

cytosol as a soluble polypeptide. The R domain was detected in <1 % of cellular protein, similar to the fraction of cells that were permeable to ethidium homodimer-1 in live/dead cell assays, therefore the cytoplasmic aspect of CFTR is probably labelled by sulfo-NHS-SS-biotin in a subpopulation of leaky cells that are undergoing normal cell turnover.

To study the trafficking of small quantities of immature protein to the cell surface more definitively, we inserted a target sequence for enzymatic biotinylation in the fourth extracellular loop of CFTR and $\Delta F508$ -CFTR. These constructs were specifically labelled on the cell surface and protected intracellularly within the lumen of the secretory pathway. Low temperature and chemical chaperones restored some channel activity but yielded little band C; most enzymatically-biotinylated $\Delta F508$ -CFTR that reached the plasma membrane was the immature "band B" glycoform. We then used live cell imaging to identify possible routes of CFTR trafficking. Secreted proteins usually move from exit sites in the endoplasmic reticulum (ER) to the cis-Golgi via tubulovesicular structures which comprise the ER-Golgi intermediate compartment (ERGIC), however total internal reflection fluorescence (TIRF) microscopy revealed ER-localized CFTR surprisingly close to the plasma membrane (<200 nm). The lectin ERGIC-53, a cargo carrier for glycosylated proteins, was used as an ERGIC marker. Two colour TIRF imaging revealed a close association between CFTR-mCherry and ERGIC-53-EGFP in mobile vesicles that were transported towards the cell periphery. These results suggest a mechanism for trafficking CFTR channels to the plasma membrane which bypasses the Golgi apparatus and may explain delivery of immature, core-glycosylated CFTR to the cell surface.

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C102

Nucleotides and phosphatase substrates bind at separate sites on pig kidney Na,K-ATPase

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Besides ATP, the sodium pump can hydrolyse phosphatase substrates like *p*-nitrophenyl phosphate (pNPP) in the presence of K ions; ATP, ADP, and their analogues act as low-affinity competitive inhibitors of the K⁺-phosphatase activity. Covalent block of an N-domain pocket (1) by FITC abolishes high-affinity ATP binding, ATP phosphorylation and the Na,K-ATPase activity, but the phosphatase activity and the low-affinity nucleotide effects remain (2,3). FITC modification aside, this effect on pNPP hydrolysis reflects the prominent low affinity ATP activation and ADP inhibition of the Na,K-ATPase activity. In fact, with our purified Na,K-ATPase preparations we now realise that ADP can be a better inhibitor (K_i of 0.27-0.42 mM) than ATP is an activator (K_{0.5} of 0.6 mM). We wished to find out,

therefore, whether the low affinity ATP and ADP effects on the Na,K-ATPase activity resulted from direct binding at the phosphatase substrate site.

We applied two kinetic tests. If the substrate concentration is held fixed, increasing inhibitor concentrations should yield linear Dixon plots (1/v vs. [i]) if substrate and inhibitor compete for a unique site. However, we find that at 0.6 mM pNPP, the Dixon plot is clearly hyperbolic with ATP concentration, with a K_{0.5} of 8.6 mM. This is diagnostic of partially competitive inhibition (4): at the saturating concentration, ATP will have unhindered access to the nucleotide site whilst pNPP may still bind at a distinct phosphatase site from time to time. The high fitted K_{0.5} value should signal the ATP binding affinity at the nucleotide site when pNPP occupies the phosphatase site (4). The second test involves the use of 2 inhibitors, to decide whether they bind at the same or different sites to cause the inhibition. We plotted the reciprocal of the K⁺-phosphatase activity at a fixed 6 mM pNPP, as a straight line against 4-methylumbelliferone phosphate concentration (0-3 mM); this is another phosphatase substrate and a full competitor. The same was done again, but now in the presence of 4 mM ADP. Both straight lines were clearly convergent on the left, which indicates that the ternary complex is possible, of the pump with 4-methylumbelliferone phosphate and ADP. Mutual exclusion of the two inhibitors would have shown as roughly parallel straight lines (5). Finally, we did the converse experiment, and measured Na,K-ATPase activity at a fixed 0.25 mM ATP. The reciprocal of the ATPase activity was plotted against 0-2 mM ADP, with and without 15 mM pNPP. The straight lines were again clearly convergent on the left. Taken together, these results strongly suggest that the K⁺-phosphatase substrates bind elsewhere and not at the locus where nucleotides modulate the pump activities. Long-range effects should be the main cause for the mutual decrease in binding affinities.

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C103

Interaction between purinergic and cholinergic signalling in urinary bladder smooth muscle

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Introduction: The urinary bladder has two major functions, the storage and voiding of urine. The first requires relaxation of the

urinary bladder detrusor smooth muscle during bladder filling; the latter requires coordinated contraction. Urinary bladder smooth muscle (UBSM) contraction occurs through the initiation of action potentials within the parasympathetic nerves leading to the bladder, which evokes the release of the excitatory neurotransmitters acetylcholine (ACh) and adenosine trisphosphate (ATP). The general thought is that ACh and ATP act independently through muscarinic (M2, M3) and purinergic (P2X1) receptors, respectively, to increase excitability and contractility [1, 2].

