ANOVA with post-Hoc Tukey correction was used to determine statistical significance (considered as p<0.05).

Results: Blood BHB rose from 0.1 to 2.2 mM (p<0.01) following KE ingestion, reaching 3.2 mM after 2 h of exercise. BHB concentration remained unchanged on CHO throughout exercise (0.1 ±0.05 mM, p>0.01 vs. KE). Blood lactate concentrations were significantly lower on KE vs. CHO (p<0.05). FFA were identical for the first 60 min of exercise but increased on CHO vs. KE by 2 h (p<0.05). No differences in cortisol, blood glucose or insulin were observed between CHO and KE. Mean exercise RQ was significantly lower on KE vs. CHO (0.89±0.02 vs. 0.97±0.02, p<0.05). IMTAG and glycogen were not significantly different between conditions at baseline, however IMTAG fell by 24% on KE vs. 1% on CHO (p<0.01) after 2 h of exercise. There was a direct relationship between IMTAG oxidation and % of slow type muscle fibre content (r = 0.65, p<0.05) on KE. Conclusion: Nutritional ketosis is able to harness the innate metabolic response to starvation, increasing IMTAG oxidation 20-fold during exercise in the presence of normal muscle glycogen, co-ingested carbohydrate and elevated insulin. These findings may have important implications not just for endurance exercise performance, but for conditions of dysregulated fatty acid oxidation such as insulin resistance.

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

PC218

The effect of exercise intensity on circulating blood ketone body clearance

P.J. Cox, T. Kirk, D. Dearlove and K. Clarke

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Introduction: Ketone bodies have energetic advantages over other fuel substrates, and may alter oxidative fuel selection in working muscle by providing an alternative carbon source for respiration[1]. However the relationship between exercise intensity and the clearance (metabolic disposal) of ketone bodies from the bloodstream is unknown.

Methods: After an overnight fast, 8 highly trained male endurance athletes (age 28.6 y, VO2 max 5.2 L/min) drank 0.573g/kg BW of ketone ester (KE), in a randomised crossover design. After 10 min, athletes performed 45 min of bicycle exercise at fixed intensities of 40% Wmax (Low), or 75% Wmax (High) on separate study days. Identical studies were also performed at rest. Blood samples were obtained via an intravenous catheter at regular intervals during exercise, and assayed for β-hydroxybutyrate (BHB), lactate, glucose and fats (FFA). Indirect calorimetry (Cortex, Metalyser) was performed at identical times to quantify respiratory quotient and VO2. Urinary excretion of BHB was determined from urine collections before and after exercise. A 3-way repeated measures ANOVA with post-Hoc Tukey corrections was used to determine statistical significance (considered as p<0.05).

Results: Blood BHB rose from 0.1 to 2.7 ± 0.1 mM (p<0.01) 10 min following KE ingestion. BHB concentration increased
at rest reaching ~6mM after 45 min. In contrast, circulating BHB concentrations fell with the onset of exercise, and were significantly reduced on High (2.4 ± 0.1 mM) vs. Low (3.3 ± 0.2 mM) intensity (p<0.05). BHB AUC was reduced in both high (50%) and Low (30%) vs. rest (p<0.05) after 45 min of exercise. An exponential decrease in BHB AUC was observed with increasing VO_{2} (r² = 0.7, p<0.01). Only a small fraction of circulating BHB (0.1 ± 0.04 g) was eliminated in the urine. As expected, mean exercise lactate concentrations were greatest on High (3.6 ± 0.5 mM) vs. Low (1.2 ± 0.1 mM, p<0.05) and rest (1± 0.1mM, p<0.05). No differences in blood glucose and FFA were observed between study conditions. RQ and VO_{2} increased with increasing exercise intensity as would be expected (p<0.05 rest vs. low, High, and High vs. Low).

Conclusion: Conventional calorimetric equations[2] to calculate substrate oxidation during exercise do not account for ketone bodies as an energy source. These data demonstrate an exponential increase in ketone blood ketone body clearance with increasing oxygen consumption, suggesting the hierarchy of muscular fuel selection was altered during incremental exercise in the presence of ketosis. Urinary elimination of ketone bodies in exercise was negligible and cannot explain the increase in metabolic disposal.

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

PC219

A ketone ester drink sustains exercise performance whilst reducing muscle glycolysis

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Introduction: We developed a nutritional ketone ester drink that mimics the physiology of starvation by providing ketones as a fuel source[1]. Ketone bodies have energetic advantages over other fuel substrates, and may alter oxidative fuel selection in working muscle due to their evolutionary role in conserving glucose[2]. We tested whether ketone bodies could alter glycolysis during highly glycolytic exercise: a physiological state of metabolic inflexibility where glucose is the preferred substrate[3].

Methods: After providing informed consent, and following an overnight fast, 10 highly trained male volunteers (age 28.4±y, VO_{2 max} 5.4 L/min) drank isocaloric quantities of carbohydrate (CHO), fat (FAT), or ketone ester (KE)(0.573 g/kg BW), in a randomised blinded crossover design. After 25 min, athletes performed 1 hour of bicycle exercise at 75% W_{max}. Muscle biopsies of the Vastus Lateralis were obtained before and after exercise. Muscle metabolites were extracted via the Folsch method, and analysed using a triple quadrupolar mass spectrometer (Waters, UK). 3-way repeated measures ANOVA with post-Hoc Tukey corrections were used to determine statistical significance (considered as p<0.05).

Results: Blood ketones rose from 0.1 to 3.4 mM (p<0.01) following ketone drinks. Intramuscular ketone concentrations were 3 fold greater on KE vs. FAT/ CHO. Intramuscular glycolytic intermediates, glyceraldehyde-3-phosphate, 2,3-phosphoglycerate and pyruvate, were significantly lower on KE vs. CHO/FAT (p<0.05). Fructose-1,6-bisphosphate, dihydroxyacetone phosphate (DHAP), and 1,3-bisphosphoglycerate were the same at rest. After 60 min exercise at 75% W_{max} the sum of intramuscular glycolytic intermediates increased 1-2 fold over the resting conditions (p<0.05). However, with the exception of DHAP, intramuscular glycolytic intermediates were ~40% lower on KE vs. FAT/CHO (p<0.05) at the same workload, whereas FAT vs. CHO were not different.

Conclusion: Nutritional ketosis represents a novel physiological state, providing a new fuel for oxidative respiration, whilst decreasing muscle glycolysis during highly glycolytic exercise. This suggests that re-evaluation of the hierarchy of muscular fuel selection in exercise is required in the presence of ketosis. Acute nutritional ketosis may have utility in other physiological or pathological conditions where glycolytic demands are high and substrate energetic performance is paramount.

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Variations in resting serum testosterone concentration in healthy young sportsmen and untrained male adults

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Testosterone is an anabolic steroid that has been associated with a variety of factors such as increase in skeletal muscle mass, spatial abilities, muscle development and physical exercise (Pillay, 2006). Physical activity is routinely associated with most of these factors and these factors have the potential to vary amongst athletes in their various competitive sports. Therefore, we sought to examine and quantify the existence of attainable variations in resting serum testosterone (ST) concentration in different groups of sportsmen (6 types of sports) and in untrained control human subjects. Eighty-one (n=81) healthy male participants comprising of 21 controls, 10 footballers, 10 athletics, 10 karate, 10 volleyballers, 10 basketballers and 10 baseballers participated voluntarily in this study after acquiring written consent and ethical approval. Their Mean±SEM age, weight and height are 22.0±0.8years, 64.1±2.2kg and 1.74±0.3 cm respectively. Participants rested while in sitting position for about 30 minutes during which blood pressures and heart rates were taken. 5mLs of venous blood was drawn from the antecubital fossa of the participants (aseptically) into plain bottles between 8:00 and 10:00am after overnight fasting. Serum was collected, stored at -250C after centrifugation (at 3000 rpm for 5 minutes), and later assayed using enzyme linked immunosorbent assay (ELISA) kit for serum testosterone (ST) concentrations. ST values from the 7 groups were recorded as Mean±SEM and compared using One-way ANOVA set at 95% significance. From our findings, the values of serum testosterone (ng/ml) was 8.5±0.5 in
controls, 6.0±0.6 in footballers, 8.9±0.5 in athletics, and 8.1±0.8 in basketballers, 9.5±0.5 in volleyballers, 6.6±0.5 in baseballers and 6.9±0.6 in karates respectively. There was a significant difference (p<0.05) in the resting ST of the footballers vs volleyballers with no significant difference (p>0.05) in other groups; with an overall p value of 0.0072 (p<0.05) in all the experimental groups. Thus, this data revealed variations that may exist in resting ST levels in 6 different sporting activities and in untrained male participants. The results of this study may be influenced by increased muscle mass or metabolism, stress, intensity of the sport, and the use of exogenous steroids. In conclusion, resting ST varies significantly in sporting activities and in untrained young healthy male humans necessitating that special care should be taken whenever the serum testosterone is to be interpreted in athletics.


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**PC221**

**Role of oxygen (O₂) and cyclooxygenase (COX) products in muscle fatigue in healthy young (Y) and older (O) men**


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In Y men, breathing 40%O₂ and inhibition of COX with aspi-rin, alone or together caused similar attenuation of post-con-traction hyperaemia by ~60% and 6.9% in Y men, breathing 40%O₂ in recovery from MVE provides sufficient additional O₂ to muscle fibres to reduce fatigue in a second contraction, even during COX inhibition, which reduces muscle blood flow. By contrast, in healthy O men, breathing 40%O₂ in recovery is similarly beneficial, but uptake of sufficient O₂ to improve recovery from fatigue is more easily compromised when O₂ delivery is attenuated by COX inhibition. PGs generated during contraction do not seem to affect muscle contraction or recovery from fatigue in Y or O men.


Junojo et al. (2014). J FASEB 28:1, 1106.4

Testa M et al. (2007). J Appl Physiology 103, 1412-1418

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**PC222**

**Type 3 Inositol (1,4,5)-Trisphosphate Receptor expression level modulates migratory capacities of breast cancer cells**

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As the most common lethal cancer in women worldwide, breast cancer remains a research priority. If the involvement of ion channels in cell signaling pathways leading to cancer is now well established, the role of inositol (1,4,5)-trisphosphate (IP₃) receptors (IP₃R) remains enigmatic. In this context, we investigated the involvement of the three IP₃Rs (IP₃, 2 & 3) in migration processes of human breast cancer cells. Migratory behavior of cancer cells was measured by transwell migration assays and circularity indices determination on three breast cancer cell lines: the low-migrating MCF-7 cell line (1 ± 0.66, N=3) and the highly migrating and invasive MDA-MB-231 (15.43 ± 1.33 for MDA-MB-231 (N=3, P=0.001) vs MCF-7) and MDA-MB-435s cell lines (25.48 ± 1.59 for MDA-MB-435s, N=3, P=0.0006 vs MCF-7 and P=0.012 vs MDA-MB-231). Using Q-PCR and western-blot approaches, we demonstrate that a higher IP₃R3 expression level is correlated to a stronger migration capacity of the cell lines. IP₃R & 2 appear equally expressed in MCF-7 like in MDA-MB-435 cells. Gene silencing of IP₃R3 (siR3) in MDA-MB-231 and 435s leads to a significant decrease of their migration abilities (0.57 ± 0.13 vs. 1 ± 0.24 (N=3, P=0.04) for siR3 and siC conditions in MCF-7, 0.53 ± 0.15 vs 1 ± 0.05 (N=3, P=0.03) for siR3 and siC conditions in MDA-MB-231, and 0.08 ± 0.17 vs 1 ± 0.06 (N=3, P=0.001) for siR3 and siC conditions in MDA-MB-435s) without changing their proliferation rate. Conversely, stable overexpression of IP₃R3 in MCF7 cells significantly increases their migration capacity, this latter effect being completely reversed by siR3. The additive effect of ATP increasing migration in these IP₃R3 overexpressing cells was also abolished by IP₃R3 silencing. Interestingly this IP₃R3 dependent modulation of migration capacities was not observed with IP₃R1 or IP₃R2 silencing in all cell lines. As a key effector in migration process, we then...
investigated the calcium signaling in these three cell lines. Calcium imaging assays reveal an increasing calcium resting ratio according to cellular migration capacities (0.59 ± 0.015 for MCF-7 (n=81), 1.22 ± 0.009 for MDA-MB-231 (n=134) and 1.45 ± 0.01 for MDA-MB-435S (n=67)). IP3R silencing causes a drastic modification of the temporal feature of ATP (5µM)-induced Ca2+ signaling in the three cell lines, displaying a pattern of sinusoidal Ca2+ oscillations instead of a plateau phase. This calcium signature was specific to IP3_R3 as it could not be observed by IP3_R1 or IP3_R2 silencing. It can therefore be hypothesized that the migration capacity of cells could be related to the temporal feature of the IP3_R3 dependent Ca2+ signal. Altogether, our results demonstrate that IP3_R3 expression level modulates the migration capacity of human breast cancer cells and led us to propose IP3_R3 as a key target in cancer migration processes.

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The blood pressure lowering effect of the aqueous calyx extract of Hibiscus sabdariffa may occur via a sympathetic nervous system dependent mechanism

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The aqueous calyx extract of Hibiscus sabdariffa (HS) lowers blood pressure (BP) in animals and man due to its anthocyanin, polyphenol and hibiscus acid contents. This study tested the hypothesis that the hypotensive effect of HS may occur through a sympathetic nervous system (SNS) dependent mechanism. Following ethical approval and informed consent, the Harvard step test (HST) was performed in healthy subjects (n=14) to activate the sympathetic nervous system (SNS) before and after the oral administration of HS tablets (15mg/Kg). The BP and pulse rate (PR) responses were measured. Mean arterial pressure (MAP; taken as representative BP) was calculated. Experiments were performed in accordance with the Principles of the Declaration of Helsinki. Results are expressed as mean ±SEM. Paired t test was used for statistical analyses and P<0.05 was considered significant. HST without HS resulted in a significant rise in MAP and PR (112.6 ± 2.7mmHg and 97.7 ± 2.5/min) from the basal values (98.5±2.3mmHg and 76.6±2.0/min; P<0.001 and 0.01 respectively). In the presence of HS, HST-induced changes (ΔMAP=14.2±2.6 ΔPR= 11.4±3.5) were significantly dampened compared to its absence (ΔMAP= 24.1±2.5 ΔPR= 20.1±3.1; P<0.001 and 0.01 respectively). The HST-induced increases in BP and PR suggest SNS activation. These were dampened by HS suggesting that its hypotensive effect may occur through the inhibition of systemic vascular resistance mediated by the SNS.

Mojiminiyi, F.B.O. et al., Fitoterapia 2007; 78: 292–297

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Hot-water immersion increases leg perfusion in peripheral arterial disease

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Peripheral Arterial Disease (PAD) commonly manifests symptomatically as intermittent claudication (walking-induced muscle pain), primarily in the calf muscles. Claudication is likely caused by insufficient perfusion, but the specific perfusion dynamics are not well understood. In healthy humans, passively induced elevations in local tissue temperature acutely stimulate vasodilation in both skin and muscle microvasculature (1) and increased perfusion in conduit arteries (2). Chronically, increased perfusion and the related blood flow and shear stress profile are important for vascular health; increased antegrade shear stimulates beneficial adaptations (3). The aim of this study was to determine if lower-limb heating in patients with PAD could increase lower-limb inflow and muscle perfusion.

Eight patients with PAD (6 male, age 69 ± 5y) and nine controls free from PAD (8 male, age 71 ± 6y) underwent hot-water immersion (30 min immersed to the waist in water at 42.43°C). Popliteal artery perfusion was assessed before, during the last 3 min and 30 min after immersion, using high-resolution ultrasound. Haemodynamics of the medial gastrocnemius muscle were monitored throughout using near-infrared spectroscopy. Popliteal artery blood flow at baseline was lower in PAD (28 ± 13 ml/min) than in controls (50 ± 11 ml/min); it increased approximately three-fold in PAD and two-fold in controls during water immersion (PAD: +71 ± 49 ml/min, p < 0.01; controls: +92 ± 59 ml/min, p < 0.01), and remained elevated at 30 min after immersion (PAD: +20 ± 20 ml/min, p = 0.04; controls: +31 ± 31 ml/min, p = 0.02). At the muscle, oxyhaemoglobin volume increased to a lesser extent in PAD than in controls (+261 ± 119 uM vs. +502 ± 246 uM; interaction: p = 0.01), while deoxyhaemoglobin volume was not significantly altered in either group (PAD: +10 ± 42 uM, controls: +13 ± 57 uM). Overall, the increased perfusion of the muscle during hot-water immersion was evident by an increase in total haemoglobin volume for both groups, more so in the controls (PAD: +271 ± 94 uM, p = 0.01; controls: +524 ± 270 uM; interaction: p = 0.01). These perfusion changes were reflected in an overall increase in tissue oxygenation index of the medial gastrocnemius muscle of ~7.4 ± 8.1% in PAD (p = 0.02) and ~7.3 ± 9.5% in controls (p = 0.03).

