PS C1

Non-contractile cells with thin processes resembling interstitial cells of Cajal found in the wall of guinea-pig mesenteric arteries

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Cells similar to the interstitial cells of Cajal (ICCs) were reported in lymphatic vessels (McCloskey *et al.* 2002) and portal vein (Povstyan *et al.* 2003). This work describes the morphology, calcium dynamics and immunohistochemistry of non-contractile cells with thin processes, named arterial ICC-like (AIL) cells, found in enzymic dispersions of arteries.

Guinea-pigs were humanely killed by cervical dislocation followed by exsanguination. Single cells were obtained by enzymic digestion of mesenteric arteries, loaded with a calcium sensitive fluorescent dye and imaged using a laser scanning confocal microscope. In some experiments the cells were voltage-clamped to record membrane current simultaneously with cell imaging. In immunohistochemical experiments the cells were fixed, and incubated with primary antibodies against a target molecule and then with fluorescent secondary antibodies.

AIL cells had an irregular elongated shape and numerous thin (often less than 1 μm wide) processes with lengths up to ~60 μm. Some of the processes were observed to grow in length (average speed ~0.15 μm min^{-1}) and this elongation was blocked by 10 μM latrunculin B, an inhibitor of actin polymerization. Staining with BODIPY phalloidin, a fluorescent dye selective for F-actin, showed F-actin to be present in the processes of AIL cells. Imaging of intracellular ionised calcium with fluo-4 using a laser scanning confocal microscope showed global or local calcium transients lasting several seconds in ~28 % of AIL cells. When membrane current was recorded simultaneously, the calcium transients were found to correspond to long-lasting transient outward currents, which occurred at potentials positive to −40 mV. Unlike myocytes, AIL cells did not contract in response to 1 mM caffeine or 5 μM noradrenaline, although they responded with a $[Ca^{2+}]_i$ increase. The segments of intact arteries did not stain for *c-kit*, a marker of ICCs. Single AIL cells stained positive for vimentin, desmin and smooth muscle myosin, suggesting their close relationship to smooth muscle. However, the numerous processes and lack of contractile ability suggest they have some specialised function.

McCloskey KD *et al.* (2002). *Cell Tissue Res*, 77–84.

Povstyan OV *et al.* (2003). *Cell Calcium* **33**, 223–239.

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All procedures accord with current UK legislation

PS C3

Characterisation of calcium-activated chloride currents in murine smooth muscle myocytes

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Calcium-activated chloride currents (I_{ClCa}) contribute to smooth muscle excitation–contraction coupling and are also thought to play an important role in rhythmic electrical activity. This current has been studied extensively in a variety of smooth muscle models but remains to be fully characterised in a murine model, which is important for possible future experiments on animals that have been modified genetically. Therefore the aim of this study was to investigate I_{ClCa} in the mouse portal vein (mPV), a tissue that has been shown to display the current in preliminary studies (Britton *et al.* 2002).

Female BALB-c mice (6–8 weeks) were humanely killed by cervical dislocation and the PV was immediately removed and placed in 100 μM Ca^{2+} physiological salt solution. Single cells were dispersed using enzymatic digestion following the removal of fat and connective tissue.

Using the conventional whole cell patch clamp technique and a pipette solution containing 126 mM CsCl, 10 mM Hepes, 4 mM $MgCl_2$, 5 mM Na_2ATP and 5 mM EGTA, depolarisation from −60 mV evoked an inward current typical of the L-type Ca^{2+} current (I_{Ca}). In 1.5 mM Ca^{2+} external solution the maximum current was 28 ± 3 pA (mean ± S.E.M., $n = 20$), which increased to 67 ± 7 pA ($n = 19$) in 10 mM Ca^{2+} external solution and 100 ± 12 pA ($n = 11$) in BAY K8644 but was inhibited by nicardipine (5 ± 2 pA, $n = 7$). The $V_{0.5}$ for inactivation of this current was −25 ± 1 mV.