Methods: Electrical field stimulation (EFS) was used to stimulate nerves in isolated mouse UBSM strips. The resulting changes in force and calcium signalling were measured with myography and the calcium-sensitive dye Fluo-4, using a laser scanning confocal microscope. Purinergic and muscarinic receptors were inhibited with α,β -methylene ATP/suramin and atropine, respectively.

Results: P2X1 receptors are cation channels, whereas M2, M3 receptors are coupled to G-proteins [3]. Consequently, because purinergic signalling acts through an electrical mechanism, one might predict that it would be activated earlier following EFS than cholinergic signalling. Calcium imaging experiments on intact UBSM strips showed the presence of at least three different calcium events during and 2-3s after EFS; 1) Elementary purinergic events, which reflect ATP release from nerve varicosities and activation of P2X1 receptors [4]; 2) Fast "global" calcium flashes with calcium entry through voltage-dependent calcium channels [4] and 3) Slower calcium waves, which likely reflect activation of IP3 receptors. Calcium flashes occurred during and after EFS. Inhibition of muscarinic receptors eliminated calcium flashes that occurred after EFS without effecting calcium flashes during stimulation. In contrast, inhibition of purinergic receptors eliminated calcium flashes during stimulation and greatly augmented the number of flashes after stimulation. These results indicate that rapid purinergic signalling suppresses the slower cholinergic-mediated increases in excitability and hence calcium entry. Therefore, inhibiting P2X1 receptors should slow force generation but lead to overall elevation in force. Indeed, in the presence of purinergic inhibitors, both the rise time and the decay time of EFS-induced contractions were prolonged, indicating increased UBSM contractility.

Conclusion: Under physiological conditions, rapid purinergic signalling represents not just an independent contraction-inducing pathway, but it also suppresses slower cholinergic effects on UBSM. The consequence is that changes in purinergic signalling affect subsequent cholinergic-mediated contractility through changes in calcium signalling.

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C104

Spike potential activity and standard transient depolarisations in the aging guinea pig prostate

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Purpose: To characterise the spike potential activity and standard transient depolarisations in the aging guinea pig prostate gland.

Materials and Methods: Membrane potential recordings were made in the guinea pig stroma using conventional single micro-electrode recording techniques.

Results: Three type of spontaneous electrical activity were recorded within the aging guinea pig prostate gland. Out of a total of 86 successful impalements 46.5% consisted of spike potential activity, 43% consisted of slow wave activity and standard transient depolarisations contributed 10.5%.

Spike potentials were classified as hyper-active (frequency $\geq 15 \text{ min}^{-1}$) or active (frequency $\leq 15 \text{ min}^{-1}$). The resting membrane potential was similar between hyperactive $-41.54 \pm 3.19 \text{ mV}$ ($n=11$), and active $-42.45 \pm 1.10 \text{ mV}$ ($n=27$, $p>0.05$) cells. Hyperactive spikes had a total amplitude of $49.18 \pm 4.44 \text{ mV}$ and a duration of $7.63 \pm 0.92 \text{ ms}$, similar to what was observed in active spikes. In contrast, active cells displayed a frequency of $5.06 \pm 0.63 \text{ min}^{-1}$, significantly different to hyperactive cells ($362.05 \pm 151.82 \text{ min}^{-1}$ $p<0.05$). The amplitude of the after-hyperpolarisation was also significantly different across both groups; $17.80 \pm 1.98 \text{ mV}$ (hyperactive cells, $n=912$) and $9.96 \pm 1.05 \text{ mV}$ (active cells, $n=412$ $p<0.05$). Spike potential activity was not affected by the addition of $1 \mu\text{M}$ TTX or $1 \mu\text{M}$ Atropine ($n=5$ $p>0.05$), although was abolished upon the addition of $1 \mu\text{M}$ Nifedipine ($n=7$, $p<0.05$).

Standard transient depolarisations (STDs), ($n=32$), were recorded in the presence of slow waves ($n=10$), spikes ($n=13$) and were also observed in quiescent cells ($n=9$). STDs displayed similar membrane potentials across the three groups: $-53.62 \pm 1.31 \text{ mV}$ (in the presence of slow waves), $-48.46 \pm 2.29 \text{ mV}$ (spikes) and $-51.49 \pm 1.70 \text{ mV}$ (quiescent cells). STD frequency appeared to be highest when occurring alone ($24.55 \pm 6.48 \text{ min}^{-1}$) compared to those in the presence of slow waves or spikes. In contrast, both the duration ($49.06 \pm 5.36 \text{ ms}$) and amplitude ($9.09 \pm 0.94 \text{ mV}$) were significantly larger in STDs displayed with spike potentials ($p<0.05$). The addition of $1 \mu\text{M}$ Nifedipine ($n=5$) did not significantly affect any of the measured parameters ($p>0.05$).