A single bout of lower-limb heating by hot-water immersion significantly increased popliteal arterial inflow and acutely improved muscle perfusion in the lower limbs of patients with PAD, although the magnitude is somewhat lower to that seen in healthy controls. Lower-limb heating may have therapeutic potential for PAD patients as has previously been suggested (4); therefore the functional and long-term adaptive effects of repeated lower-limb heating in atherosclerotic arteries should be explored.

Neural hemodynamic balance in the supine and upright posture: Impact of healthy human aging

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Supine neural hemodynamic balance is present in healthy young, but not older men (Hart et al., 2009). However, humans spend two thirds of their time upright; extending these observations to the upright posture will be important to ascertain the impact of sympathetic activity in human hypertension. We compared the neural hemodynamic balance in healthy young and older men without any sign of hypertension (24 hour ambulatory blood pressure (BP) monitoring), comorbidities or classic cardiovascular risk factors. Cardiac output (Qc, C.O.), diastolic BP became less pronounced after the acute transition into the state upright posture (all P<0.05). Similar to the supine posture, burst incidence in the young group was not statistically related to Qc or TPR in any steady-state upright posture (all P>0.05). Similar to the supine posture, MSNA burst incidence in the older group was not related to Qc or TPR in any upright posture (all P>0.05). In the young group, the relationship between MSNA burst incidence and diastolic BP became less pronounced after the acute transition into the state upright posture (all P>0.05). In contrast to the supine condition, MSNA burst incidence in the older group was not related to diastolic BP in any upright posture (all P>0.05).

Young men displayed neural hemodynamic balance in the supine posture, which was partially attenuated whilst upright. Neural hemodynamic balance was not apparent in the healthy older men in either posture. Interestingly, in this group of hemodynamically stable older men, MSNA was unrelated to BP in the steady-state condition. However, as Qc varied markedly between the older men and BP was normal, hemodynamic balance must have been maintained, at least in part, by non-neural mechanisms.


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Does the duration of stimulus alter the cerebral blood flow-to-carbon dioxide responsiveness measure?

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The responsiveness of the cerebrovasculature to alterations in arterial content of carbon dioxide (pCO₂) is a common functional test to assess brain health (i.e., CBF-CO₂ responsiveness). The most common and simplest test to assess CBF-CO₂ responsiveness is the inhalation of one concentration of CO₂ via a Douglas bag open circuit (e.g., 5% CO₂ in air). Across the studies using this approach a range of durations have been used to induce this stimulus-response, from 1 to 5 min. However, given the known kinetics of this response, a 1 min response test may represent something different to that of a 5 min test. Further, given the interaction between the ventilatory and vascular responses with the open-circuit technique, the ventilatory response may also affect the consistency between the different stimulus durations for the CBF-CO₂ responsiveness measure. The purpose of this study was to examine whether 1, 2, 4 and 5 mins of CO₂ stimulus gave the same CBF-CO₂ responsiveness measure. Fourteen healthy volunteers were recruited. Following a full familiarisation visit, participants attended the laboratory for one experimental trial. Participants lay supine for ~20 min while being instrumented, following which they completed a 5 min resting baseline. In a randomised order, participants then completed four durations (1, 2, 4 and 5 minutes) of 5% CO₂ (in air) inhalation via the open-circuit Douglas bag method, with 5 min recovery between. Bilateral middle cerebral artery velocity (MCAv, transcranial Doppler), mean arterial pressure (MAP, Finometer), ventilation (VE) and partial pressure of end-tidal carbon dioxide (PetCO₂) were recorded continuously. CBF-CO₂ responsiveness and ventilatory sensitivity to CO₂ (VE-CO₂) were calculated as change from baseline (relative and absolute, respectively) per mm Hg change in PetCO₂. We compared values calculated from the final 30 s of each CO₂-stimulus duration. A repeated-measures ANOVA (Bonferroni corrected) was used to test differences between durations.

We observed no statistical difference (p=0.15) across the different durations of CO₂-stimulus for CBF-CO₂ responsiveness (bilateral Doppler data pooled (mean±SD): 2.4±1.2, 2.4±1.1, 2.3±1.1, 2.3±1.1 %MCAv/mm Hg PetCO₂, for 1, 2, 4 and 5 min respectively), despite observable differences in the MCAv-response profile between durations. For example, a steady-state profile typified the 4 and 5 min tests, whereas the 1 min test tended to peak in the final seconds of the stimulus. Further, while grouped CBF-CO₂ responsiveness data appear consistent across durations, there was variation within individuals.
across the four stimulus durations; CoV ranging from 8 – 45% between individuals. In contrast, VE-CO₂ sensitivity increased ∼3-fold from the 1 min to the 4- and 5-min test durations (0.3±0.2, 0.6±0.3, 0.9±0.3, 1.0±0.4 Lmin⁻¹/mm Hg, for 1, 2, 4 and 5 min respectively; p<0.01).

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**Difference in carotid pulsatile wall tension explain variability in muscle sympathetic outflow between young men but not women. Preliminary observations in humans**

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In the supine resting condition, muscle sympathetic nerve activity (MSNA) is higher in men than women. Furthermore, cardiac output is related to MSNA in young men, but not in young women. Increased pulsatile sinus pressure evokes sustained reductions in sympathetic activity (Chapleau, et al., 1989) and depressor response (Mendelowitz and Scher, 1988). Thus, carotid-pressure interactions may partially explain within and between sex differences in neural outflow to skeletal muscle.

To begin to assess this question, six young men and four young females were studied in the resting supine condition. Muscle sympathetic nerve activity and heart rate (ECG) were obtained continuously during 6 minutes of spontaneous breathing. Blood pressure was obtained in duplicate by elecsotrophymgomanometry. Simultaneously, carotid sinus pulsatil- ity was measured continuously for the first minute using high-resolution ultrasonography. Beat-by-beat systolic and diastolic sinus diameters were obtained using wall tracking software and averaged. Carotid tensile force during diastole was calculated as the product of diastolic blood pressure and diastolic cartid radius and expressed as diastolic static wall tension (SWT_dia, mmHg x mm). Pulsatile wall tension (PWT) was calculated as PWT (mmHg/mm) = (pressure x radius) systole – (pressure x radius) diastole. These data are preliminary, thus data are expressed as mean differences and effect sizes (ES). Muscle sympathetic nerve activity (Δ19, beats100 hb⁻¹), ES 4.6), mean arterial pressure (Δ7, mmHg, ES 1.20) and SWT_dia (Δ4, mmHg/mm, ES 1.8, Figure) seemed greater in men than women. Average pulse pressure (Δ0.5, mmHg, ES <0.1) alongside PWT (Δ1, mmHg, ES 0.2) seemed similar between sexes. These observations were similar when hemodynamics was scaled to height.

No relationship was observed between SWT_dia and muscle sympathetic nerve activity in men or women (R<0.1). In young men, PWT was negatively related to muscle sympathetic nerve activity (R²=0.63, Figure) whereas in young women the relationship was positive but very weak (R²=0.27, Figure). These preliminary data suggest that carotid-pressure pulsatility, even in the resting state, is related to basal sympathetic outflow in young males. In young females, carotid-pressure interactions seem to play a smaller role.

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**Bipedal vs quadrupedal locomotion in human**

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Introduction - Why does human want to walk and run in two legs? Through the evolutional processes of human bipedality, biomechanical function of limbs has been progressively evolved out of quadrupedalism. Due to human hands being free from locomotion, legs engage in motor activities. Human has gained the gluteus muscles in order to run with two legs free from locomotion, legs engage in motor activities. Human motion have not been studied.

Methods - Healthy young individuals participated in this study, and they performed the 3-min walking (2km/hr) on the treadmill with the order of bipedal and quadrupedal conditions followed by the 3-min recovery. Expired air was obtained throughout the exercise and was analyzed by a gas analyzer in order to determine energy expenditure (EE), oxygen consumption (VO₂), carbon dioxide production (VCO₂) and respiratory exchange ratio (RER). VO₂ and VCO₂ were used to calculate substrate oxidation rate. Blood lactate, glucose, salivary amylase, ratings of perceived exertion (RPE) were measured.
A salivary amylase was used for quantifying the physiological stress or fatigue caused by exercise. Results - There was over a twofold increase in VO₂ in quadrupedal walking as compared with bipedal walking. Blood lactate was not changed after the bipedal walking as compared to rest, but significantly increased after the quadrupedal walking and maintained high level during the recovery. Conclusion - These results suggest that quadrupedal walking in human requires large amount of energy from the glycolysis energy pathway, while human bipedalism reduces locomotor costs with saving glycolytic energy.

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Haematological indices of apparently healthy students of Igbo ethnicity of Abia State University Uturu, Nigeria

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Adequate nutrition is a prerequisite for normal blood cells production. Prior to early 1984 when government feeding subsidy to students in institutions of higher learning in Nigeria and centralized canteen system of feeding was the norm before it was abrogated, balanced diets was provided for students. Lower values of blood cells in communities in developing countries always come from populations of low socio-economic background (Ezeilo, 2002). Studies have shown that values of well nourished Africans are indistinguishable from those of Caucasians (Green and Ezeilo, 1978). This study made up of two parts I and II were carried out among 500 apparently healthy students of Igbo origin (265 males and 235 females aged 16 - 30 years) over a period of two years to compare their blood indices -haemoglobin concentration, packed cell volume, mean corpuscular haemoglobin concentration, white blood cells count, total white blood cell s differential count. The subjects were selected after a structured questionnaire was administered and these blood indices were determined by standard laboratory procedures with each subject serving as control and test three months later. The results showed that haemoglobin concentration decreased after three months in both Parts I and II for both the males and females when compared with the control. Packed cell volumes were reduced from 40.2 to 29.4% (Part I) and 42.6 to 30.3% (Part II). Mean corpuscular haemoglobin concentration in females decreased from 31.5 to 30.3g/dL (Part I) and 32.8 to 30.3g/dL (Part II) and for males, from 33.6 to 30.9g/dL (Part I) and 33.5 to 30.8g/dL (Part II). Total white blood cell decreased in both Parts I and II respectively (p < 0.05). White blood cell differentials showed that neutrophils counts decreased, eosinophils increased while the rest remained the same in both parts. The ABO blood group pattern were O - 56.9%; A - 34.3%; B - 7.1%; and AB - 1.7% while for the Rh positive, it was 94% and negative - 5%. The results for part I and II followed a similar trend. The observed differences between the controls and the tests could be attributable to the poor campus diet as the indigent student feed according to their financial capacity and from restaurants as against when the government subsidized feeding with a centralized canteen system and perhaps will enhance a better blood indices pattern


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Corticotrophin-releasing hormone neurons in the Paraventricular nucleus: Evidence for glucose sensing and projection to autonomic relay areas

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Maintenance of glucose homeostasis is essential for health, and is tightly regulated by peripheral and central counter-regulatory mechanisms. The mechanisms underlying central counter-regulation are not fully understood, although the hypothalamus is thought to play a key role. Corticotrophin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVH) regulate cortisol release via the pituitary-adrenal axis and may also project to the autonomic nervous system, although this has not been definitively established(1). In addition, although glucose-sensitivity has been shown for uncharacterized PVH neurons in the rat, it is unclear whether CRH neurons contribute to hypothalamic glucose sensing(2). We used a genetically modified mouse strain expressing TdTTomato under the control of the CRH promotor(3) to identify CRH neurons. After terminal anaesthesia with isoflurane, hypothalamic slices were cut in 10mM glucose for recordings of CRH neuronal properties and sensitivity to glucose levels. Data is presented as mean±S.E.M., unpaired t-test. Whole cell recordings (n=68 CRH neurons) revealed two distinct populations: group 1 (n=59) fired spontaneous action potentials in 10mM glucose (7.3±0.67 Hz.), whilst group 2 were silent under the same conditions (n=9). Group 2 also had lower input resistance (385±63 vs 753±51 MΩ, p<0.0001) and larger cell capacitances (51.1±6.4 vs 30.4±2.8 pF, p<0.001). We observed heterogeneous responses to a glucose step from 10 to 2.5mM. Of the group 1 population, 8/23 neurons showed a reversible fall in firing frequency (glucose excited) and a further 2/23 neurons showed an increase (glucose inhibited). Of the group 2 neurons tested were depolarised by the step, indicating glucose-induced inhibition (n=4). Recordings in the presence of TTX to block synaptic inputs suggest many CRH neurons (7/11) are also intrinsically glucose inhibited, depolarising by 7.5±1.2mV on stepping to 2.5mM glucose. The group 2 neurons were located in more caudal and ventral parts of the PVH, areas associated with autonomic projecting neurons(4). To establish whether PVN CRH neurons project to brainstem autonomic control centres we stereotaxically injected a canine adenoviral vector(5) into the nucleus of the solitary tract/dorsal vagal motor nucleus complex (recovery surgery performed under ketamine 70mg/kg and medetomi-
dine 0.5mg/kg i.p anaesthesia, n=2). This enabled retrograde tracing to the PVH and revealed the presence of CRH neurons with brainstem projection in the caudal region (11-13% of the autonomic projecting neurons co-localised with TdTomato, range).

These data indicate that subpopulations of CRH neurons are glucose sensitive and have the necessary anatomical connectivity to play a role in the counter-regulatory response to hypoglycaemia.


Bru T et al 2010 Viruses. 2(9), 2134-53.

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An experimental investigation into the anti-ulcer effect of risperidone in male wistar rats

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Risperidone is a second-generation atypical antipsychotic drug currently used for the management of schizophrenia, delusional psychosis and psychotic depression, an action ascribed to its being a dopamine antagonist possessing anti-serotonergic, anti-adrenergic and anti-histaminergic properties. However, there are indications that risperidone has gastroprotective effect. This study was carried out to examine the effect of risperidone on stress-induced and indomethacin-induced ulcers in the rats. Rats were treated with risperidone (0.1mg/kg, 0.3mg/kg and 0.5mg/kg) orally once daily for 21 days before assessing for ulcer using water immersion restraint stress (WIRS) according to the method of Byun et al., 2007, starvation (Elegbe, 1978) and indomethacin-induced ulcer (Elegbe, 1978) models. The animals were handled in accordance with principles of laboratory animal care and use (NIH, 1985). Gastric lesions were scored and assessed using previous methods (Alphin and Ward, 1967; Elegbe and Bamgbose, 1976). Morphometric studies were performed using Olympus light microscope (x100) fitted with Casio digital camera and Motic plus China, 2000 software. Statistical analysis of data was done by one-way analysis of variance (ANOVA) and Student’s t-test for paired data using Graphpad prism software version 5 and were expressed as Mean ± SEM (Standard Error of Mean). Risperidone caused a significant dose-dependent reduction in gastric ulcer scores [0.1mg/ kg (3.5±0.2), 0.3mg/kg (1.9±0.3), 0.5mg/kg (1.2±0.2)] compared with control (5.6±0.3) in WIRS; [0.1mg/kg (4.0±0.3), 0.3mg/kg (2.3±0.2), 0.5mg/kg (1.8±0.2)] compared with control (6.1±0.3) in starvation and [0.1mg/kg (4.9±0.3), 0.3 mg/kg (2.0±0.2), 0.5 mg/kg (1.3±0.2)] compared with control (6.4±0.4) in indomethacin-induced ulcer models. The findings suggest that risperidone has gastric anti-ulcer property. However, more detailed studies are necessary to confirm the relevance of this finding and its implications in clinical settings.


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An experimental investigation into the anti-ulcer effect of risperidone in male wistar rats

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The study group consisted of 23 females, and 13 males with a weight of 33.5±6.7Kg and BMI centile of 55.6±30.8. The room temperature during IRT was 22.1±0.7°C. FFQ: The C, Sw and V food groupings were reliable and parent-child agreement was predominantly poor. FFQ: The C, Sw and V food groupings were reliable and parent-child agreement was fair–good. A significant correlation was observed between FFQ scores for V and right-sided TSCR (Table 1). After adjustment for confounders, this relationship no longer persisted and a significant correlation between both C and Sw FFQ scores was observed (Table 1). No significant relationships were observed between FFQ scores and TSCR.

For the first time we have shown that as preference for sweet foods/carbohydrate increases, BAT thermal activity decreases independent of BMI centile. The acute and chronic effects of dietary composition should now be prospectively studied to identify foods capable of BAT stimulation and obesity prevention.

<table>
<thead>
<tr>
<th>Group</th>
<th>Pearson’s r (left TSCR)</th>
<th>P value</th>
<th>Pearson’s r (right TSCR)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>0.22</td>
<td>NS</td>
<td>0.31</td>
<td>NS</td>
</tr>
<tr>
<td>Sweet food</td>
<td>0.36</td>
<td>NS</td>
<td>0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Vegetable</td>
<td>0.32</td>
<td>NS</td>
<td>0.34</td>
<td>0.047</td>
</tr>
</tbody>
</table>

Table 1 – Relationship between supraclavicular temperature (TSCR) and food preferences *adjusment for BMI centile and ambient temperature.


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PC234

Natural sweetener agave inhibits gastric emptying by a glucagon-like peptide-1 (GLP1) receptor-dependent mechanism


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Enteroendocrine cells of the gut sense chemical components of ingested food and secrete several gastrointestinal satiation peptides, including glucagon-like peptide-1 (GLP1) and cholecystokinin (CCK), which influence gastric emptying, reduce food intake and increase satiety. In humans, hexose sugars were shown to release CCK release and delay gastric emptying by a CCK1 receptor-dependent mechanism. Several artificial sweeteners, used in the management of diabetes and obesity, were suggested to have no effect on gastric emptying and less effect on satiety hunger. The present study was conducted to compare the gastric emptying rate of agave nectar, a fructose-rich herbal liquid sweetener, with the rate of glucose and fructose, and to evaluate the involvement of CCK and GLP1 receptors in agave-induced alterations in gastric emptying. Under anesthesia (Ketamine 100 mg/kg and Chlorpromazine 3-5 mg/kg; intraperitoneally) Gregory cannulas were fitted onto the gastric corpus of female Sprague-Dawley rats (n=8). Following recovery, 3 ml of saline or test meals (agave, glucose or fructose) at 12.5, 25 and 50 % concentrations (containing phenol red as a non-absorbable dilution marker, 60 mg/l) were instilled into the gastric fistula on different days. The rate of gastric emptying was determined from the volume and phenol red concentrations recovered after 5 min. GLP1 (exendin 9-39; 30 μg/kg), CCK1 (Devazepide; 1mg/kg) or CCK2 (Y M 022; 1mg/kg) receptor antagonist was injected subcutaneously before the experimental. When compared to saline, gastric emptying of fructose was significantly delayed (p<0.01-0.001) at all 3 concentrations, while glucose and agave delayed gastric emptying at 25% and 50% concentration (p<0.05-0.001). At 50% fructose concentration, delayed gastric emptying was significantly reversed by both CCK1 and GLP1 receptor antagonists (p<0.01-0.001), while 50% glucose-induced delay in gastric emptying was abolished by the CCK2 receptor antagonist (p<0.05). GLP1 receptor antagonist significantly facilitated gastric emptying rate that was delayed by 50% agave (p<0.05). The results of the study revealed that glucose, fructose and agave all delayed gastric emptying contributing to satiety, while higher concentrations of agave are required to reach a similar delay in gastric emptying. This inhibitory effect of agave on gastric emptying rate appears to be mediated by GLP1 receptors.


Supported by a grant from Marmara University Research Fund (SAG-A-131113-0418).

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PC234

K(ATP) currents in INS-1 rat insulinoma cells recorded via automated patch-clamp

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Motivation: Shifts in nutritional habits and relative abundance of refined high-calorie foods in modern postindustrial societies resulted in increased prevalence of both type I and type II diabetes mellitus, causing significant morbidity and mortality and incurring increasingly higher healthcare and economic costs. A promising therapy is autologous transplantation of insulin-secreting b cells obtained via redifferentiation of patient’s own induced pluripotent stem cells (iPSC) (1), including for juvenile diabetes supplementary means to prevent autoimmune attack by effector T cells (2). The complex insulin secretion machinery comprises the interplay of several
ion channels: cell depolarization via ATP inhibition of inward rectifier K(ATO) channels upon glucose entry is followed by opening of voltage-dependent Ca^{2+} channels triggering exocytosis of insulin (3). The assessment of these ion channels in b cells or correspondent cell lines applying automated patch-clamp provides an efficient tool for analyzing these processes and identifying therapeutic strategies.

Methods: Whole-cell patch-clamp experiments were performed in the rat insulinoma cell line INS-1 (4), kindly provided by Prof. Baltrusch and Dr. Schultz from the Institut für Medizinische Biochemie, Universität Rostock, using the CytoPatch™ equipment (5), with dual-channel quartz pipette tips 2 mm in diameter. Depolarizing voltage steps (-70 to +40 mV for 300 ms) or ramps from -100 to +100 mV were applied regularly, while K+ concentration was increased from 2.5 to 20 mM. The internal solution contained either 5 mM ATP or was ATP-free.

Results: Automated whole-cell recordings utilizing the INS-1 cells have been established. Depolarizing steps elicited large delayed rectifier and transient outward currents, which were strongly inhibited by either 4-aminopyridine 1 mM or tetraethyl ammonium 5 mM. With ATP-free internal solution we recorded large inward rectifying currents, displayed as difference of traces at 20 mM and 2.5 mM external K+, amounting to -200 pA/pF at -100 mV, with a reversal potential close to the K+ equilibrium potential in the specified ionic conditions.

Conclusion: Automated patch-clamp technology utilizing the CytoPatch™ can be successfully used via whole-cell experiments to investigate the signaling pathways and ionic currents involved in insulin secretion. It is therefore an appropriate tool to be applied for quality control and validation of iPSC-derived pancreatic b cells in view of clinical applications and to screen against modulators of those currents for pharmacological purposes.


urinary $\text{Na}^+/\text{K}^+$ ratio (Hedayati et al., 2012). Aldosterone regulates the renal reabsorption of $\text{Na}^+$ and the secretion of $\text{K}^+$ (Wiederholt et al., 1972) thus a major determinant of urinary $\text{Na}^+/\text{K}^+$ ratio. This study aims to assess gender difference in urinary $\text{Na}^+/\text{K}^+$ ratio and aldosterone excretion in urine before and after furosemide administration. We investigated $\text{Na}^+/\text{K}^+$ ratio and aldosterone excretion in 24hour (24h) urine and 12h (7am-7pm, D; and 7pm-7am, N) urine samples with and without furosemide administered. Ethical approval was obtained for 20 healthy subjects (10 males and 10 females) aged 20-30 years for this study. On the first day, 12h (D), 12h (N) urine were collected. Thereafter, 20mg oral dose of furosemide (I) was given to subjects twice at 7am & 7pm on the second day. 12h (D and N) urine samples were collected. 24h urine samples were obtained from both 12h samples. $\text{Na}^+$ and $\text{K}^+$ were analyzed using flame photometry; while urine aldosterone concentration was analysed using the enzyme immunoassay method (DRG International, Inc, USA). Values are expressed as Means ± S.E.M and analyzed using unpaired Student’s t-test. Females had higher baseline $\text{Na}^+/\text{K}^+$ ratio than males in 12h-D (6.09±1.03 vs. 2.9±0.28; P<0.001) 12h-N (7.78±0.49 vs. 4.28±0.3; P<0.001) and 24h (6.6±0.65 vs. 3.63±0.24; P<0.001) samples. Administration of furosemide caused diuresis and significant increase (P<0.001) in the urinary Na$^+$/K$^+$ ratio in the timed urine samples (12h-D, 12h-N and 24h) in the male subjects. Although the baseline urinary aldosterone excretion in all timed urine samples were similar in both males and females, the 24h urinary aldosterone excretion after furosemide administration increased only in males (P<0.05). We conclude that there is a gender related difference in the urinary Na$^+$/K$^+$ ratio and that this difference was abolished by furosemide administration. Hedayati, SS., Minhajuddin, AT., Jiaz, A., Moe OW., Elsaayed EF., Reilly, R.F. and Huang C-L. Clin. J. Am. Soc. Nephrol., CJASN 7(2): 315-22, 2012. Wiederholt, M., Behn, C., Schoormans, W. and Hansen L. J. Steroid Biochem., 3(2): 151-159, 1972.

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bility was determined by using the three cell viability assays; Alamar Blue, MTT and the Neutral Red (NR) assay. Cell-free supernatants were collected and analysed for Interleukin-6 (IL-6) and IL-8 release by ELISA. Data expressed as Mean ± SD of n=4-8 determination in quadruplicate. *p≤0.05 vs. control.

Results: Both RT-PCR, and Western Blot showed MC<sub>1-7</sub> and MC<sub>1-3</sub> expression on C-20/A4 cells. Cell viability analysis: IL-1β stimulation led to a maximal cell death of 35% at 6h (Alamar Blue), and 40% and 75% with MTT and Neutral Red respectively at 24h compared to control. The three cell viability assays have different cellular uptake pathways, which accounts for the variations observed in cell viability in response to the concentration of IL-1β, and time. Cytokine analysis by ELISA: IL-1β (5000pg/ml) stimulation for 6 and 24h showed maximal IL-6 production 292.3 ±3.8 and 275.5 ±5.0 respectively, and IL-8 production 353.3 ±2.6 and 598.3 ±8.6 respectively. Pre-treatment of cells with α-MSH and D[Trp<sup>6</sup>-γ-MSH caused significant reductions in both IL-6 and IL-8 respectively following IL-1β stimulation at 6h.

Conclusion: MC<sub>1-3</sub> are expressed on C-20/A4 cells, activation by melanocortin peptides led to an inhibition of IL-1β induced cell death and pro-inflammatory cytokine release.


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PC239

Hepatoprotective activity of aqueous leaf extract of *Moringa Oleifera* following administration of lead acetate in Wistar rat

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Lead is a common environmental pollutant capable of causing acute and chronic illness (Vaziri, 2008). Generation of reactive oxygen species (ROS) has been postulated to be one of the possible mechanisms by which lead induces various toxic effects (Pande et al., 2001). It has been reported that lead increased the level of lipid peroxides and alter the antioxidant defense system in the hepatic tissues (Sandhir and Gill, 1995). Moringa oleifera leaves are rich in Vitamin A, B, C, α-tocopherols, flavonoids and carotenoids (Makkar and Becker, 1996). These vitamins and compounds act as antioxidants and help to stop lipid peroxidation chain reactions generated by free radicals from cell membranes (Nordberg and Arnér, 2001). Based on the observation that free radicals were generated during the pathogenesis processes induced by lead exposure, it has been presumed that supplementation of antioxidants could be an alternative method for chelation therapy (Flora et al., 2003). Therefore, the aim of this study was to investigate the hepatoprotective effect of aqueous leaf extract of *Moringa oleifera* following lead acetate administration to wistar rats. Twenty five adult male wistar rats were assigned into five equal groups (n=5). Group A served as control; group B animals were administered with 500ppm of lead acetate orally via drinking water daily; group C animals were administered 50mg/kg body weight of Silymarin (a known hepatoprotective drug) orally via drinking water prior to the administration of 500ppm of lead acetate; group D and E animals were administered orally with 400mg/kg (low dose) and 800mg/kg body weight (high dose) of aqueous leaf extract of *Moringa oleifera* respectively prior to the administration of 500ppm of lead acetate. The experimental period lasted for 28 days and all animals were cared for according to the guidelines of the National Institute of Health, (NIH, USA). The results revealed a significant elevation (P<0.05) of malondialdehyde (MDA) and significant reduction (P<0.05) in superoxide dismutase (SOD), catalase (CAT) and Glutathione (GSH) activity in group B (lead treated group only) compared to group A (control) and significant improvement (P<0.05) in SOD, CAT and GSH activity in groups D and E (treated with low and high doses of Moringa oleifera and lead respectively) when compared to group B but no difference (P>0.05) when compared to group A (control). Histopathologic studies of sections of the liver showed congestion of the central vein, enlargement of sinusoids' leneration of the hepatic plates in group B while group D & group E appeared normal when compared to the control. These observed changes in liver antioxidants activity and histology suggest that *Moringa oleifera* has hepatoprotective properties at low&high doses.


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PC240

Semenc characteristics and sperm morphology of *Pistia stratiotes* Linn. (Aracea) protected male albino rats (Wistar strain) exposed to sodium arsenite

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The sperm protective potential of *Pistia stratiotes* Linn (Aracea) in arsenic-exposed rats was carried out using twenty-four male albino rats (225 to 228g) aged between 14 and 16 weeks old. They were grouped in to 4 (A-D), each group containing 6 rats. Group A animals were orally treated with 100mg/kg ethanol leaf extract of *Pistia stratiotes* Linn. daily for 14 days. B (sodium arsenite 2.5 mg/kg body weight), C (*Pistia stratiotes* extract for 14 days and single dose of sodium arsenite (SA) on the 14th day, D (0.1 ml Propylene glycol) orally by gavage. Samples were collected by standard procedure from all the animals twenty-four hours after the last treatment, after which they were sacrificed by cervical dislocation. The rats were anaesthe-
tized with diethyl ether before sacrifice, the mid caudoventral abdominal incision was made with sterilized scissors, permitting instant access to the testis once pushed upward from the scrotum. The testes were then separated from the epididymides. The right and left epididymides were trimmed off the body of the testes and semen sample collected from the tail of the epididymis through an incision.

It was observed that group B had a more significantly lower (P<0.05) percentage motility (26.7±6.67%) when compared across the groups while group A had a significantly (P<0.05) higher mean value (63.3±3.33%). The sperm motility of rats in group D was significantly higher (P<0.05) than groups B and C. This implies that P. stratiotes extract had no adverse effect on the sperm motility of the rats and also ameliorates the adverse effect of arsenite on sperm motility. The mean value obtained for sperm viability, semen volume and sperm count followed a similar pattern although, the difference was not significant (P>0.05) for semen volume and the sperm count of rats across the groups. The total sperm abnormality obtained across the groups ranges between 10.44 and 14.27% with group B treated with sodium arsenite (SA) having the highest value when compared with groups A treated with Pistia stratiotes extract and D treated with Propylene, although, the differences were not significant (P>0.05). The study concluded that ethanol leaf extract of Pistia stratiotes has no negative effect on sperm motility, viability and morphology and also protected spermatozoa against arsenic reproductive toxicity in wistar strain albino rats and may potentially play an important role in the protection of populations with chronic sodium arsenite exposure.


Chattopady S., Sampa P., Ghosh D.M., and Jogen,(2003),Effect of dietary co-administration of sodium-selenite on sodium arsenite induced ovarian and uterine disorders in mature albino rats. Toxicol. Sci. 75; 412-422.


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Probiotic Lactobacillus rhamnosus GG conditioned media enhances acute ROS production, but reduces nitric oxide in J774 murine macrophages

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Macrophages as professional phagocytes have been considered prominent participants in the response to acute infection and play a significant role in preventing infecting bacteria multiplyng and damaging the host environment. Macrophage may also contribute to the inflammatory process by excessive production of reactive oxygen species (ROS) and nitric oxide (NO) which although beneficial in killing bacteria may result in deleterious consequences, especially following an interaction with superoxide radicals to form reactive nitrogen species such as peroxynitrite. Formation of free radicals in macrophages may be regulated by probiotic bacteria (Kumar et al., 2007). However, the mechanism of regulation is not clear. Thus, studies were carried out to determine the role of probiotic in the acute regulation of both ROS and NO production in the murine J774 macrophages. In this study a cell free Lactobacillus rhamnosus GG culture medium (LGG-CM) was used since it has been shown that LGG releases a number of soluble factors which are responsible for beneficial health effects in the hosts.

J774 macrophages were loaded with either H2-DCFDA for monitoring reactive oxygen species or with DAFFM-DA for nitric oxide. Acute free radicals production was measured on a fluorescence microplate reader and changes were analysed by cumulative sum (CuSuM) calculations. The fluorescence measurements were taken every two minutes for the first 60 minutes to monitor free radicals production during ingestion period and from 60 minutes to 280 minutes to monitor free radicals production during digestion period (de-koning ward et al., 1998) by J774 macrophages. The fluorescence was measured at 485 nm excitation and 528 nm emissions. Low concentration of LGG-CM (10% LGG-CM) or LPS did not cause any significant change in basal levels of ROS or NO production. In contrast, high concentration of LGG-CM (75% LGG-CM and 100% LGG-CM) significantly enhanced ROS generation (p<0.01) but also significantly reduced NO (p<0.05) level in both ingestion and digestion phase of phagocytosis. ROS production in ingestion phase is significantly higher that the production in digestion phase (p<0.001). These effects of LGG-CM were not altered in the presence or absence of E. coli.

A balanced production of NO and ROS is necessary for normal phagocytic function of macrophages. Our findings suggest that probiotics may accelerate bacterial killing by potentially enhancing ROS production and may additionally reduce deleterious effects associated with excessive NO by suppressing production of the latter. In this study a pulse of excessive ROS production to LGG-CM seems to be targeted for rapid digestion of E.coli. Therefore, the ability of probiotic to balance NO and ROS generation can be a novel approach in improving the intestinal homeostasis.


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Long-term potentiation in the dentate gyrus of C57Bl/6j mice: Induction and expression

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Long-term potentiation (LTP) was first discovered at the perforant path-dentate granule cell synapse. However, this form of synaptic plasticity has not been extensively studied in this pathway in the mouse. LTP is a striking form of synaptic plasticity that is believed to underlie the expression of learning and memory (Bliss and Lomo, 1973). Understanding the basic features and mechanisms underlying the induction and expression of these forms of synaptic plasticity in wild type mice will be essential in understanding any pathological changes in disorders such as Alzheimer’s disease. Following decapitation, acute transverse hippocampal slices were prepared from 21-29 day old male C57Bl/6j mice. Extracellular stimulation was applied to either the medial or lateral perforant pathway to evoke dendritic field potentials recorded from the dentate gyrus. Pairs of stimuli were applied 50 ms apart to confirm correct electrode placement, as the interaction between the two field excitatory postsynaptic potentials is different in the two pathways. Paired-pulse facilitation characterised the lateral perforant pathway, while paired-pulse depression was evident in the medial perforant pathway. Two different conditioning paradigms were then applied to induce LTP (referred to here for simplicity as tetanus (TET): single train of 40 pulses at 100 Hz; or high-frequency stimulation (HFS): 8 trains of 8 pulses each at 200 Hz, inter-train interval 1.5 s).