Conclusions: In summary, the main type of spontaneous electrical activity recorded in the aging guinea pig prostate was spike potentials, which was grouped into hyperactive and active states displaying slightly different properties. Spike potentials were insensitive to $1 \mu\text{M}$ TTX and $1 \mu\text{M}$ Atropine, although were

abolished by Nifedipine which suggests the role of L-type calcium channels. Finally, STDs were unaffected by addition of Nifedipine suggesting that mechanisms other than calcium entry via L-type calcium channels are responsible for the generation and maintenance of this activity.

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C105

Mechanisms of hypoxic pulmonary vasoconstriction in isolated rat intrapulmonary arteries

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Whereas the systemic circulation dilates in response to hypoxia, the pulmonary responds with a biphasic constriction. The latter is termed hypoxic pulmonary vasoconstriction (HPV; von Euler & Liljestrand, 1946). Physiologically, HPV maintains the ventilation-perfusion ratio during alveolar hypoxia. Pathologically, ongoing HPV contributes to pulmonary hypertension. Most groups require precontracting agonists, such as PGF₂α, to elicit HPV in isolated arteries. However, the mechanisms underlying the agonist-induced contraction may overlap with those of HPV, so we have characterised HPV in unprecontracted arteries. Intrapulmonary arteries (500–800 μm dia.) from male Wistar rats were mounted on a myograph. Drugs were applied 20 min before hypoxia (95% N₂/5% CO₂; pO₂=15–20 Torr). All data were analysed using repeated measures 2-way ANOVA with Holm-Sidak's post-hoc test. The L-type Ca²⁺ antagonists diltiazem (10 μM; n=5) and nifedipine (3 μM; n=5), and the store-operated Ca²⁺ channel antagonist La³⁺ (100 μM; n=4) had no significant effect on HPV. Furthermore, HPV persisted in Ca²⁺-free Krebs containing EGTA (200 μM; n=4; n.s.). Both ryanodine (100 μM; n=4; P<0.05) and the lysosomal H⁺-ATPase inhibitor concanamycin A (1 μM; n=5; P<0.05) strongly suppressed HPV. Although it has been proposed that cyclic ADP-ribose (cADPR) and AMP-activated protein kinase (AMPK) mediate hypoxia-induced Ca²⁺ release from the SR (Evans et al., 2005), neither the cADPR antagonist 8-bromo-cADPR (30 μM; n=4) nor the AMPK inhibitor compound C (10 μM; n=4) had a significant effect on HPV. The rho-kinase inhibitor Y-27632 (1 μM) markedly attenuated HPV (n=5; P<0.05) and the hypoxia-induced biphasic (at 2 and 30 min) increase in phosphorylation of the myosin phosphatase targeting subunit MYPT-1 (n=6; P<0.05) and the monophasic increase (at 30 min) in phosphorylation of the 20 kDa regulatory myosin light chain (MLC20; n=6; P<0.05). Superoxide dismutase (SOD; 200 units ml⁻¹; n=6; P<0.05) but not catalase (200 units ml⁻¹; n=5; n.s.) suppressed HPV. Removal of the endothelium strongly inhibited HPV (n=5; P<0.05). These data are consistent with a model in which HPV in these arteries is dependent upon the endothelium, but does not require cADPR, AMPK or extracellular Ca²⁺ influx. Conversely, HPV appears to involve superoxide, Ca²⁺-sensitisation via rho-kinase and intracellular Ca²⁺ release from concanamycin and ryanodine sensitive compartments.

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C106

A possible role for transient receptor potential (melastatin) 8 (TRPM8) channels in human cutaneous blood flow

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TRPM8 has previously been identified as a cold- and menthol-activated ion channel which gives rise to detection of cold sensation in the innocuous temperature range (Peier et al., 2002). Recently, their presence has been reported in rat pulmonary artery and aorta (Yang et al., 2006). We have recently shown that TRPM8 agonists raise intracellular calcium and cause contraction in isolated vascular smooth muscle cells (VSMCs) and relaxed segments from several rat arteries (Melanaphy et al., 2008), although vasodilatation is induced in precontracted arteries (Melanaphy et al., 2007). In this study we have examined a possible role for TRPM8 channels in controlling cutaneous blood flow in humans.

Following approval from the QUB Medical School Ethics Committee, forearm cutaneous red cell flux (cRCF) was measured by means of a laser Doppler probe in 10 healthy volunteers (5 male, 5 female) aged 21.0 ± 0.3 years (mean ± S.E.M.) during 30 minutes of passive application of menthol (3% in 25% ethanol vehicle) to the probe chamber. Before application of menthol, cRCF was relatively low (18 ± 2 flux units). After approximately 14 minutes (median 14, range 10–22 minutes) application of menthol, cRCF increased considerably in 9/10 subjects, so that cRCF had risen by 534 ± 138% (P<0.01, n=10, paired Student's t-test, performed on raw data) above baseline after 30 minutes. No significant increase was seen with vehicle alone, applied concurrently. There were no significant differences between males and females in the effects of menthol. However, these dilator effects were markedly reduced by prior iontophoretic application of atropine (10 mM in sterile water, 1 x 30 seconds at 75 μA, 12 minutes prior to acetylcholine), or prior passive application of L-NAME (100 nM in sterile water, 30 minutes application), the inhibitor of nitric oxide production (250 ± 76 %, P<0.05 and 352 ± 59%, P=0.061, respectively).

This study suggests that TRPM8 channels are active in control of human cutaneous blood vessels, causing vasodilatation of constricted vessels. Its effects may be mediated, in part, by production of nitric oxide. Additional details of this mechanism await further experimentation.

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C107

Kisspeptin-GPR54 signalling is essential for NMDA-induced luteinizing hormone secretion in the mouse

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BACKGROUND: The gonadotrophin-releasing hormone (GnRH) neuronal network is defined as the GnRH neurons and associated assembly of brain cells responsible for controlling GnRH release into the pituitary portal circulation. Among the known neurotransmitters that alter GnRH neurons, the classical excitatory amino-acid glutamate partly through the activation of NMDA receptor, and the recently discovered family of kisspeptins that bind GPR54 receptor, potentially stimulate GnRH release and thus luteinizing hormone (LH) secretion from the anterior pituitary. GnRH neurons express GPR54 and are directly stimulated by kisspeptins whereas glutamate may activate GnRH through NMDA receptors in an indirect manner.

HYPOTHESIS: The NMDA-induced GnRH/LH release requires the kisspeptin-GPR54 system.

PROTOCOL: To define a functional hierarchy for the action of kisspeptins and NMDA on GnRH neurons, we investigated the effect of peripheral injection of NMDA on plasma LH concentration. We used Gpr54-null or Kiss1-null mice of both sexes, and wild-type littermates at 25 days old. Each animal received a single i.p. injection of 0.3mg NMDA, and blood was collected 10 min after the injection, from the the vena cava post mortem. Hormone assays were performed for LH and growth hormone (GH) plasma levels.

RESULTS: All wild-type animals positively responded to NMDA injection and exhibited an LH release after NMDA injection (0.45 ± 0.13 ng/ml or 1.19 ± 0.16 ng/ml in females or males respectively) whereas none of the Gpr54-null or Kiss1-null mice exhibited LH release after NMDA injection (individual values for all mice below 0.33 ng/ml; the sensitivity of the LH assay). However, we found that all animals of both genotypes and sexes positively responded to NMDA with an increase in plasma GH levels. The failure of mice lacking a functional GPR54-kisspeptin system to release LH after NMDA stimulation demonstrates that either GPR54 or kisspeptin is required to relay the NMDA signalling that leads to GnRH neuronal activation. Our data also show that the GPR54-kisspeptin system is specifically involved in the NMDA activation of the GnRH/LH release as NMDA still generates the neuroendocrine activation of GH release.

CONCLUSION: NMDA-induced GnRH/LH release is dependant of the GPR54-kisspeptin system. Kisspeptin neurons represent primary afferents to the GnRH neurons as NMDA receptors-activating glutamatergic neurons are likely to represent secondary afferents upstream to kisspeptin neurons. The direct activation of kisspeptin neurons by NMDA is under investigation.

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C108

Exposure to a high fat, and low carbohydrate diet *in utero* and during lactation modulates hepatic mitochondrial complex activity in adult mouse offspring

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Evidence suggests that altered maternal nutrition increases susceptibility to the metabolic syndrome in adult offspring. An increase in the prevalence of metabolic syndrome has been linked to an increase in incidence of non-alcoholic fatty liver disease (NAFLD), which can range from fatty liver (steatosis), to non-alcoholic steatohepatitis (NASH) and fibrosis. Since mitochondrial dysfunction impairs fatty liver homeostasis, increases reactive oxygen species and triggers lipid peroxidation mitochondrial dysfunction it is a likely candidate for NAFLD disease progression. To investigate the effect of maternal nutrition on mitochondrial dysfunction and development of NASH, we determined changes in electron transport chain (ETC) enzyme complex activity in liver tissue from mouse offspring that were exposed to a high fat or control diet both pre and post-natally. Female C57 BL/6J black mice were randomly assigned to either a high fat diet (HF- 45% kcal fat, 20% kcal protein, 35% kcal carbohydrate, n=10) or standard laboratory chow diet (C- 21% kcal fat, 18% kcal protein, 63% kcal carbohydrate, n = 10). Dams were fed 4 weeks prior to conception, during gestation and lactation. At weaning the offspring were assigned either HF or C diet, generating 4 experimental groups: HF/HF (n= 12), HF/C (n = 12), C/HF (n = 12), C/C (n = 12), which represents prenatal/postnatal diet respectively. Offspring food intake and weights were recorded at weekly intervals. Locomotor activity and blood pressure was measured at 13 weeks of age. At 15 weeks offspring were killed and tissues were weighed and collected. Hepatic mitochondrial complex (I, II/III, and IV) activity was measured by spectrophotometer following addition of relevant cofactors (rotenone, antimycin reduced cytochrome c respectively). Results were standardized using citrate synthetase activity. Histological analysis of liver specimens stained with haematoxylin and eosin (H and E) were used to determine levels of steatosis.