In the lateral perforant pathway TET, but not HFS, induced significant potentiation at 51-60 minutes compared to baseline (114±4%, n=9 and 102±6%, n=6 mice, respectively). In contrast, in the medial perforant path, LTP was induced only using the HFS conditioning (111±5%, n=12 mice), while TET failed to induce significant potentiation (100±4%, n=10 mice). Paired-pulse ratio was monitored throughout the recordings to assess locus of expression of LTP. There was no correlation between the change in paired-pulse ratio following induction and the magnitude of LTP in the medial perforant pathway (r2=0.15, p>0.05), suggesting a postsynaptic locus of expression at these synapses. In the lateral perforant pathway, paired-pulse ratio changes correlated with the magnitude of LTP (r2=0.55, p<0.05), indicating a presynaptic component to the expression of LTP. Treatment with D-2-amino-5-phosphonopentanoate (D-AP5, 25 mM) blocked the induction of LTP (100±6%; n=5 mice) in the medial perforant path. While an NMDA-dependent mechanism of LTP induction was shown in the medial perforant path, our results suggest the presence of a significant presynaptic component in the expression of LTP in the lateral perforant pathway. We demonstrate here that the conditioning protocols contribute to different forms of LTP expressed in these pathways in the mouse and may represent different functions of the pathways.


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Carbon monoxide prevents Aβ induced rises in microglial intracellular Ca²⁺

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Calcium ion (Ca²⁺) imbalance is a potential trigger for apoptosis and has been implicated in Alzheimer’s disease. Carbon monoxide (CO) gas has recently been shown to provide neuroprotection against amyloid beta (Aβ(1-42) peptide (1). Our study aimed to investigate the interplay between intracellular Ca²⁺ and Aβ(1-42) in microglia cells. Using the BV-2 microglia cell line we determined cell viability and intracellular calcium concentrations in the presence of Aβ(1-42) alone and in addition of the carbon monoxide releasing molecule (CORM-2). Cell viability XT assays showed that Aβ(1-42) (48hrs) displayed no microglia toxicity using a range of concentrations (100pM-1µM, 1µM: 94.8 ± 1.1 % cell viability, mean ± SEM, P<0.05, Student’s paired t test, n=48 from 4 replicates). Further viability studies revealed that CORM-2 (1µM-100µM) was also not toxic to microglia cells (116 ± 12% cell viability, P>0.05, Student’s paired t test, n=24 from 2 replicates). To investigate intracellular Ca²⁺ levels we employed the Fluo-4 fluorescent probe. BV-2 cells pre-exposed to Aβ(1-42) peptide showed a dose dependent increase in intracellular Ca²⁺ levels, significant at 1µM (12.4 ± 1.5% increase, P<0.05, Student’s paired t test, n=100 from 10 replicates). This increase was not observed in the presence of a scrambled Aβ peptide (0.5 ± 0.01% increase, P>0.05, Student’s paired t test, n=40 from 4 replicates), highlighting specificity for Aβ(1-42). Interestingly in the presence of 10µM CORM-2, the Aβ(1-42) induced intracellular Ca²⁺ rise was significantly suppressed (12.4 ± 1.5% reduced to 96 ± 2.5% in the presence of CORM-2, P<0.05, Student’s paired t test, n=20 from 4 replicates). Further experiments using the inactive form of CORM (ICORM) failed to suppress the increase in intracellular Ca²⁺ levels in the presence of 1µM Aβ(1-42) (15.6 ± 1.6%, P>0.05, Student’s paired t test, n=20 from 4 replicates) suggesting that suppression was predominantly mediated by CO. These results highlight the potential for CO to be used to modulate microglia physiology to provide a novel approach in tackling neurodegenerative diseases.


This work was supported by The Alzheimer’s Society.

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

Carbon monoxide modulation of microglia viability: Role for glycolysis

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Microglia are vital cellular components of brain physiology and indeed pathology. Carbon monoxide has been shown to be neuroprotective in the face of an array of stressful stimuli (1). Here we investigated the role of carbon monoxide on microglia under conditions of metabolic stress. BV-2 microglia cells were exposed to the glycolytic inhibitor, deoxyglucose (DOG), and viability quantified using the XTT assay. Experiments revealed a time dependent increase in cell death, at 3 hours there was no significant cell death when cells were only exposed to DOG compared to vehicle (10mM, 91.6 ± 3.7% cell viability, mean ± s.e.m., P>0.05, Student’s paired t test, n=24 from 2 replicates). Whereas, 24 hour incubation showed that DOG significantly decreased cell viability in a dose dependent manner, significant at 100µM (10mM, 53.9% cell death, P<0.05, Student’s paired t test, n=54 from 5 replicates). In addition the carbon monoxide (CO) donor, CORM-2 (1-100µM), had no significant effect on cell viability over a 24hr time period (116 ± 12%, P>0.05, Student’s paired t test, n=24 from 2 replicates). To determine the role of CO under conditions of metabolic stress, CORM-2 was applied in the presence of DOG. A biphasic response was observed; at low concentrations of DOG (1µM), 10µM CORM increased toxicity (DOG alone: 96.9 ± 9% cell viability, DOG in the presence of CORM: 81.9 ± 2.8%, one-way ANOVA followed by Bonferroni post-test, P<0.05, n=60 from 5 replicates). In contrast at higher concentrations of DOG (10mM) CORM suppressed toxicity (DOG alone: 45.6 ± 1.1% cell viability, DOG in the presence of CORM: 56.3 ± 2.4%, one-way ANOVA followed by Bonferroni post-test, P<0.05, n=60 from 5 replicates). Our data reinforces that microglia rely on glycolysis to maintain cell viability. Furthermore we highlight that CO can modulate microglia cell viability in conditions where glycolysis is compromised. Given that glycolysis is perturbed in neurological disorders, further research into these gases mediators is required before promoting them as potential therapies.


This work was supported by The Physiological Society.

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

On the electrical response of the retina to light stimuli

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The eye is a complex sensing system. It converts the light stimulus that reaches the retina to electrical signals and also functions as a processor of the data. Understanding the biological visual system has contributed already to major tasks in computerized image processing and machine vision, including medical imaging.

In this work, the retinal response to light stimuli at the ganglion cells was investigated and analyzed. A retina of a rat (rattus norvegicus aged 4-6 weeks, after dark adaptation, n=24) was used, and experiments of extracellular recordings in vitro were carried out. We have used isoflurane and CO2 for anesthesia and euthanasia. The eye was enucleated and the retina was extracted and placed on a microelectrode array (MEDA), allowing recording from up to 60 electrodes in parallel. Throughout the recording, the retina was superfused with Ringer’s solution similar to extracellular liquid at 300C.
Two sets of experiments were performed. At the first set of experiments, the retina was stimulated with uniform flash illumination. In each stimulus at this set, the pulse duration was 2, 1, 0.5, 0.1 or 0.05 sec. The additional controlled parameter was the dark duration between pulses, as 3.5 or 10 sec. The effect of those parameters on retinal response was analyzed according to three parameters: the response time (between the beginning of the stimulus and the start of the response), the frequency peak (the highest firing rate) and the time-constant of the adaptation process. The second set of experiments was performed with a checker-board pattern stimulus of various resolutions, from a 2X2 checker-board up to a 10X10 checker-board.

At both experiments the electrical signals were processed by a dedicated algorithm to improve the signal to noise ratio. For the first set experiments, using uniform illumination, we found that the optimal pulse of 0.1 sec led to maximal firing rate and minimal delay. With longer dark duration, the retina was slower. The time-constant of the adaptation was not affected by the pulse duration and the dark duration. At the second set of experiments, recognition of the checker-board stimulus was successfully performed, such that each recorded point could be characterized as an ON or OFF cell. The resolutions that could be recognized were 2X2 and 3X3. Higher resolutions were not recognized correctly and tended to elicit results closer to those obtained with uniform light. In this experiment, it was found that the number of ON cells was much higher than OFF cells.

The conclusions of this research may contribute to the development of artificial bionic vision and in supporting future research in computerized machine vision.

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PC247

Retinal ganglion cell dendropathy is an early marker of cell death in mouse retinal explants

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The murine retinal explant is a popular model for the study of neuronal degeneration and repair (1). Therapeutic efficacy is usually quantified by cell counts/viability markers, which provide limited information regarding mechanisms leading to cell death. Predicated on the evidence that dendropathy precedes cell loss in most neuronal degeneration (2, 3), we postulated that in the retinal explant dendrite analysis may provide a more sensitive measure of neuronal health than cell counts alone. Explants from adult C57/B16 mice were cultured as wholemounts at 37°C, 5% CO₂ for up to 14 d. Frozen sagittal sections were nuclear stained and immunohistochemically stained for the apoptotic marker active caspase-3. Retinal ganglion cells (RGCs) were labelled diostically (DiI/DIO) for the quantification of dendritic arbours by Sholl analysis (4).

The effect of brain-derived neurotrophic factor (BDNF) was investigated by incubation with BDNF (100 ng/ml) for 3 d initiated at 0 d (3 d total) or at 3 d (6 d total). Values are means ± S.E.M. Nuclei counts, BDNF Sholl area under the curves (AUCs) and non-BDNF branching indexes were compared by ANOVA with TUKEY post-hoc, active caspase-3 staining and non-BDNF Sholl AUCs were compared by Mann-Whitney with Bonferroni correction, and BDNF branching indexes were compared by independent samples T-test. The ganglion cell layer (GCL) nuclei count significantly decreased by 14 d (65±6 vs.104±5 cells/mm at 0 d, p<0.005, n=18 retinas) with a corresponding 45.6% increase in active caspase-3 in the GCL (2.69±1.24 vs. 1.85±0.52 +ve cells/mm at 0 d, p>0.05, n=16 retinas). Significant dendropathy was observed at 1 d, quantified as a 36.6% reduction (1136±94 vs.1737±116 at 0 d, p<0.001) in Sholl AUC and a 43.0% reduction (645±66 vs. 1133±95 at 0 d, p<0.05) in branching index (n=178 cells). BDNF treatment resulted in a 61.5% increase (1365±180 vs. 845±134 for control, p<0.05) in Sholl AUC and an 80.3% increase (909±130 vs. 504±83 for control, p<0.05) in branching index relative to controls (n=36 cells). Delayed BDNF treatment resulted in a 136% increase in Sholl AUC (1166±147 vs. 495±74 for control, p<0.001) and a 107% increase (610±86 vs. 295±43 for control, p<0.005) in branching index relative to controls (n=36 cells). The delayed BDNF Sholl AUC increased relative to 3 d Sholl AUC (56.6%,1166±147 vs. 745±82 for 3 d, p<0.005). We report that in adult mouse retinal explants, RGC dendropathy precedes cell loss by at least 7 d. We demonstrate that dendrite analysis provides a sensitive method for monitoring neuronal health and a sensitive readout for the effects of therapeutic interventions. This assay facilitated the identification of a treatment window when neuronal cell structure and function may be recovered.


This work was funded in part by the Biotechnology and Biological Sciences Research Council (UK).

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PC248

Spontaneous vibration of cochlear basilar membrane:

A model of transient receptor potential coupled with the tip link vibration

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A mechanism of spontaneous generation of cochlear basilar membrane vibration by hair cells was examined under non-firing hair cell conditions simulated using a transient receptor potential (TRP) channel model equipped with the tandem repeats of outer hair cells driven by the membrane potential based on the Hodgkin-Huxley (HH) equations for their channels including TRP channel. We proposed a model of the TRP channel opening coupled with the tip link vibration, which resulted spontaneous oscillations of the membrane potential.
Brain angiotensin type 1 receptors are involved in Cushing response to increased intracranial pressure in normotensive rats

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**Introduction:** Aute increase in intracranial pressure (ICP) may result from cerebral hemorrhage, brain trauma, edema or acute hydrocephalus and leads to compression of brain structures and decreased cerebral blood flow [1]. The diminished cerebral perfusion is compensated for by Cushing reflex which maintains cerebral blood flow under conditions of high ICP via elevation of blood pressure (BP) [2]. Robust evidence shows that brain angiotensin type 1 receptors (AT1Rs) play an important role in pressor response to physiological and pathological stimuli [3].

**Aim:** Thus, we sought to find out how AT1Rs participate in the hemodynamic response to acutely increased ICP.

**Methods:** Male Sprague-Dawley rats, 14-18 weeks of age, were implanted with arterial catheter for recording of blood pressure (BP) followed by implantation of two steel cannulae in the lateral cerebral ventricles (LCV). The cannula placed in the right LCV was used for intrabrain infusion for increasing ICP and for recording of ICP, while the one in the left LCV was used for administration of investigated substances. The animals were divided into 2 groups: control group LCV infused with 0.9% NaCl (n=6); and experimental group LCV infused with losartan, AT1Rs antagonist (n=6). After recording baseline BP and ICP, saline (10 µL/30sec) or losartan (10 µg/10µl/30 sec) was infused into LCV. After 5 min ICP was gradually increased by intracerebroventricular infusion of 0.9% NaCl by syringe pump at the rate of 60 µl/min till obtaining ICP of ca. 100 mm Hg. All procedures and measurements were performed under urethane (1.5g/kg b.w.) anaesthesia.

**Results:** The pre-treatment BP and ICP were similar in control and losartan animals (95 +/- 7 mmHg, 5.8 +/- 2.8 mmHg; 97 +/- 8 mmHg, 7 +/- 1.4 mmHg, respectively). LCV administration of saline (60 µl/min) resulted in similar increase in ICP in the control group (98 +/- 2 mmHg) and in the losartan one (99 +/- 2). In the control group increase in ICP resulted in significant elevation of BP in comparison to baseline values (paired Student t-test, p<0.05), which is typical for Cushing reflex. In the experimental group, pre-treatment with losartan diminished BP increase in response to high ICP and significantly reduced blood pressure change in comparison to the control group (unpaired Student t-test, p>0.05).

**Conclusions:** Our results indicate that brain angiotensin type 1 receptors participate in pressor Cushing reflex response to acute ICP increase. This new finding suggests that pharmacotherapy with angiotensin receptor blockers may adversely modulate BP control under conditions of acute intracranial hypertension.

Dennis LJ, Mayer SA. Diagnosis and management of increased intracranial pressure. Neurol India 2001; 49 Suppl:1:S37.


The study was funded by student grant nr 1MA/NM1/14 from the Medical University of Warsaw.

**Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.**
Optogenetic drive of thalamocortical neurons can block and induce experimental absence seizures in freely moving animals

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Absence Seizures (AS), a feature of many idiopathic generalised epilepsies, are characterised by a loss of consciousness and concomitant bilateral, synchronous 2.5 – 4Hz ‘spike and wave’ discharges (SWD) in the EEG. It is well established that AS expression relies on the integrity of both cortical and thalamic structures, given that 1) cortical initiation sites have been identified in both patients (frontal cortex) and animal models (perioral somatosensory cortex); 2) cortical and thalamic cells fire synchronously during AS and 3) spike and wave activity can be induced by electrical stimulation of the thalamus. However, the precise contributions of distinct neuronal populations in thalamic and cortical territories to the generation, maintenance and termination of AS remain unknown. This study investigated whether AS can be suppressed or induced by modulating the firing of discrete cell populations within the thalamocortical network, using a well-established genetic model of AS, the Genetic Absence Epilepsy Rats from Strasbourg (GAERS).

Adult male GAERS (n=4, 250-350g) were fully anaesthetised (2.5% isoflurane inhalation), injected with 1μl pAAV-CaM-KII::hChR2(H134R)-mCherry (1.14 x 10^9 GC/μl) into the ventrobasal thalamus and implanted with fiber optic-tetrode microdrives targeted to the same region, in accordance with UK legislation and local ethical guidelines. A minimum of 3 weeks post-injection, 473nm laser pulses were delivered to freely moving animals upon detection of SWD using a closed-loop system, or during seizure-free periods of active wakefulness (AW), quiet wakefulness (QW) and slow wave sleep (SWS). Driving excitation of ChR2+ thalamocortical neurons, using light stimulations as brief as 100ms, was able to block spontaneously occurring AS (mean 3-20Hz power ± SEM. Sham trials 356.4 ± 26.2 AU, versus laser trials 178.6 ± 27.4 AU, ANOVA p<0.001). Interestingly, single, brief stimulations (5-200ms) of the same population of thalamocortical neurons were also able to induce AS that were indistinguishable from natural AS in their EEG signature (~7Hz), multi-unit firing and concomitant behavioural arrest. Induction of AS was highly dependent on arousal state, whereby seizures could be induced during QW (54.2 ± 20.8%, median induction rate for 5ms pulses ± SEM), but less so during AW (14.1 ± 1.6%, ANOVA p=0.05). During SWS, neither single nor patterned stimulation of thalamocortical neurons was able to induce AS (0 ± 0%, ANOVA p<0.05). The occurrence of both spontaneously occurring and light-induced AS during QW (5ms pulses: 79.3 ± 5.7%) was markedly reduced by administration of ethosuximide (100mg/kg i.p; 49.4 ± 6.1%, T-test p<0.01). This work provides further insight into the precise role of thalamocortical neurons in the generation, maintenance and termination of AS.

Supported by the Wellcome Trust (grant 091882)

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Lateral habenula regulates temporal pattern organization of rat exploratory behavior and acute nicotine-induced anxiety in hole-board

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Nicotine is one of the most addictive drugs of abuse. Tobacco smoking is a major cause of many health problems worldwide, and is the first preventable cause of death (Di Giovanni, 2012). Several findings show that nicotine exerts significant aversive as well as the well-known rewarding motivational effects (Fowler and Kenny, 2014). Less certain is the anatomical substrate that mediates or enables nicotine aversion. Here we have focused on nicotine-induced anxiety-like behavior in unlesioned and lesioned lateral habenula (LHb) rats. Firstly, we showed that acute nicotine induces anxiogenic effects in rats at the doses investigated (0.1, 0.5, and 1.0 mg/kg, i.p.; n = 10 for each group) as measured by the hole-board apparatus, and manifested in behaviors such as decreased rearing and head-dipping and increased grooming. No changes in locomotor behavior were observed at any of the nicotine doses given. T-pattern analysis (Casarrubia et al., 2015) of the behavioral outcomes revealed a drastic reduction and disruption of complex behavioral patterns induced by all three nicotine doses, with the maximum effect for 1 mg/kg (n = 10). Lesion of the LHb (n = 7) induced a significant anxiogenic effect, reduced the mean occurrences of T-patterns detected, and strikingly reverted the nicotine-induced anxiety to an anxiolytic effect when compared to sham-lesioned rats (n = 10). We suggest that LHb is critically involved in emotional behavior states and in nicotine-induced anxiety, most likely through modulating serotonergic/dopaminergic nuclei.

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

PC252

Divergent angiotensin receptor signaling in a mouse model of post-traumatic stress disorder (PTSD)

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Background: Independent of their beneficial effects on hypertension and cardiovascular related disease, angiotensin receptor type 1 (AT$_1$R) blockers can improve stress-related symptoms (Saavedra JM et al., 2012). AT$_1$R receptor-mediated actions can be counteracted directly or indirectly by the angiotensin receptor type 2 receptor (AT$_2$R). Our recent studies in a mouse model of PTSD have shown that AT$_1$R blockade increases the extinction (learned inhibition) of a traumatic fear memory and that AT$_1$R mRNA expression is reduced in fear related brain regions of animals treated with the AT$_1$R antagonist losartan (Marvar et al., 2014). These data imply that downstream AT$_1$R signaling events maybe important in consolidation of fear memory extinction. Therefore we investigated the acute effects of AT$_1$R inhibition on fear memory and baseline anxiety in mice. Methods: We performed classical Pavlovian fear conditioning pairing auditory cues with foot shocks and examined fear extinction behavior and cardiovascular responses in the presence of the AT$_1$R antagonist PD 123319. Results: Twenty-four hours following fear conditioning, PD 123319 (15 mg/kg IP) was administered prior to fear memory extinction. The PD treated group exhibited significantly less freezing behavior (F$_{10,300} = 1.9; p<0.05$) during fear expression and contrary to our previous results with the AT$_1$R antagonist losartan, there was no effect during extinction retention, an index of long-term fear memory. Moreover, qPCR data revealed that mRNA expression in the central amygdala of AT$_1$R and angiotensin converting enzyme 2 (ACE2) are elevated following fear conditioning, whereas the AT$_1$R gene expression pathways are unaltered. Conclusion: These data indicate that AT$_1$R and AT$_2$R may have divergent effects on short and long-term fear memory formation. Further studies are required to understand the differential regulation of angiotensin receptor signaling in PTSD.


National Institutes of Health K99 / R00

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PC253

Caffeine activates a sodium–calcium exchange current in pyramidal neurons of the hippocampus

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Caffeine is the most widely consumed behaviorally active substance in the world. It exhibits a variety of stimulatory effects upon the CNS. In low doses, caffeine produces a state of behavioral arousal & increased alertness; higher concentrations may bring about CNS hypexcitability characterized by restlessness & tremor, while toxic levels are associated in some cases with focal & generalized seizures (Dunwiddie et al. 1981). The mechanism(s) by which it produces these effects is not well understood although much is known of the role of caffeine in a number of well-defined pharmacological effects related to the blockade of adenosine receptors, inhibition of phosphodiesterases as well as caffeine-induced [Ca$^{2+}$]$^+$ elevation in central neurons (Fredholm et al. 1999). The Na$^+$ / Ca$^{2+}$-exchanger (NCX) in the plasma membrane of neurons appears to be the rapid extrusion of high concentrations of Ca$^{2+}$ that has entered neurons through surface channels, or has been released from the endoplasmic reticulum (Annunziato et al. 2004). Thus, the aim of this work was to measure caffeine-induced NCX currents in isolated pyramidal neurons of the hippocampus.

Saline

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Nicotine 0.5

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Nicotine 1

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<td>dip</td>
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Fig. 2 – Percent distribution of T-patterns containing hole-exploratory behavioral components (edge-sift and/or head-dip) in unlesioned (saline, nicotine 0.1, 0.5 and 1 mg/kg) and in LHB lesioned groups. * = p < 0.0001 compared to the unlesioned saline group; + = p < 0.0001 compared to the same group saline condition.

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

PC253

Caffeine activates a sodium–calcium exchange current in pyramidal neurons of the hippocampus

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Caffeine is the most widely consumed behaviorally active substance in the world. It exhibits a variety of stimulatory effects upon the CNS. In low doses, caffeine produces a state of behavioral arousal & increased alertness; higher concentrations may bring about CNS hypexcitability characterized by restlessness & tremor, while toxic levels are associated in some cases with focal & generalized seizures (Dunwiddie et al. 1981). The mechanism(s) by which it produces these effects is not well understood although much is known of the role of caffeine in a number of well-defined pharmacological effects related to the blockade of adenosine receptors, inhibition of phosphodiesterases as well as caffeine-induced [Ca$^{2+}$]$^+$, elevation in central neurons (Fredholm et al. 1999). The Na$^+$ / Ca$^{2+}$-exchanger (NCX) in the plasma membrane of neurons appears to be the rapid extrusion of high concentrations of Ca$^{2+}$ that has entered neurons through surface channels, or has been released from the endoplasmic reticulum (Annunziato et al. 2004). Thus, the aim of this work was to measure caffeine-induced NCX currents in isolated pyramidal neurons of the hippocampus.

235P
All experiments were performed in accordance with the guidelines set by the National Institutes of Health for the humane treatment of animals & the Animal Care Committee of Bogomoletz Institute of Physiology. The Wistar rats (P 14) were deeply anesthetized using sevoflurane & decapitated. The NCX currents were measured in acutely vibroisiliated CA1 & CA3 pyramidal neurons from hippocampal slices (400-500μm) using whole-cell patch-clamp technique (V_m = -80 mV) in combination with extracellular solution switches. Values are means ± SEM, compared by Student’s t-test.

Application of caffeine (10 mM, 5 sec) caused the inward current of maximal amplitude 50 ± 7 pA (n = 16) & 61 ± 7 pA (n = 9) in acutely isolated CA1 & CA3 pyramidal neurons respectively. These currents were suppressed by NCX mineral blocker Ni²⁺ (5 mM) on 87 ± 5 % (n = 20, p < 0.001). Cd²⁺ (15 μM – blocking concentration of voltage-gated calcium channel currents) didn’t decrease the caffeine-induced current. So, the observed caffeine-generated depolarizing current is associated with NCX activity in Ca²⁺ extrusion mode due to the electrolytic nature of a 3 Na⁺ for 1 Ca²⁺ exchange process. Thus, caffeine causes depolarization of hippocampal pyramidal neurons, a response which appears to be induced by activation of an inward Na⁺/Ca²⁺-exchange current, as Ca²⁺ is extruded through the Na⁺/Ca²⁺-exchanger, likely following local calcium release from intracellular stores.


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PC254

Changes in brain state influence urodynamic parameters during cystometry in urethane-anesthetised rats

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Urine production is reduced during sleep, largely due to absence of fluid intake and increased secretion of ADH. Functional bladder capacity also increases, enabling larger volumes of urine to be stored (Negoro et al., 2013). This effect may be centrally-mediated. Micturition is dependent on the functional integrity of a spino-midbrain-spinal loop that relays in the caudal ventrolateral periaqueductal grey (vPAG) (Stone et al., 2011). We therefore investigated 1) whether urodynamic parameters change during alterations in brain state and 2) whether such effects involve the PAG.

The urethane anaesthetised rat shows spontaneous cyclical changes in EEG waveforms that are considered to reflect changes in sleep-like brain states (Clement et al., 2008). We used this preparation to make urodynamic measurements during continuous cystometry whilst recording neuronal activity in the PAG.

Male urethane anaesthetised Wistar rats (1.4g Kg⁻¹ i.p.) were instrumented to record blood pressure, heart rate, tracheal air flow and cortical EEG and for intravenous infusion of fluids. Rectal temperature was maintained at 37ºC. Following a laparotomy the dome of the bladder was pierced with a needle to allow infusion of saline and simultaneous recording of bladder pressure. Bipolar electrodes were inserted into the external urethral sphincter (EUS) to record EMG. Single unit activity in the PAG was recorded using an insulated tungsten electrode. Infusion of saline into the bladder (6ml h⁻¹) evoked repeated cycles of filling and voiding (0.3±0.02 min⁻¹). Each void was characterized by a steep rise in bladder pressure and development of rhythmic bursting activity in the EUS.

During the experiment the EEG cycled spontaneously between high amplitude low frequency (1Hz) waves (‘activated’ state similar to slow wave sleep) and low amplitude, high frequency activity waves (‘activated’ state similar to rapid eye movement sleep), mean period 22min. There was no detectable change in depth of anaesthesia.

On transition from the ‘activated’ to ‘deactivated’ state the threshold bladder pressure for initiating a void increased from 11.2±0.6 to 14.0±0.9 SEM mmHg (p<0.01, paired t-test) accompanied by a decrease in end filling bladder compliance (0.18±0.02 to 0.11±0.04 ml mmHg⁻¹, p<0.05, log transformed values). Of 10 spontaneously active units in the vPAG, which showed excitatory or inhibitory responses time locked to voiding, the basal firing rate of 7 also cycled in parallel with changes in EEG. Firing decreased from 9.8±3.2 to 2.0±1.3Hz (p<0.05) as the EEG cycled from the low amplitude ‘activated’ state to synchronized slow wave activity. All changes were reversed as the EEG cycled back to the ‘activated’ state.

We suggest that the micturition reflex is reset centrally during slow wave sleep in a manner that promotes continence, via a mechanism mediated via the cvlatPAG.


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PC255

The non-synaptic anti-epileptic effect of leucurogin

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Intense epileptic seizures are accompanied by an extracellular increase of K⁺ and decrease of Ca²⁺. Under this circumstance, the synaptic transmission must have been blocked and the seizure is sustained by non-synaptic mechanisms. Therefore, the searching of anti-epileptic drugs acting on non-synaptic targets is being considered as a powerful strategy to control seizures that nowadays are refractory to the available drugs. There is increasing evidence that integrins may participate in epileptogenesis and, therefore, may be considered a non-synaptic target. In the present work we investigate the effect of leucurogin (Leuc), a recently identified disintegrin, on the non-synaptic epileptiform activity (NEA) induced in the
dentate gyrus (DG) of a rat (Wistar, male, 100-120g, n = 8) hippocampal slices. The NEA was induced by bathing slices with high-K+ and low-Ca++. The extracellular potential (EP) and intrinsic optical signal (IOS) were used to record the epileptiform events. EPs were quantified according to the parameters: DC-shift (DC), event duration (ED) and population spikes amplitude (PS). Twenty minutes after NEA induction, slices were bathed with solutions containing Leuc. EP recordings performed at the granule cell layer of DG show that with the dosage ~ 33μg/ml of Leuc the NEAs were suppressed. Comparison between the electrophysiological parameters before and after Leuc application shows non-significant differences. Values are means ± S.E.M. (DC – before: 7.80±0.68 mV, after: 7.57±0.67 mV; ED – before: 35.82±2.35 s, after: 37.63±3.14 s; PS – before: 0.43±0.04 mV, after: 0.38±0.05 mV; n = 40; compared by paired t-test; p<0.05). The IOS shows that during the blockage the entire layer had no NEA. Our data show that Leuc is a potential non-synaptic anti-epileptic drug, which is reversibly recovered after washout. Future work must be addressed to unravel the mechanism responsible for the anti-epileptic effect of Leuc. This perspective may open new avenues on the seizure control.

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PC256

Elucidating the reversibility of ataxia

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Dominant heterozygous mutations as well as a recently identified homozgyous nonsense mutation in the SPTBN2 gene (1), encoding β-III spectrin, are implicated in human cerebellar ataxia. A mouse model lacking β-III spectrin (KO), published by our lab in 2010, mimics the progressive human phenotype displaying motor deficiencies as well as Purkinje cell (PC) degeneration and death (2). Recent immunohistological analysis of cerebellar brain slices from 12 month old mice using anti-calbindin antibody has shown significant PC loss in the posterior cerebellum of KOs when compared to wild-type (WT) animals (55.5±7.8% PCs of WT, N=3, p<0.01, t-test). These findings were supported by the observation that KO PC dendritic trees in posterior lobules filled with Alexa 568 show significant reduction in the surface area occupied (WT 13889±972.3μm² and KO 6512±1786μm², N=3, n=8, p<0.01, t-test). We have used a recombinant adenovirus (rAAV) approach to elucidate whether reintroduction of β-III spectrin to the posterior cerebellum can halt, alleviate or reverse the disease phenotype. Unfortunately the full length (FL) β-III spectrin cDNA is too large to be packaged but using HEK 293 cells we have identified that C-termius (C-trm) of β-III spectrin localises at the plasma membrane and does not alter the distribution of FL protein. Furthermore we have previously reported increased sodium currents in primary hippocampal cultures transfected with FL β-III spectrin (3) and C-trm β-III spectrin was found to enhance sodium currents to the same degree as FL (untransfected -886.2±117.2pA, FL-1726±277.8pA and C-trm-1712±250pA at -10mV step, N=6, n=20, p<0.01 when compared to untransfected cells, one-way ANOVA). AAV1/2 particles expressing C-trm of β-III spectrin under the control of the enhanced synapsin promoter and GFP from an internal ribosome entry site were introduced via stereotaxic injection into mouse cerebellum (anesthesia induced by 2% isoflurane inhalation at flow rate of 1 l/min). We observed that 5 months after surgery KO animals had similar levels of transduced cells surviving to WTs (WT 204±64.2 cells per animal, KO 109±57.8, N=4) but with higher percentage of transduced cells residing in the posterior lobules (WT 28.3±16.7% and KO 62.3±16.7%). The motor phenotype of β-III spectrin KO mice using the elevated beam task was found not to be reversed (0.25±0.08 slips for WTs and 1.8±0.32 for KOs, p<0.01, two-way ANOVA), however the motor deficits did not deteriorate beyond 2 months of viral expression. Nevertheless, the weak behavioural effect is likely due to a low viral titre and small number of PCs transduced in this study. Ongoing work is examining whether injection at an earlier age and with more viral particles shows more therapeutic promise. Data reported as mean±SEM. N indicates the number of animals/cultures, n indicates the number of cultures.


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PC257

Single-dose curcumin administration has antiincoceptive and anti-allodynic effects in mice

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Curcumin (Cc), one of the main ingredients in curry powder, inhibits the cyclooxygenase 2 pathway (1), restores mitochondrial functions and decreases lipid peroxidation (2). Recent studies (3–5) indicate that chronic Cc administration has analgesic and antinociceptive effects, but less is known about acute Cc administration. The aim of this study was to assess the effect of single-dose Cc on nociception and allodynia. Male BALB/c mice (n=32) were divided in groups of eight: groups C1 and C2 received single-dose 120 mg/kg b.w. Cc dissolved in olive oil and groups O1 and O2 - equivalent volume of olive oil. All substances were administered via gavage. Groups C1 and O1 were assessed for nociception by means of Tail Flick (TF) and Hot Plate (HP); groups C2 and O2 were assessed for mechanical and thermal allodynia by means of von Frey (vF) and dynamic plantar (Hg) tests. Baseline values were recorded for each test. After gavage, HP and TF were performed at 30, 60, 120, 180 and 240 minutes and vF and Hg at 2 and 4 hours. Paired and unpaired Student t tests were performed with the...
aid of SPSSv20 software in order to assess the results. Cut-off was set at 15s for HP, 12s for TF, and 20s for Hg and vF. Values are expressed as maximum possible effect \([\text{MPE}(\%) = \frac{(\text{treated} - \text{baseline}) \times 100}{(\text{cut-off} - \text{baseline})}\] Single-dose Cc administration induced a long-lasting antinociceptive effect, with increased response latencies that started after 30 minutes in the TF test and after 3 hours in the HP test and lasted until the end of the experiment \((p<0.05\) in both paired and unpaired t test). MPEs for TF were 21.4% at 30 minutes, 34.1% at 60 minutes, 18.8% at 120 minutes, 23.6% at 180 minutes and 14.3% at 240 minutes. MPEs for HP were 8.5% at 30 minutes, 18.1% at 60 minutes, 24.6% at 120 minutes, 50.9% at 180 minutes and 54.7% at 240 minutes. Cc also induced a persistent increase in Hg and vF test latencies, with MPEs of 18.1% for vF and 20.4% for Hg at two hours and 11.9% and 38.0% at 4 hours \((p<0.05)\) in both paired and unpaired t test at 2 and 4 hours after administration. The results indicate that even a single curcumin dose has antinociceptive effects. Taking into account Cc pharmacokinetics, our results indicate that Cc exerts its analgesic effect through both peripheral and central mechanisms. Thus, the increase in thermal latencies in TF is probably due mainly to peripheral changes, while thermal and mechanical latencies from HP, Hg and vF test are consequence of both peripheral and central effects. Further studies are required for understanding Cc’s analgesic mechanisms and for translating the results in clinical practice.