In liver tissue from both the HF/C and HF/HF offspring mitochondrial complex I, II/III and IV activity was significantly reduced when compared to both the C/C and C/HF ($p < 0.05$) groups. The greatest decrease was observed with Complex I, where a 3.2 and 3.7-fold reduction in activity levels were seen

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PC139

Disruption of an integrin-containing muscle adhesion complex causes muscle protein degradation in *Caenorhabditis elegans*

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Continuous transmission of mechanical signals (mechanotransduction) is required for maintenance of muscle protein mass (1). Of the many factors that cause muscle wasting in human beings, spaceflight and immobilization are thought to induce wasting via decreased use, possibly via decreased mechanotransduction. However, the nature and scope of the mechanical linkage to intracellular signalling pathways regulating protein mass are largely unknown. Proteins making up worm focal adhesions (known as dense bodies) are present in decreased amounts following spaceflight (2-3); these changes correlate with a post-flight movement defect (2). We conducted a series of experiments on the ground in order to examine the significance of this correlation.

We find that acute treatment of adult *C. elegans* with RNAi against one of these genes, *unc-97* (PINCH/LIM-domain), causes degradation of a reporter protein in muscle cytosol. Acute RNAi treatment against any of another eight genes, whose products are likewise conserved members of an integrin-containing muscle adhesion complex, also causes degradation. Suggesting specificity in the regulation of degradation, we find that RNAi against the gene for another complex member, *unc-95* (LIM-domain), fails to cause muscle protein degradation even though it causes a movement defect. Experiments using temperature-sensitive mutations in two of these genes, either *unc-112* (MIG-2) or *unc-52* (Perlican), confirm that disruption of this complex causes degradation, and further show that the extramuscular ligand (UNC-52/Perlican) is required to prevent degradation. In these mutants movement becomes uncoordinated and muscle structure is disrupted following temperature shift. In *C. elegans*, Acetylcholine Receptor and opposed Insulin Growth Factor Receptor-Fibroblast Growth Factor Receptor signalling networks control muscle protein degradation via proteasome and non-proteasome dependent mechanisms, respectively (4-5). Drugs, mutations, and RNAi treatments targeted at these networks, and known to block degradation in *C. elegans* muscle, fail to block degradation triggered by acute loss of members of the muscle attachment complex. Current goals include determining the identity and regulation of the relevant protease(s).

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PC140

Generalized disruption to relaxation by Cumene Hydroperoxide in rat aorta

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Increased oxidative stress is a common finding in diseases such as diabetes (1). Cumene hydroperoxide is an organic compound commonly used to induce free radical formation in vitro to mimic oxidative stress (2). We examined the effect of this compound on the relaxation induced by a variety of agonists in the rat aorta. Segments of the aorta from Sprague Dawley rats mounted in organ baths were incubated for 30 or 120 minutes in physiological salt solution containing 100 or 300 μ M of Cumene hydroperoxide (CHP) before concentration-response curves were constructed for acetylcholine (Ach, endothelium-dependent relaxant), sodium nitropruside (SNP, non endothelium-dependent nitric oxide donor), Levromakalim (ATP-sensitive potassium channel opener), Naringenin (large conductance potassium channel opener) and forskolin (activator of adenylyl cyclase-Protein kinase A pathway). Relaxations to all agonists were severely impaired by CHP (100 or 300 μ M) following exposure for as little as 30 minutes. The concentration-response curves for Ach, SNP, levromakalim, Naringenin and forskolin were all significantly ($p < 0.05$, t-test and ANOVA) shifted to the right of respective controls. PD₂ for levromakalim and forskolin were significantly ($p < 0.05$, unpaired t-test and ANOVA) increased, while the E_{max} for all the agonists except for forskolin were significantly ($p < 0.05$, unpaired t-test and ANOVA) reduced.

The results show that CHP non-selectively alters the mechanisms of relaxation of the rat aorta resulting in generalized impairment in responses. This suggests that CHP may not be an ideal agent for induction of oxidative stress intended to target a specific cellular pathway.

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PC141

Vascular contractility in streptozotocin diabetic rats: the role of protein kinase C in adrenergic agonist and calcium sensitivity

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In diabetes significant changes in vascular function occur which are associated with increased frequency and severity of vascular disease. Diabetes causes elevation of protein kinase C (PKC) activity (Evcimen & King 2007), one of the main elements in sensitizing myofilaments to Ca^{2+} (Woodsome et al., 2001; Soloviev et al., 2005). We hypothesized that PKC-mediated increase in the Ca^{2+} -sensitivity of myofilaments and/or agonist sensitivity in α -adrenoceptor/G-protein coupled pathways in vascular myocytes may be involved in diabetes-associated alteration of vascular contractility. Smooth muscle isometric tension measurements were performed using either intact or chemically permeabilised (60 μM eserin) isolated vascular rings obtained from thoracic aorta and tail artery of humanely-dispatched male Sprague-Dawley rats. 12-week streptozotocin-treated rats (diabetic - measured plasma glucose, MPG, 26.7 ± 1.2 mM, $n=16$), and age-matched controls (MPG - 5.0 ± 0.2 mM, $n=7$) were used. Aortic rings showed no difference in sensitivity to $\alpha 1$ -adrenoceptor agonist phenylephrine (0.1 nM–1 mM) between control and diabetic rats (pD_2 (-log EC_{50}) = 7.35 ± 0.15 , $n=8$ and 7.46 ± 0.17 , $n=10$, respectively; mean \pm S.D., unpaired Student's *t*-test). Pretreatment of tissue with the potent PKC inhibitor, chelerythrine (1 μM), significantly shifted concentration-response curves to the right for aortic rings from both groups (pD_2 = 5.76 ± 0.23 , $n=6$, $P < 0.001$ in control and 6.89 ± 0.21 , $n=8$, $P < 0.05$ in diabetes, respectively). In contrast to aorta, diabetic tail artery showed significantly higher sensitivity to phenylephrine (pD_2 = 6.36 ± 0.17 , $n=11$) compared to control (pD_2 = 5.78 ± 0.12 , $n=8$, $P < 0.02$). However, inhibition of PKC had no effect on agonist sensitivity in tail artery (control pD_2 = 5.47 ± 0.23 , $n=11$; diabetic pD_2 = 6.01 ± 0.06 , $n=8$). Furthermore, in permeabilized aorta rings no differences were found in smooth muscle myofilament Ca^{2+} -sensitivity (pCa_{50}) between control and diabetic vessels (6.12 ± 0.01 vs. 6.14 ± 0.01 , respectively, $n=8$), or following PKC inhibition (control 6.13 ± 0.01 , $n=6$; diabetic 6.14 ± 0.01 , $n=9$). Similar results were obtained in permeabilized rat tail artery rings where pCa_{50} values were 5.96 ± 0.02 in control ($n=6$) compared to 5.90 ± 0.02 ($n=6$) in diabetic vessels. Chelerythrine had no effect on Ca^{2+} -tension relationships (control 5.93 ± 0.02 , $n=11$; diabetic 5.81 ± 0.12 , $n=9$). Thus, in tail artery, but not in aorta, diabetes mellitus is associated with an increased sensitivity to phenylephrine. Moreover, in rat aorta there is significantly smaller chelerythrine-induced desensitization of the $\alpha 1$ -adrenoceptor mediated contractions in diabetic vessels compared to control. These differences were unlikely to be due to differential myofilament Ca^{2+} -sensitivity or its modulation by PKC.

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC142

Oxytocin-induced oscillations of isometric force and cytoplasmic Ca in slices of human myometrium subjected to inhibition of Ca channels

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Oxytocin-induced release of Ca ions from sarcoplasmic reticulum (SR) and sensitisation of contractile proteins to Ca mediate the oxytocin-induced potentiation of myometrial contractions. Solitary transients and sustained oscillations of cytoplasmic Ca in response to oxytocin have been previously described, respectively, in freshly isolated and cultured human myometrial cells [1, 2]. In the present study, we employed the thin myometrial slices technique recently developed in our laboratory to investigate the tissue-level effects of oxytocin on human myometrium. Myometrial biopsies were taken from patients undergoing Caesarean section with informed written consent and approval from the Local Ethics Committee (REC-05/Q2802/107). Ca signalling events were recorded from Fluo-4 loaded slices using a Zeiss LSM 510 META confocal microscope at 1 and 4 frames/s. Isometric force was measured in parallel experiments using standard organ bath. Application of nifedipine (1 μM) to slices exhibiting phasic contractions led to immediate abolition of spontaneous activity. Subsequent addition of 100 nM oxytocin produced an initial, transient rise in force followed by high frequency oscillations in >50% of preparations. Confocal microscopy of myometrial slices subjected to identical experimental protocol revealed asynchronous oscillations in cytoplasmic Ca at the beginning of oxytocin application which tended to synchronise at later stage of oxytocin application. We quantified the synchronicity of oscillations as standard deviation of time delays between the start of oxytocin application and the first peak of Ca in different cells within the field of view. The standard deviation was 33.62 s at the beginning, decreasing to 2.56 s within 10 to 15 minutes of oxytocin application. This synchronisation was a likely cause underlying oscillations in isometric force observed in the organ bath experiments. Addition of the gap junction inhibitor carbenoxolone (200 μM) prevented synchronisation of Ca oscillations indicating that gap junctions are involved in this process.

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PC143

Agonist-induced Ca^{2+} -sensitisation of tone spanning pCa-force relationship of permeabilised human arteries *in vitro*