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PC258

**Single versus repeated administrations of disulfiram on nociception and visceral pain in mice**

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Disulfiram (Dis) is widely used in the treatment of alcoholism (1), also it has been reported that disulfiram changes perception on some tests involving thermonociception (2) and also induces mitochondrial changes through at least two mechanisms (3).

The purpose of our study was to assess the effect of a single Dis dose versus repeated administrations (19 days) on thermonociception and visceral pain in mice. Thermonociception was evaluated by hot plate (HPT) and tail flick (TFT) tests and visceral pain by the writhing test.

Methods: BALB/c mice \((n=62)\) were divided into 2 groups AG \((n=32)\) receiving a single dose of Dis \((50 \text{ mg/kgc})\) by gavage or an equivalent volume of olive oil and CG \((n=32)\) with daily administrations for 19 days of Dis \((50 \text{ mg/kgc})\) or same volume of olive oil. In AG, after baseline determination, thermonociception was evaluated every 2 days for the next 19 days by HPT and TFT. In the 19th day, writhing tests was performed one hour after the daily administration both for Dis \((n=8)\) and control group \((n=8)\).

The maximum possible effect was expressed as \([\text{MPE}(\%)=(\text{treated} - \text{baseline}) \times 100/(\text{cut-off} - \text{baseline})]\), where the cut-off for TFT was set at 12s and for HPT at 15s. ANOVA test was used for statistical analysis.

Results: On HPT, a single administration showed analgesic effect after one hour and persisted throughout the experiment with the MPE at 240 min \((\text{MPE}=39.84\% \pm 24.87\%, p<0.01)\), on tail-flick a decrease in response time was noted with significance after the first hour \((p<0.05)\). In the writhing test there was a decrease in the number of writhes with no statistical significance.

In the CG, on HPT changes were noted from day 9 showing an antinociceptive effect \((p=0.02)\) maintaining until the last day. No significant changes were noted on TFT or in the number of writhes compared with control.

Conclusions: Our data demonstrate that both acute and chronic Dis administrations impair thermonociception but have no effect on the visceral pain. One interesting finding is that only one dose has an analgesic effect on HPT while cumulative doses reverse this effect into a persistent hyperalgesia after 3 days of treatment. On the TFT, single or repeated doses of disulfiram produced hyperalgesia. Even further biochemical studies must to be conducted to clarify the mechanism of action we could suppose that transitory/permanent disruption of mitochondrial aldehyde dehydrogenase as well as impairment of norepinephrine release after acute and chronic disulfiram administration are responsible for thermonociception changes.

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Anti-nociceptive effect of taurine and caffeine in sciatic nerve ligated Wistar rats: Involvement of autonomic receptors

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Neuropathic pain has been defined as pain caused by a lesion of the peripheral or central nervous system (or both), manifesting with sensory symptoms and signs (positive and negative sensory phenomena) (Backonja, 2003). Neuropathic pain is often associated with the appearance of abnormal sensory signs, such as allodynia (pain as a result of a stimulus which does not normally provoke pain) or hyperalgesia (an increased response to a stimulus which is normally painful) and often reported as having a lancinating or continuous burning character (Bridges et al., 2001).

In this study, we investigated the effects of co-administration of taurine and caffeine on thermally induced pain in sciatic nerve ligated rats as well as the roles of autonomic receptors. Neuropathic pain was induced by chronic constriction injury in line with the modified method of Bennett and Xie (1998). In brief, each rat was deeply anaesthetized with intraperitoneal injection of 50mg/kg ketamine (Kaur et al., 2012) and the skin of the lateral surface of the left thigh was incised and a cut was made directly through the biceps femoris muscle to expose the sciatic nerve. Once exposed, the sciatic nerve was tightly ligated with silk 4-0 thread at two sites with about 1mm gap (kumar et al., 2010). The anti-hyperalgesic effect of combined systemic (i.p.) administration of taurine and caffeine were assessed using tail flick tests and hot plate test for two weeks. To determine the involvement of autonomic nervous system, we examined how administration of cholinergic (Atropine and hexamethonium) and adrenergic (prazosin and propranolol) receptor blockers altered the combined effect of taurine and caffeine. Likewise, the serum level of oxidative stress marker malondialdehyde (MDA) was evaluated. The results showed that co-administration of taurine and caffeine attenuated thermal hyperalgesia in sciatic nerve ligated rats as shown by significant (p<0.05) increase in tail and paw withdrawal latencies in the treated groups compared to the ligated control group after two weeks of administration. The anti-nociceptive effects were reversed by pre-treatment with cholinergic blockers especially atropine while the adrenergic blockers spared the effects of taurine and caffeine. Also, the increase in tissue level of MDA induced by sciatic nerve ligation was significantly attenuated by combined administration of high dose of taurine and caffeine.Ilt can be concluded that co-administration of taurine and caffeine attenuates thermal hyperalgesia in sciatic nerve-ligated rats and this effects involves cholinergic system. The findings suggest that co-administration of taurine and caffeine might be useful for the treatment of neuropathic pain.


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Functional identification of cortical and subcortical areas associated with the increase in muscle sympathetic nerve activity in obstructive sleep apnoea in awake humans

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Introduction: Muscle sympathetic nerve activity (MSNA) is greatly elevated in patients with obstructive sleep apnoea (OSA) during daytime wakefulness, leading to hypertension. By recording MSNA concurrently with functional Magnetic Resonance Imaging (fMRI) of the brain we aimed to identify the central processes responsible for the sympathoexcitation.

Methods: Spontaneous fluctuations in MSNA were recorded via tungsten microelectrodes inserted into the peroneal nerve in 17 OSA patients and 15 age-matched controls while lying in a 3T MRI scanner. Blood Oxygen Level Dependent (BOLD) contrast gradient echo, echo-planar images were continuously collected in a 4 s ON, 4 s OFF protocol. BOLD signal intensity, measured every 1 s during the 4 s OFF period, was coupled to the bursts of MSNA, measured every 1 s during the 4 s ON period. Fluctuations in BOLD signal intensity covaried with the intensity of the concurrently recorded bursts of MSNA from the preceding 4 s. Results: MSNA-coupled BOLD signal intensity in the dorsolateral PFC, medial PFC, dorsal prefrontal cortex, and prefrontal cortex were higher in OSA than in controls, while in the RVLM, dorsolateral pons and medullary raphe it was lower. All of these changes were reversed following 6 months of continuous positive airway pressure, which caused a significant fall in MSNA towards control levels. Conclusions: We conclude that the elevated MSNA in OSA results from functional changes within suprabulbar regions known to be directly or indirectly involved in the modulation of sympathetic outflow via the brainstem, as well as from functional changes within the brainstem itself.

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Electrophysiological assessment of oligodendrocyte precursor cells and oligodendrocytes derived from human pluripotent stem cells

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The derivation of regionally-defined cellular phenotypes from human pluripotent stem cells (hPSCs) presents a platform
for the assessment of human physiology and disease in vitro. We have developed a protocol that generates enriched populations of oligodendrocyte (OL)-lineage cells from multiple hPSC lines. Within 1 week of OL differentiation, approximately 10-20% of the cells in culture were found to be platelet-derived growth factor receptor α (PDGFRα)-positive OL precursor cells (OPCs) and between 60-80% were OLs that were positive for mature marker, myelin basic protein. The membrane properties of rodent OPCs and OLs are well described and demonstrate distinctive intrinsic membrane properties and ion channel expression. In this regard, we have examined the membrane properties of hPSC-derived OPCs and followed these through to their maturation into OLs. Depolarization of OPCs induced large, sustained outwardly rectifying currents that were substantially reduced in OLs. Our data indicate a change in the ion channel expression profile of OLs compared to those expressed in OPCs. Passive membrane properties were measured as indicators of development. For OPCs compared to OLs input resistances of 2786 ± 228 MΩ (OPC, n = 11) vs 1508 ± 122 MΩ (OL, n = 12), whole-cell capacitances of 5.7 ± 0.5 pF (OPC, n = 11) vs 29.6 ± 3.7 pF (OL, n = 11), and resting membrane potentials of -34 ± 1 mV (OPC, n = 11) vs -44 ± 1.3 mV (OL, n = 12) indicate maturation of OPCs into OLs. We next examined the properties of AMPA receptors (AMPARs) expressed in hPSC-derived OPCs and OLs. AMPARs can be composed of 4 potential subunits, GluA1-4 of which the GluA2 subunit is developmentally post-transcriptionally edited post-transcriptionally, such that an ion channel-lining glutamine (Q) is RNA-edited to arginine (R). This imparts distinctive biophysical and pharmacological properties to AMPARs which include low Ca2+-permeability and reduced single-channel conductance. Using non-stationary fluctuation analysis we found that the single-channel conductance of AMPARs expressed in OPCs was 8.2 ± 0.9 pS (n = 7) and in OLs was 3.2 ± 0.5 pS (n = 7). This is consistent with a developmental switch in AMPAR composition from GluA2(R)-lacking to GluA2(R)-containing AMPARs in OPCs and OLs, respectively. This was confirmed by assessing block of AMPAR-mediated currents by NASPM which inhibited currents in OPCs 54 ± 9.8% (n = 8) but by only 28.1 ± 5.6% in OLs (n = 7). Thus, ion channel expression profiles exhibited by hPSC-derived OPCs and OLs are in good agreement with native OL-lineage cell development seen in rodents and offer the potential to assess the physiological properties of OL-lineage cells derived from patients suffering from demyelinating diseases.

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**PC262**

**Direct measurement of chloride transport by prestin (SLC26A5) indicates decoupling between antiporter and actuator functions**

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Mature outer hair cells of the mammalian cochlea express a molecular actuator, prestin, which determines cochlear sound amplification. Prestin (SLC26A5) is a low efficiency electro-ergic chloride-bicarbonate antiporter (Mistrik et al, 2012) and we have previously suggested that the mechanoenzyme properties of prestin arise from transitions between substrates of the transport cycle (Muallem & Ashmore, 2012). Although fluxes of other radiolabelled anions have been used as substitutes for chloride (Bai et al, 2009) we report here using direct chloride imaging techniques on whether chloride transports is separable from prestin’s mechanoenzyme role. In order to image chloride fluxes, we have used either YFP or pHluorin (a pH-sensitive enhanced GFP) linked to the cytoplasmic side of prestin and expressed in CHO cells. Elevated intracellular chloride was signalled by a fluorescence quenching and changes in pH were minimized. Transfected cells, characterised by a ring of plasmamembrane fluorescence, were imaged at 0.2 Hz while being continuously superfused with differing levels of chloride solutions in constant 23 mM bath bicarbonate. Without prestin expression there was no measurable movement of chloride in CHO cells. When prestin was expressed in the cells, changes in fluorescence depended upon the chloride gradient across the cells membrane. In order to calibrate the flux rates, cells were patch-clamped with pipettes containing either 5, 15, 45 or 130 mM Cl (Cl− replaced with gluconate) and the change in fluorescence measured on break-in to whole-cell, and determined the initial internal Cl− of the cells, held in normal extracellular Cl−, to be 18 mM. The fluxes were measured using pHluorin as the sensor and modelling the data indicated that the quench fluorescence curve had IC50 ~24 mM. To convert the fluxes to a prestin turnover, we used a mean prestin number of 2.5x10⁶ per cell. The prestin turnover so determined was 900 prestin⁻¹ s⁻¹. It is known that extracellular salicylate, a blocker of prestin’s non-linear capacitance (NLC) and actuator properties, reduces the NLC by over 90%, and is compatible with a competitive block of an intracellular chloride binding site with a Kᵢ =200 μM (Oliver et al, 2001). However 10 mM salicylate pre-applied to the to the bath reduced the YFP measured chloride flux by only 30%. There was a minimal shift in the fluorescence due to pH changes induced by salicylate. These data suggest that the hypothesis of a single mechanical step in the prestin transport cycle requires elaboration.


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**PC263**

**Characterisation and comparison of temporal release profiles of nitric oxide generating donors**

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Nitric oxide (NO) is a highly reactive and freely diffusible molecule that has been implicated in regulating a diverse range of physiological processes from synaptic plasticity to vasodilation. NO elicits these effects through different signalling pathways, which are largely dependent on the concentration of NO produced. At low and physiological concentrations, NO generation from nitric oxide synthase (predominantly neuro-
nal or endothelial nitric oxide synthase) leads to the activation of its target receptor soluble guanyl cyclase and subsequent generation of cyclic guanosine monophosphate which leads to the activation of a wide range of downstream signalling molecules such as protein kinases. At higher concentrations, such as those produced in response to pathophysiological conditions, NO release can result in post-translational modifications. These include S-nitrosylation of cysteine residues and nitration of tyrosine residues, both of which alter protein function, usually detrimentally. The study of NO signalling usually involves application of exogenous NO via various donors. However, the use of NO donors in research can be problematic due to the unknown release capacity of each donor. This has led to countless publications of contradictory findings due to the use of different donors and concentrations across studies. In order to better characterise the release profile of NO from commonly used NO donors, we measured temporal release profiles following varying storage times at 4°C and -20°C of different donors at multiple concentrations. NO release was detected in standard phosphate buffered saline over time using NO sensing electrodes. The NO microsensor chosen for this study (NOFP100; World Precision Instruments Ltd) possesses a multi-layered selective coating that eradicates non-specific detection providing reliable NO measurements. We found that diazeniumdiolate donors such as NOC-5 and PAPA NONOate initially release high levels of NO but decay substantially within days, whereas S-nitrosothiols SNP and GSNO stocks show greater stability in solution releasing consistent and lower levels of NO over several days. Furthermore, in all donors tested, the amount of released NO differs between long-term frozen and fresh stock solutions. Therefore our data provides a systematic and comprehensive comparison of NO release by different donors which provides insightful information for studying nitrergic signalling and allows a better evaluation of reported nitrergic signalling outcomes.

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PC264

A role for calcium in the release of matrix vesicles during aortic valve calcification

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Calcific aortic valve disease (CAVD) is characterised by the progressive thickening of the valve leaflets. The 5-year survival rate of inoperable patients with aortic stenosis is lower than many metastatic cancers, and there is no medication therapy that can stop its progression. Vascular calcification is particularly widespread amongst patients with chronic kidney disease (CKD), as elevated serum calcium (Ca) and phosphate (Pi) levels have reported to enhance vascular smooth muscle cell (VSMC) calcification via the release of matrix vesicles (MV), which are nano-structures that initiate mineralisation during skeletogenesis. In vitro studies employing the SV40T rat V2C cell line revealed that elevated Ca induced calcium deposition at a minimum concentration of 2.7mM (4.5 fold; P<0.01), as determined by quantitative calcium assay. Moreover, 3.6mM Ca treatment significantly increased the mRNA expression of the osteogenic markers PIT-1 (2 fold, P<0.001), Runx2 (1.2 fold, P<0.05) and Msx2 (2 fold, P<0.001). While no effect of Pi treatment alone was observed, treatment of Ca (2.7mM) and Pi (2.5mM) synergistically induced calcium deposition (414 fold; P<0.001). In further studies, MVs were harvested using ultracentrifugation from primary rat VCs that were cultured with control medium or calcifying medium containing 2.7mM Ca and 2.5mM Pi for 16 hrs. Their composition was assessed using Isobaric Tags for Relative and Absolute Quantitation (iTRAQ) Mass Spectrometry to identify potential mediators of mineralisation. The results revealed that VMCs share similarities with both chondrocyte-derived and VSMC-derived MVs, including enrichment of the calcium-binding proteins: annexins (Anx) A2 (4.8 fold), A5 (4.6 fold) and A6 (4.3 fold). These findings were further validated by western blotting analysis. Additionally, immunohistochemical studies were performed on human valves showing different degrees of calcification. Increased expression of Anx A6 in the severely calcified valve sample was observed when compared to the uncalcified control. To determine whether MVs mediate calcification in vivo, transmission electron microscopy (TEM) was used to examine MV deposition in human valves. MV-like structures were observed in the extracellular matrix of heavily calcified valve tissue, suggesting MV plays a role in the pathogenesis of CAVD. Our data establish calcium as a novel trigger of VMC calcification. These studies are the first to report extracellular vesicles resembling MVs in calcified human aortic valve tissue, suggesting CAVD is a cell mediated process regulated by vesicle release.