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Knowledge of physiological mechanisms regulating vascular tone in human resistance arteries lags considerably that in animal vessels, mainly due to limited supply of non-pathophysiological human tissue. In myometrial arteries from biopsies obtained at term (≥ 37 weeks) Caesarean section of women with uncomplicated pregnancy, agonist-induced tone development arises partly through G protein-coupled Ca^{2+} -sensitisation pathways (enhanced contractile response at a particular activating $[\text{Ca}^{2+}]_i$). [1] As in prior animal studies, agonist responsiveness of myofilaments was examined at only one submaximal $[\text{Ca}^{2+}]_i$, thus questioning the generality of the term agonist-mediated Ca^{2+} -sensitisation. In this study we characterise Ca^{2+} -sensitising actions of the thromboxane analogue U46619 in isometric, α -toxin permeabilised human myometrial arteries over a range of $[\text{Ca}^{2+}]_i$ (pCa 9 - 4.5). pCa was controlled by adjusting the ratio of K_2EGTA to CaEGTA in mock intracellular solution. A first cumulative, concentration-response (pCa-force) curve elicited concentration-dependent increases in tension, with mean \pm S.E.M. maximal tension developed in pCa 4.5 (2.3 ± 0.4 kiloPascals, $n = 7$). A second successive curve in the same arteries elicited significantly lower tensions ($P < 0.01$, repeated measures (RM) 2 way ANOVA) with mean maximal response 0.6 ± 0.2 fold that in the first curve, but with similar EC_{50} values determined from responses normalised to their own maximum force. Presence of $3.3 \mu\text{M}$ GTP, necessary for G protein-coupled, agonist-mediated signalling, did not alter pCa-force properties in first or second curves. In contrast to the reduced responsiveness of a second pCa-force curve, in arteries when the second curve was elicited in the presence of $1 \mu\text{M}$ U46619, there was significantly greater tension than that observed in the first curve without agonist in the same arteries ($P < 0.01$, RM 2 way ANOVA, $n = 10$). Mean maximal response in pCa 5.5 was 2.9 ± 3.3 fold that in the first curve, but mean EC_{50} values were significantly lower (pCa 6.3 ± 0.1 vs. 6.6 ± 0.1 , $P < 0.01$, Wilcoxon signed rank test); active tone was first elicited at pCa 7 compared with pCa 6.7 in the first curves. These second curve responses were also significantly greater than those in separate, time-matched arteries where second curves were elicited in the absence of U46619 ($P < 0.01$, 2 way ANOVA). A similar pattern of enhanced responses was observed in the presence of $0.1 \mu\text{M}$ endothelin. These findings indicate that agonist-induced Ca^{2+} -sensitisation does indeed occur over a wide range of $[\text{Ca}^{2+}]_i$, further implicating this phenomenon as a contributor to contractile tone regulation in human arteries;

this mechanism in myometrial arteries may participate in regulation of blood flow in the uteroplacental bed, important for matching fetal oxygen/nutrient demand to supply. Hudson N *et al.* (2007). *Biol Reprod* **77**, 45-52.

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PC144

17 β oestradiol-dependent acute relaxations of human arteries *in vitro*: a role for ER β ?

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An acute vasodilatory action of 17- β oestradiol in many vascular beds depends upon oestrogen receptor (ER α and ER β) activation. The role of 17- β -oestradiol in regulating human vascular tone is of interest in many physiological settings including the uteroplacental unit. Maternal serum levels of 17- β oestradiol, and uterine blood flow, increase during pregnancy and 17- β oestradiol acutely relaxes uterine arteries *in vitro*. Alternatively, little is known of 17- β oestradiol influences on placental vascular tone. As the placenta lacks autonomic regulation, arterial tone is controlled by locally derived substances one of which may be oestrogen. The placenta also offers a source of non-pathophysiological blood vessel specimens, and it was therefore pertinent to investigate if 17- β oestradiol and agonists of ER α or ER β , have a role in the regulation of placental arterial tone.

Chorionic plate arteries (200-500 μm) were dissected from placentas collected ($n=12$, with LREC approval) from normal term pregnancies. Vessels were bathed in Physiological Salt Solution (PSS, 5% CO_2 /95%air, 37°C) and isometrically constricted with the thromboxane receptor ligand U46619 ($1 \mu\text{M}$). Upon stable constriction, incremental doses (0.05–30 μM) of 17- β oestradiol, 17- α oestradiol (a putatively inactive stereo-isomer), ER α agonist propylpyrazoletriyl (PPT) or ER β agonist diarylpropionitrile (DPN) were applied. Vessels were washed with PSS, re-constricted with U46619 and exposed to a single dose (30 μM) of 17- β oestradiol, 17- α oestradiol, PPT or DPN. In a subset of experiments, the influence of 17- β oestradiol, 17- α oestradiol, PPT or DPN on U46619-induced Ca^{2+} -sensitisation (pCa 6.7) of α -toxin permeabilised arteries was studied.

Pre-constricted arteries ($n=6$) significantly relaxed in response 17- β oestradiol from $1 \mu\text{M}$ -30 μM ($p < 0.001$, 2 way anova of Arcsine transformed data, bonferroni post hoc test) and to DPN from $2 \mu\text{M}$ -30 μM (Figure 1). Similar relaxations to PPT or 17- α oestradiol were not evident. Separate single dose 30 μM exposure to 17- β oestradiol ($n=6$) or DPN also relaxed pre-constricted arteries, to $41.8 \pm 1.1\%$ or $50.5 \pm 3.9\%$ respectively ($P < 0.01$, one way anova) of U46619 max-constriction. In permeabilised arteries ($n=5$), none of the agents significantly relaxed U46619-induced tone at pCa 6.7.