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AMP-activated protein kinase is necessary for hypoxic pulmonary vasconstriction

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Hypoxic pulmonary vasconstriction (HPV) is a physiological response that diverts blood flow to oxygen rich areas of the lung from those areas deprived of oxygen (Sylvester et al. 2012). The AMP-activated protein kinase (AMPK, Hardie, 2007) has been proposed to underpin cardiorespiratory adjustments during hypoxia (Evans et al., 2006), and pharmacological studies support the view that AMPK mediates HPV (Evans et al., 2005). The aim of the present investigation was to determine by conditional gene deletion whether or not the LKB1-AMPK signalling pathway in pulmonary arterial myocytes is indeed necessary for HPV. We assessed the impact of single and dual deletion of the genes for Ampkα1 and α2 catalytic subunits, and also assessed the impact of deleting the genes for the upstream kinases that activate AMPK in response to metabolic stress and increases in cytoplasmic calcium, respectively, namely LKB1 and CAMKKβ. Patch-clamp electrophysiological experiments show that deletion of AMPK catalytic subunits markedly attenuates Kv current inhibition by hypoxia in pulmonary arterial myocytes. Moreover, non-invasive Echo Doppler ultrasound revealed that HPV in-vivo was significantly inhibited by deletion in arterial myocytes of LKB1 or the AMPKα1 and α2 subunits, but remained unaffected following global knockout of CAMKKβ. Our findings demonstrate that AMPK determines, at least in part, pulmonary vascular responses to acute hypoxia at the molecular, cellular and system level. We therefore conclude that the LKB1-AMPK signalling pathway is required for HPV.


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PC266

Altered vascular responsiveness to Kv7 channel activators in old normotensive and hypertensive rats

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The Kv7 family of voltage gated potassium channels contribute to the maintenance of vascular tone, a function which is impaired in hypertension. There are three Kv7 isoforms consistently expressed in the vasculature – Kv7.1, 7.4 and 7.5 – but it is the reduction in Kv7.4 function and expression that has been attributed to compromised vascular function. Aging is a prime risk factor for the development of cardiovascular disease, but the status of Kv7 channels in the aging vasculature is not clear.

Using male Wistar normotensive (NT) and hypertensive (SHR) rats at 3 and 12 months of age, we investigated the vasorelaxant responses to the Kv7 activators ML213 (Kv7.4 activator) and RL-3 (Kv7.1 activator) on segments of mesenteric and renal arteries (MA and RA, respectively) by isometric tension recording in order to determine any functional change in Kv7 responses with aging. Experiments were performed in accordance with the UK Animal (Scientific Procedures) Act 1986, all data are presented as mean±SEM. ML213 relaxations in both MA and RA of 12 month SHR were reduced compared to 12 month NT animals (from 53.9±11.9 to 92.9±6.8% at 300nM in MA (n=4-5, p<0.01) and from 47.5±9.4 to 91±4.1% relaxation at 10μM in RA (n=5, p<0.005 determined by 2-way ANOVA)). Relaxations to RL-3 were also compromised in 12 month SHR compared with 12 month NT (from 63.5±9.8 to 99.7±0.7% relaxation at 100nM in MA (n=3-4, p<0.01) and from 64.4±16.4 to 92.7±5.6% relaxation at 10μM in RA (n=5, p<0.01 determined by 2-way ANOVA)). With aging, 12 month NT animals showed impaired relaxations to ML213 in the RA compared to their 3 month counterparts (from 16.8±11.6 to 96.1±3.6% relaxation at 300nM (n=4-5, p<0.005) whilst relaxations to RL-3 were also reduced (from 36±11.1 to 92.7±3.6% at 3μM (n=5, p<0.005 determined by 2-way ANOVA)). In contrast, the mesenteric arteries of 12 month NT showed no significant difference in relaxations to ML213 compared to 3 month animals. These findings reveal that the functional role of Kv7 channels is altered in the vasculature with aging and impaired in 12 month SHR compared with NT animals. This data shows for the first time the changing contribution of Kv7 channels to vascular reactivity with age, which could have profound implications for our understanding of disease pathogenesis and the development of hypertension.

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Pulse waveform analysis reveals vascular contributions to Gordon Syndrome and Gitelman Syndrome blood pressure homeostasis

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The intense study of the rare monogenic diseases Gordon and Gitelman syndrome, which mirror each other phenotypically, has been instrumental to further our understanding of the WNK pathway’s role in renal control of blood pressure by natriuresis. Deletion of exon-9 in Cullin-3 (CLL3ex9) causes a severe form of familial salt-sensitive hypertension known as Gordon Syndrome, which causes renal WNK4 accumulation leading to overactivation of the thiazide-sensitive Na-Cl Cotransporters (NCC) via the WNK4 downstream effector SPAK. Conversely the salt-wasting hypotensive Gitelman syndrome is caused by mutations affecting the NCC phosphorylation sites or can be modelled by mutating SPAK to reduce NCC activation.
However recent evidence has suggested that the WNK pathway may have additional roles in maintaining vascular smooth muscle tone. *Ex vivo* aortic ring myography of SPAK knockout mice has shown reductions in vascular contractility suggesting the changes in blood pressure may not be solely due to changes in renal salt homeostasis. Ex vivo aortic ring myography of SPAK knockout mice has shown reductions in vascular contractility suggesting the changes in blood pressure may not be solely due to changes in renal salt homeostasis.

To investigate the vascular contribution of the WNK pathway to blood pressure in mouse models of Gordon Syndrome (CUL3<sup>ex9</sup> heterozygotes) and Gitelman Syndrome (SPAK<sup>L502A</sup> homozygotes) by pulse waveform analysis. Systemic blood pressure traces were generated by catheterisation of the right carotid artery with a SPR-1000 pressure transducer under terminal anaesthesia (isoflurane). Pulse waveforms were then analysed and compared to wildtype littermates (WT) using macros scripted in Lab Chart Pro 8 to extract augmentation index (AIx), a measure of arterial stiffness, and the diastolic pressure decay time constant (τ), a surrogate marker of vascular resistance. All data are mean±SEM, statistical significance was determined by two-tail t-test, **P<0.01, ***P<0.001.

As expected mean arterial pressure was elevated in CUL3<sup>ex9</sup> vs WT (93.9±1.2 vs 81.0±0.7 mmHg ***) and decreased in SPAK<sup>L502A</sup> vs WT (54.0±1.6 vs 73.7±1.8 mmHg ** *). Pulse waveform analysis revealed an increase in arterial stiffness in CUL3<sup>ex9</sup> (AIx: 50.2±1.6 vs 42.6±1.6 % **) and vascular resistance (τ: 0.65±0.01 vs 0.59±0.01 s **) (Fig1A). Whereas SPAK<sup>L502A</sup> exhibited signs of vascular relaxation compared to WT indicated by decreased AIx (21.1±1.0 vs 35.0±2.1 % ***) and τ (0.44±0.01 vs 0.56±0.02 s ** *).

In conclusion, perturbation of the WNK pathway results in changes in vascular tone compounding the hypertension of Gordon Syndrome and hypotension of Gitelman Syndrome. These findings have implications for clinical treatment of patients to reduce long term cardiovascular risk and suggest that the WNK pathway is an attractive antihypertensive therapeutic target.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Urinary bladder strips were isolated from adult male Wistar rats and suspended in 20 ml water-jacketed tissue bath containing Krebs’ solution at 37 °C and pH 7.4, constantly bubbled with 95% O2-5% CO2. Isometric contractions were recorded by using isometric force displacement transducers which were attached to a Biopac data acquisition system. After equilibration under resting tension of 0.5 g for 60-minute, bladder strips were contracted by bath applications of carbachol (CCh, 1 μM). Ivabradin (10 and 60 μM) was added to tissue bath either prior or after application of the agonist and resulting contractile activity was compared with the preceding contractile activity. Amplitude and area under force-time curves (AUC) of isometric contractions were evaluated.

Ivabradin dose dependently inhibited both the peak amplitude and AUC values of the CCh-induced contractions in a concentration-dependent manner. On the average, normalized AUC values of CCh-induced contractions was reduced to 47 ± 5.5% (P<0.05) and 35% ± 6% (P<0.05) after application of 10 and 60 μM ivabradin (n=7 each), respectively. In addition, pretreatment with ivabradin significantly attenuated the contractile responses to the CCh without significantly effecting the resting tension (30μM ivabradin pre-treatment: 590±120 mg (n=6) vs. without ivabradin pretreatment: 2565±461 mg, n=8, P<0.05).

In conclusion, results of this in vitro study demonstrated that ivabradin inhibits carbachol-induced contractions of rat bladder smooth muscle implicating that this agent may have potential to be used in the treatment of overactive bladder. Where applicable, experiments conform with Society ethical requirements.


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KCa3.1 channel dysfunction by globotriaosylphosphoglycerol accumulation inhibits the collagen synthesis in fibroblast

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Fabry disease is an X-linked recessive metabolic disorder that arises by the deficiency of lysosomal alpha-galactosidase A, resulting in an excessive accumulation of globotriaosylceramide (Gb3) in multiple organs of the body, including the cardiovascular system. Among various cardiovascular complications ascending thoracic aortic dilation and aneurysm is relatively common in Fabry patients, which is characterized by abnormal extracellular matrix composition. In addition to Gb3, increased concentrations of globotriaosylphosphoglycerol (lyso-Gb3) have recently been reported in urine and plasma of Fabry patients. Therefore, we investigated the effect of lyso-Gb3 on the pathogenesis of ascending aortic aneurysms in Fabry disease, especially focused on the biological responses related to aortic remodeling by fibroblasts. Here we report that the fibroblast proliferation, differentiation into myofibroblasts and collagen synthesis are compromised in lyso-Gb3 accumulated fibroblasts. In addition, all of these compromised responses are attributed to KCa3.1 channel dysfunction in fibroblast by lyso-Gb3 via cAMP/PKA pathways. cAMP mediated phosphorylation, which was confirmed by whole-cell patch clamping method, and reduced the membrane channel expression by the inhibition of ERK phosphorylation, which is estimated by real-time PCR and immunoblotting. In KCa3.1 dysfunctional fibroblast by lyso-Gb3, the fibroblast proliferation and TGF-beta1 stimulated myofibroblast differentiation were compromised. As a result, collagen synthesis was significantly reduced. All of these impairments were restored by KCa3.1 activator. These results suggests that the modulation of KCa3.1 could provide a means to attenuate and prevent development of ascending thoracic aortic aneurysm in Fabry disease.

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Obligatory role of Gβγ subunits for the relaxing effect of isoprorenal in rat renal arteries

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Despite the key role of Kv7.4 channels in the regulation of vascular tone, and their involvement in mediating Gs-coupled vasodilator responses [1], the factors that regulate channel activity are poorly understood. Moreover, the signals linking Kv7.4 to receptor stimulation is still unclear. In this work we studied possible interactions between Kv7.4 channels and Gβγ subunits in β-adrenergoreceptor mediated responses in rat renal arteries (RRA). Membrane currents were measured using cell-attached configuration of patch-clamp technique on single myocytes freshly isolated from RRA. The cells were patched with a cocktail of K+ channel blockers in the pipette and generated small amplitude (≈0.2 pA) currents with apparent NPo 0.025±0.009 (n=7, mean±SEM) when the cell membrane was depolarised to 0 mV. Isoproterenol (ISO, 1 μM) applied to the bath solution increased the NPo to 0.102±0.023 (n=7, p<0.01, unpaired Student’s t-test). The presence of pertussis toxin did not affect basal channel activity (0.026±0.012, n=6) and ISO induced a small increase of NPo to 0.055±0.033 (n=5), this was not statistically significant (p=0.437). Linopirdine (10 μM), a pan-Kβγ blocker, in a patch pipette abolished the single channel activity, but did not produce significant changes if applied to the bath solution (n=4). In the presence of gallein (100 μM), a molecular inhibitor of Gγ7 activity, basal NPo was negligible, and the stimulatory effect of ISO was prevented (NPo 0.002±0.001, n=5). In HEK293 cells stably expressing Kv7.4 channels addition of 2ng/ml Gγ7 subunits to inside-out patches increased NPo from 0.035±0.006 (n=14) to 0.117±0.034 (n=3, p<0.05), and 50ng/ml Gγ7 subunits increased it further to 0.343±0.062 (n=6, p<0.001). Single channel conductance of these Kv7.4 channels was calculated as 2.31 pS, which is similar to the native linopirdine-sensitive channels in RRA myocytes. The functional role of Gγ7 subunits on the activity of Kv7.4 channels was checked on RRA segments using isometric tension myography. Both linopirdine and gallein produce a similar robust contraction. Linopirdine did not produce any further contraction if added after gallein consistent with a role for Gγ7 subunits dictating Kv7.4 activity. 10μM mSRK, a cell-permeable Gγ7 binding peptide, which causes dissociation of Gγ7 subunits from α subunits without stimulating nucleotide exchange [2] relaxed pre-contracted renal arteries by 36.1±2.8% (n=7) that was prevented by linopirdine (13.1±2.2%, n=7) or gallein (13.5±5.5%, n=6). Similarly, ISO relaxations of RRA were sensitive to Kv7 blockade and gal-
le as well significantly inhibited ISO-mediated relaxations of RRA. Our study suggest the view that Gβγ subunit interaction is obligatory for effective function of vascular Kv7 channels and when compromised can lead to diminished ISO-mediated vasorelaxation.


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Spreading of micromotions in juvenile rat bladder: directionality and function of gap junctions

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Urinary bladders display spontaneous phasic activity manifested as pressure fluctuations and bladder wall movements i.e. micromotions. Phasic activity is pronounced in bladders of young animals and changes during ageing. These changes give insight into factors moderating such activity, which could be relevant in the clinical context e.g. for overactive bladder syndrome. Hence, here we characterise the effects of 18β-glycyrrhetinic acid (18β-GA), a known gap junction blocker, on whole organ pressure fluctuations and bladder wall micromotions.

Methods: 3 week old (21±2 days) Wistar rats were killed by UK Schedule 1 procedures (N=15). Bladders were dissected and catherized with 26GA venflon (Becton Dickinson), filled with ~350 mbar Krebs (NaCl 118.4mM, glucose 11.7mM, NaHCO₃ 24.9mM, KCl 4.7mM, CaCl₂ 1.9mM, MgSO₄ 1.15mM, KH₂PO₄ 1.15mM) and carbon particles were applied to the bladder surface to monitor movements. Bladders were placed in an oxygenated organ bath (150ml) in Krebs solution at 37°C. Pressure and video data were simultaneously acquired at 10Hz via LabView application (National Instruments, USA) and a camera (Prosilica EC650). After at least 30min of equilibration in isovolumetric conditions, 30µM 18β-GA was administered (or 0.1% DMSO as a drug vehicle control). We compared 5 minute periods before and after drug addition using a two-tailed paired Student’s t-test (GraphPad Prism) and calculated percentage change of each parameter. Distances between the carbon points on the bladder wall were analysed (LabView) and plotted in LabChart (ADInstruments).

Results: 18β-GA decreased spontaneous phasic pressure fluctuations: the amplitude decreased by 79.8% (N=6) (p<0.001). When pressure fluctuations were detectable the frequency remained unchanged (3/6). Movement of bladder wall persisted at 30 minutes after addition of 18β-GA in all preparations, including three experiments without detectable pressure changes (<1cmH₂O).

Quantification of distances between points on the bladder wall confirmed altered pattern of movement with 18β-GA. Not all pressure fluctuations could be explained by visualised movement: distance elongations and shortenings coincided with pressure fluctuation changes, but not all rhythmical micromotions were synchronous with pressure change. This suggests that rhythmicity of localised movements on bladder wall is independent of pressure fluctuation. Longitudinal distances showed a dynamic pattern of contractions, where one part was contracting before the other, suggesting dynamic propagation of movement in this direction.

Conclusions: In general, in spontaneously active whole bladder the movements started at the top of the bladder dome and bladder base and spreading vertically (longitudinally). Gap junctions change but do not block bladder wall movements. Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

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Differential effects of cAMP/PKA and cAMP/Epac signalling in vitro angiogenesis: Role of RhoA/Rock signalling

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Background and Aims: cAMP signalling regulates several endothelial functions such as endothelial barrier integrity, vascular tone, and both physiological and pathological angiogenesis. cAMP mediates these effects via activation of its two well characterised effectors i.e. PKA and Epac (exchange protein directly activated by cAMP). We have previously shown that activation of both cAMP-effectors stabilises endothelial barrier via activation of different signalling pathways. The aim of the present study was to analyse the effects of these cAMP effectors on angiogenesis in vitro.

Methods: The study was carried out on cultured human umbilical vein endothelial cells (HUVEC). Angiogenesis was analysed by endothelial cell tube formation and 3-D spheroid assay. cAMP analogues, 8-pCPT-2′-O-Me-cAMP (200 µM) and N⁶-Benzoyl-cAMP (50 µM) were used to activate Epac or PKA, respectively.

Results: Specific activation of either PKA or Epac induced HUVEC proliferation and collective migration (wound healing) which was accompanied by enhanced phosphorylation of Akt (at Ser473) and p42/44 MAPK. Both PKA and Epac-induced HUVEC proliferation and migration was abrogated by inhibitors of both Akt and p42/44 MAPK. Accordingly, specific activation of PKA induced endothelial cell tube formation and promoted sprouting of spheroid in 3-D collagen gels. Surprisingly, specific activation of Epac abrogated endothelial cell tube formation and VEGF-induced sprouting. Furthermore, Epac activation attenuated VEGFR2 phosphorylation at Y996. Although activation of both PKA and Epac induced Rac1 activation, however, both have differential effects on RhoA activity. PKA antagonised RhoA activity, while Epac caused an activation of RhoA. Inhibition of either RhoA activity or downstream Rho kinase (ROCK) alone resulted in increased HUVEC tube formation and sprouting and abrogated completely the anti-angiogenic effect of Epac activation. Similarly, specific activation of RhoA abrogated PKA-induced angiogenesis. The data was further confirmed by over expression of constitutive active and dominant negative RhoA using lentivirus based vectors.

Conclusion: The data of present study demonstrate that cAMP/PKA and cAMP/Epac signalling pathways have differential effects on in vitro angiogenesis. PKA activation pro-
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A potential role for arachidonic acid metabolites in gap junctions-mediated signaling between smooth muscle cells in the sustained hypoxic pulmonary vasoconstriction development

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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. The mechanisms of the sustained hypoxic pulmonary vasoconstriction (HPV) remain unclear. The aim of this study was to determine the role of gap junctions (GJs) between smooth muscle cells (SMCs) in the sustained HPV development and involvement of arachidonic acid (AA) metabolites in GJs-mediated signaling. The vascular tone was measured in bovine intrapulmonary arteries (BIPA) using isometric force measurement technique. Expression of contractile proteins was measured using Western blot technique. Mass spectrometry analysis of the tissue bath fluid was done for AA metabolites detection. Values are means±S.D., compared by Student’s T-test. 13-hours chronic hypoxia elicited endothetrometry analysis of the tissue bath fluid was done for AA was measured using Western blot technique. Mass spectrometry also detected 15,20-DiHETE in the tissue bath fluid. Taken together, our novel findings show that GJs between SMCs are involved in the presence of 20-HETE in tissue bath fluid. The sustained HPV was not dependent on Ca 2+ entry (122±16 %, n=8, P>0.05, respectively). Inhibition of GJs decreased smooth muscle myosin heavy chain (SM-MHC) expression (from 1.56±0.22 to 0.48±0.11, n=8 normalised by β-actin expression, P<0.05), and myosin light chain phosphorylation (from 0.52±0.15 to 0.18±0.11, n=8, P<0.05) in BIPA. Mass spectrometry also detected 15,20-DIHETE in the presence of 20-HETE in tissue bath fluid. Taken together, our novel findings show that GJs between SMCs are involved in the sustained HPV in BIPA and AA metabolites passing through GJs appear to mediate SM-MHC expression and contribute to the sustained HPV development.

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The vascular responses to cerebral parasympathetic stimulation, and changes associated with ageing

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The pterygopalatine ganglion (PPG) is the major source of cerebrovascular parasympathetic innervation. Stimulation of parasympathetic nuclei releases nitric oxide (NO) from post-ganglionic nitrergic nerves, inducing cerebral vasodilatation (1). It has been shown, in young (Y) rats, that PPG stimulation induces significant cerebral vasodilatation (increase in cerebrovascular conductance; CVC) resulting in increases in cerebral blood flow (CBF), with no change in arterial blood pressure (ABP) (2).

Our findings in Y rats agree with these observations. However, we have evidence these dilator fibres are functionally important. We showed the increase in cerebral blood flow (CBF) beyond the upper limit (UL) of autoregulation (in response to increased ABP) is associated with a vasodilatation (fall in cerebrovascular resistance; CVR). Infusion of the NO synthase inhibitor L-NAME prevented the UL being reached and so abolished the dilatation seen at a comparable ABP. These observations suggest an active, NO-mediated dilatation as opposed to a pressure-passive increase in CBF when the UL is reached in Y rats. This increase in CBF and fall in CVR at the UL was blunted in old (O) compared to Y rats.

We therefore hypothesise that the increase in CBF beyond the UL may be mediated by an increase in parasympathetic, nitrergic nerve activity to the cerebral vessels, and that this mechanism is impaired with age. In Alfaxan-anaesthetised (12-30mg.kg.hr⁻¹ i.v.), male O Wistar rats (52-58 weeks old, n=8), we isolated and stimulated the PPG at 3, 10, 30 and 60Hz (2-4V for 90 seconds) before and after infusion of L-NAME (10mg.kg⁻¹ i.v.). Data is presented as mean±SEM.

The responses were blunted in O compared to Y rats. The higher frequencies induced significant increases in CBF, with a maximum increase of 0.36±0.07ml.min⁻¹ at 10Hz (paired t-test, P<0.05). However, this was associated with a concomitant increase in ABP of 17±3mmHg * (paired t-test, P<0.05) and only a small, non-significant, increase in CVC (0.019±0.002 to 0.02±0.002ml.min⁻¹, mmHg, paired t-test, P>0.05).

Infusion of L-NAME significantly reduced the increase in ABP in response to PPG stimulation at 10Hz; CVC remained unchanged, resulting in a reduced increase in CBF. These data suggest that PPG-induced dilatation is substantially blunted in O rats, the ABP increase accounting for the increase in CBF. We suggest that the increase in CBF seen beyond the UL is an active, NO-mediated dilatation, caused by nitrergic, parasympathetic nerve activation. This may be part of a pressure-release safety mechanism in response to increases in ABP, which may otherwise compromise the cerebral vasculature. This mechanism appears to be blunted in old rats, which may have implications for the increased risk of haemorrhagic stroke associated with ageing.


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Dorsomorphin inhibits in vitro angiogenesis and ameliorates thrombin-induced endothelial barrier failure

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Background: Dorsomorphin also known as compound C is a cell-permeable pyrazolopyrimidine derivative, widely used as an adenosine monophosphate-activated protein kinase (AMPK) inhibitor to characterise the role of AMPK in various physiological processes, however, its AMPK-independent effects have also been reported. In cell culture model, minimally a 40 μM concentration of dorsomorphin is required to inhibit AMPK. In the present study we investigated the effects of low dose (0.1-10 μM) dorsomorphin on endothelial cell (EC) proliferation, in vitro angiogenesis, and barrier function.

Methods: The study was carried out on cultured human umbilical vein endothelial cells (HUVEC). Angiogenesis was analysed by endothelial cell migration (wound assay), 3-D tubes and spheroid formation. EC barrier function was analysed by measuring flux of albumen through EC monolayers cultured on filter membranes. Metformin (2 mM) and A-769662 (10 μM) were used to specifically activate AMPK.

Results: Dorsomorphin was unable to inhibit AMPK phosphorylation (activation) induced by metformin and A-769662 both in ECs and human smooth muscle cells at concentration range of 0.1-10 μM. However, at these concentrations, dorsomorphin inhibited EC proliferation, migration, tube formation, and sprouting in a concentration-dependent manner. Both metformin and A-769662 also had moderate inhibitory effects on EC proliferation, migration, tube formation, and sprouting. However, combining dorsomorphin either with metformin or A-769662 had additive effect on EC proliferation, migration, and sprouting suggesting an AMPK-independent phenomenon. Interestingly, dorsomorphin antagonized thrombin-induced EC hyperpermeability in a concentration-dependent manner accompanied by the inhibition of RhoA/Rock signalling and EC contractile machinery. This EC barrier protective effect was not affected by the presence of AMPK activators.

Conclusion: The data of present study demonstrate that long-term treatment of ECs with dorsomorphin inhibits EC proliferation and angiogenesis. However, short-term exposure antagonises thrombin-induced EC hyperpermeability in AMPK-independent manner, presumably via inhibition of RhoA/Rock signalling. Moreover, the study warns that this agent should be used with caution to demonstrate the effects mediated by AMPK activation.

The technical support by S. Schäfer, D. Reitz, and H. Thomas is gratefully acknowledged.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Characterisation of the chemerin signalling pathway: CCX832 is a selective antagonist for the human CMKLR1 receptor

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Targeting G-protein coupled receptors (GPCRs) has made significant advances in cardiovascular disease treatment, but there is still no cure. Orphan GPCRs with unknown functions could prove to be future drug targets. Hypertension is a risk factor for coronary artery disease and the single most important cause of stroke. Chemerin is a ligand for CMKLR1 and plays a role in inflammation and vascular endothelial dysfunction. Chemerin has also been proposed as the ligand for orphan receptor GPR1, which our group has recently confirmed. The BRIGHT cohort study identified GPR1 as a candidate gene for essential hypertension and chemerin is purported to contract rat aorta via CMKLR1. Having previously found that chemerin causes vasoconstriction of human saphenous vein (hSV), we hypothesise that chemerin has a role in modulating human vascular tone through one or both of these receptors. The aims of this study were to confirm the selectivity of CMKLR1 antagonist, small molecule CCX832 (1) and to delineate which receptor is mediating chemerin’s vasoconstrictor response in human vessels.

β-Arrestin recruitment assays (DiscoverRx) were performed with either GPR1- or CMKLR1-transfected CHO-K1 cells. Concentration response curves were constructed for chemerin peptides; C13 (145-157) and C9 (149-157), ± CCX832 (3nM-3μM). Total and non-specific binding, using unlabelled C13, C9 and CCX832, of [125I]-Chemerin(145-157) to GPR1- or CMKLR1-transfected CHO-K1 cells or hSV homogenate were carried out for 1 hour at RT. The cellular distribution of both receptors was studied in hSV tissue and human aortic smooth muscle cells (hASMCs), by immunohistochemistry and qPCR. All values are quoted as mean ± S.E.M.

CCX832 was functionally active at the CMKLR1 receptor causing a rightward shift of the chemerin response with an affin¬ity of logKᵢₐₛ = 8.19 ± 0.02 (n=3), however no effect was seen at the GPR1 receptor. Further confirmation of CCX832 selectivity came from radiolabelled binding experiments. These studies revealed that CCX832 binds to the same site on the CMKLR1 receptor as [125I]-C9, however it does not bind to the C9 binding site on the GPR1 receptor. Having characterised the antagonist, studies are now ongoing to delineate which receptor mediates the response. Both receptors co-localised to smooth muscle α-actin on hSV (n=3). Radiolabelled binding of [125I]-C9 on hSV homogenate was not blocked by CCX832, suggesting that C9 is binding predominately to GPR1 in hSV tissue. This study confirms that chemerin has vasoactivity in human vessels. CCX832 is a selective CMKLR1 receptor antagonist and this compound will allow further studies to investigate the relative role of each receptor in chemerin-mediated vascular constriction.

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Ascorbate intake among maladapted (CMS+) highlanders


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Introduction: A recent research experiment identified that maladapted (CMS+) highlanders exhibit elevated systemic biomarkers of oxidative-nitrosative stress and impaired vascular structure-function (relative to well-adapted controls; CMS−). This may be due to the depletion in ascorbate given that it is the primary water soluble chain-breaking antioxidant in the circulation. This may be the outcome of inadequate dietary intake, hence the focus of the present study.

Methods: Thirty-six male highlanders with CMS (n = 22, CMS+; age 57 ± 10) and without CMS (n = 14, CMS−; age 52 ± 12) participated in the study. Participants were interviewed to collect a 24 hour structured dietary recall using a portion size photo atlas2. The stages followed in the UK Low Income Diet and Nutrition survey were used3. The dietary questionnaires were analysed using NetWISP dietary analysis software (Version 4.0, Tinuviel Software; Anglesey, UK). Data were tested for normality using Shapiro-Wilks tests. Kruskal-Wallis and Mann-Whitney tests to compare the groups were performed. Significance level was established at P < 0.05 and data are expressed as mean ± standard deviation (SD)

Results: Consumption of ascorbate (Vitamin C) is clearly deficient in the maladapted male highlanders (CMS+, 47 ± 35) versus the well-adapted controls (CMS−, 66 ± 33). The intake demonstrates the (borderline) significant differences between the two groups (P < 0.07); though consumption is below the recommendations (table).

Conclusions: These findings are the first that describe antioxidant intake in the population affected by Monge’s disease (CMS+). Furthermore, the result supports our hypothesis that compared to controls (Bolivian non-diseased native highlanders), CMS patients are characterised by an inadequate intake of dietary antioxidants. This is an independent variable that has previously been associated with augmented oxidative-nitrosative stress and vascular endothelial dysfunction subsequent to impaired antioxidant defence.

Table: Ascorbate intake (Vitamin C) in CMS+ vs. CMS−

<table>
<thead>
<tr>
<th>Vitamin C (mg)</th>
<th>CMS+</th>
<th>CMS−</th>
<th>RDA1 (mg/day)</th>
<th>CMS+ vs. CMS−</th>
</tr>
</thead>
<tbody>
<tr>
<td>47 ± 35</td>
<td>66 ± 33</td>
<td>60</td>
<td>0.07</td>
<td></td>
</tr>
</tbody>
</table>

Mean ±SD; † = difference between groups (P < 0.05). 1Recommended Dietary Allowance.


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

**Carbon dioxide mediated cerebral vasomotion: A role for prostaglandins?**


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Cerebrovascular regulation during perturbations in arterial CO₂ is thought to occur solely at the level of the pial vessels. However, recent evidence implicates large extracranial cerebral blood vessels in this regulatory process. Although the mechanisms governing CO₂ mediated vasomotion remain unclear, animal and human studies support a powerful role of prostaglandins. Thus, we examined two hypotheses: 1) vasomotion of the ICA would occur in response to both hyper and hypocapnia; and 2) pharmacological inhibition of prostaglandin synthesis would reduce the vasomotor response of the ICA to changes in end-tidal CO₂ (P₄CO₂). Using a randomized single-blinded placebo controlled study, subjects (n=10; 1 female) were tested on two occasions separated by 10±8 (mean±standard deviation) days. Before and 90-minutes following either oral indomethacin (INDO; 1.2mg/kg) or placebo capsule, concurrent measures of beat-by-beat blood flow, velocity and diameter of the ICA were made at rest and during steady state stages (4 min) of iso-oxic hypercapnia (+3, +6, +9mmHg above baseline) and hypocapnia (-3, -6, -9mmHg below baseline). End-tidal gases were tightly controlled via dynamic end-tidal forcing. To examine if INDO effects ICA vasomotion in a permissive manner (i.e., independent of prostaglandins) a subset of subjects (n=5) were tested before and 45-minutes following oral ketorolac (20mg). During pre-drug testing in the INDO trial, the ICA dilated during hypercapnia at +6mmHg (4.72±0.45 vs. 4.95±0.51mm; P<0.001; 1-way ANOVA) and +9mmHg (4.72±0.45 vs. 5.12±0.47mm; P<0.001; 1-way ANOVA), and constricted during hypocapnia at -6mmHg (4.95±0.33 vs. 4.88±0.27mm; P<0.05; 1-way ANOVA) and -9mmHg (4.95±0.33 vs. 4.82±0.27mm; P<0.001; 1-way ANOVA). The slope of the vasomotor responses (i.e., mm × mmHgP₄CO₂⁻¹) were not different between the pre-INDO, pre-placebo and placebo trials for both hyper and hypocapnia (1-way ANOVA). Despite a reduced ICA diameter at all stages following INDO (P<0.05; 1-way ANOVA), dilation of the ICA was still observed at +6mmHg (4.50±0.54 vs. 4.75±0.52mm; P<0.05) and +9mmHg (4.50±0.54 vs. 4.61±0.50mm; P<0.01); however, INDO reduced the magnitude of dilation by 67±28% (0.045±0.015 vs. 0.015±0.012mm × mmHgP₄CO₂⁻¹). In the Ketorolac condition, there was no effect of the drug on the vasomotor response to hyper or hypocapnia. We conclude that: 1) changes in P₄CO₂ mediate vasomotion of the ICA, with the vasomotor response greatest in the hypercapnic range; 2) inhibition of non-selective prostaglandin synthesis via INDO markedly reduces the vasomotor response to changes in P₄CO₂; and 3) INDO may be acting via a mechanism(s) independent of prostaglandin synthesis inhibition to reduce CO₂ mediated vasomotion as Ketorolac administration had no apparent effect.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
Figure 1. Representative trace of changes of intracellular calcium in an A7r5 cell during incubation with CaP crystals

We want to thank Dr Matthias Epple and Diana Kozlova (Inorganic Chemistry and Center for Nanointegration Duisburg-Essen (CeNIDE), University of Duisburg-Essen, Essen, Germany), who kindly provided the CaP crystals used in this study.

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