In summary, the acute relaxation of human placental vascular tone by 17- β oestradiol may, in part, be due to signalling via

ER- β but does not appear to involve direct activation on smooth muscle of Ca^{2+} -desensitising pathways. It remains to be seen if similar actions are evident in other human arterial vascular beds but nonetheless places oestradiol as a potential endocrine regulator of human placental vascular tone.

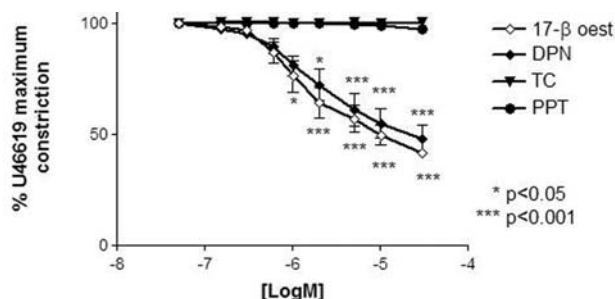


Figure 1: Dose-response curves to 17- β oestradiol, PPT and DPN with matched time controls. Significant relaxation (*) was achieved when exposed to 17- β oestradiol or DPN.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC145

Mathematical modelling of electrical action potentials in a uterine smooth muscle cell

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Uterine smooth muscle cells (USMCs) undergo ionic channel remodelling during gestation to facilitate spontaneous action potential (AP)-driven contractions during labour. Electrical activity of USMCs is related to the intracellular Ca^{2+} dynamics which in turn controls their contraction. Simple models of $[\text{Ca}^{2+}]_i$ dynamics and mechanics of USMCs are available but a rigorous model for the membrane excitation is lacking. The aim of this study was to construct a mathematical description of an USMC at the late pregnant stage with biophysically detailed membrane electrophysiology.

Mathematical models for thirteen ionic currents were developed based on voltage-clamp experimental data of late pregnant rat and human tissues in the literature. Several inward currents were considered: L-type Ca^{2+} current, attributed to be the major inward current, fast Na^+ current, T-type Ca^{2+} current and an hyperpolarisation-activated current. The outward currents include: fast A-type transient K^+ current, two voltage-gated K^+ currents (I_{K1} and I_{K2}), Ca^{2+} -activated K^+ current and a sustained background current. Ca^{2+} -activated Cl^- current and a non-specific cation current, each have reversal potentials within the reported AP amplitude range, are also included. All currents are modelled as ohmic resistors and their conductance kinetics described by Hodgkin-Huxley-type first order ordinary differential equations. Currents of a Na^+ - Ca^{2+} exchanger and a Na^+ - K^+ pump are also included to allow incorporation of $[\text{Ca}^{2+}]_i$ dynamics (via the membrane channels, the Na^+ - Ca^{2+} exchanger and the plasma membrane Ca^{2+} -ATPase) modified from a simple uterine excitation-contraction model [1].

The model was initially validated by the ability to produce bursting APs by an external stimulus. In Figure 1(a), repetitive APs were evoked by a current clamp consistent with experimental recordings from myometrium of pregnant rats [2]. Further model parameterisation was evidenced by the ability to closely simulate the effects of oestradiol, which modulates the amplitude and activation properties of individual Ca^{2+} and K^+ currents, to alter AP configuration to a tonic-like plateau [2-4]. In conclusion, a novel mathematical model of the electrical action potential of USMCs has been developed. This provides a powerful tool to investigate the ionic mechanisms underlying the physiological genesis of electrical excitation governing labouring contractions.

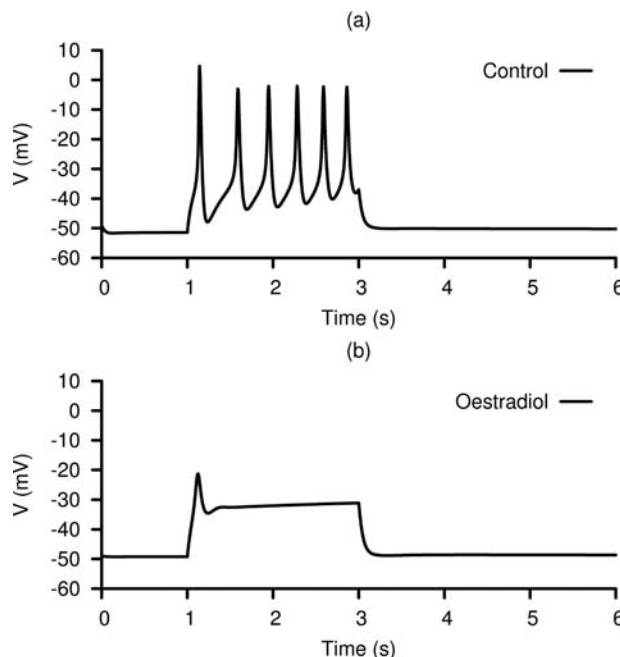


Figure 1. Simulated membrane action potential (V) of a USMC of pregnant rat in the absence (a) or presence (b) of oestradiol [2]. Current clamp applied at time = 1 – 3 s.

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC146

Free radicals on dopaminergic neurons in medial preoptic area and sexual behavior of streptozotocin-induced diabetic male rat

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