I_{ClCa} was studied using the perforated patch technique by adding 240 μg ml^{-1} of amphotericin to the pipette solution. In 70 % of the 51 cells studied, depolarisation from −60 mV evoked I_{Ca} followed by a tail current (I_{TAIL}) upon repolarisation. The amplitude of I_{TAIL} was extremely variable from cell to cell averaging 120 ± 30 pA in 1.5 mM Ca^{2+} external solution (range from −13 pA to −300 pA, $n = 12$) and 190 ± 54 pA in 10 mM Ca^{2+} external solution ($n = 11$). Modulators of I_{Ca} such as nicardipine and BAY K 8644 abolished and augmented I_{TAIL}, respectively. I_{TAIL} was also abolished by membrane rupture leading to intracellular dialysis with EGTA. I_{TAIL} was inhibited by niflumic acid (mean inhibition was 55 ± 8 % and 73 ± 9 % for 10 μM and 20 μM, respectively) and had a reversal potential (−4 ± 4 mV) close to the theoretical chloride equilibrium potential. Replacement of the external anion with thiocyanate, isethionate and glutamate changed the reversal potential to −46 ± 4, 18 ± 5 and 30 ± 2 mV, respectively ($n = 4$).

This study shows that mPV myocytes display robust I_{ClCa} that is activated as a result of Ca^{2+} influx through dihydropyridine-sensitive voltage-dependent calcium channels.

Britton FC *et al.* (2002). *J Physiol* **539**, 107–117.

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All procedures accord with current UK legislation

PS C4

Signal transmission between two cell types isolated from the media of rabbit portal vein

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Recent studies in our laboratory have demonstrated the presence of interstitial cells (ICs) in the wall of rabbit portal vein (Povstyan *et al.* 2003); ICs are considered to play a pacemaker role in the gastrointestinal tract and urethra (Sanders *et al.* 1999, Sergeant *et al.* 2000). Following enzymatic dispersion of portal vein fragments, isolated ICs, as well as ICs contacting other ICs or smooth muscle cells (SMCs) were obtained. We therefore investigated whether any signal transmission from IC to IC or from IC to SMC is possible through contacts which survive the isolation procedure. This was explored by combining stimulation of an IC under tight-seal voltage clamp with simultaneous imaging of $[Ca^{2+}]_i$ in the IC and in adjacent IC(s) or SMC(s). Imaging was performed using fast *x–y* laser-scanning confocal microscopy of fluo-3 fluorescence.

A section of portal vein about 20 mm long, upstream from the anastomosis of its right and left branches was removed from male New Zealand rabbits (2–3 kg) immediately after they had been humanely killed by an overdose of pentobarbitone injected into the ear vein. For scanning electron microscopy, isolated cells were fixed with cacodylate buffer, post-fixed in 1 % osmium tetroxide, dehydrated and mounted on aluminum stubs. For viewing under a Zeiss EM 940 electron microscope, specimens were shadowed with a thin layer of evaporated gold that gives the secondary electron image seen on the monitor.

Contacts that survived the isolation procedure between the processes of ICs and SMCs, were observed in electron micrographs. Depolarization of an IC under voltage clamp resulted in increase in fluo-3 fluorescence in the IC without apparent latency, while a rise of $[Ca^{2+}]_i$ in an adjacent SMC occurred also but with a delay of up to ~3 s. Thus, any evoked change in $[Ca^{2+}]_i$ in a SMC required a long-lasting depolarization of the IC connected to it. Long-lasting spontaneous depolarizations were observed in isolated ICs under current clamp conditions. Finally, when the same protocol as was used to study IC-SMC pairs was applied to small fragments of surviving IC network, it was revealed that signal transmission from IC to IC occurred almost instantaneously.

These results suggest that the IC network may serve as a generator and/or conductor of low frequency electrical signals (such as slow wave), which act as SMC pacemakers.

Povstyan OV *et al.* (2003). *Cell Calcium* **33**, 223–240.

Sanders KM *et al.* (1999). *Neurogastroenterol Motil* **11,** 311–338.

Sergeant GP *et al.* (2000). *J Physiol* **526,** 359–366.

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All procedures accord with current UK legislation

PS C5

Ca^{2+} sparks and excitability in phasic ureteric smooth muscle

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Local SR Ca^{2+} release events, sparks, have been shown to influence excitability in tonic vascular smooth muscle (Herrera & Nelson, 2002), but little is known of their role in phasic muscles. In the present study we have therefore investigated the role of Ca^{2+} sparks in the control of excitability in guinea-pig ureter.

Guinea-pigs were humanely killed with CO_2. Fluo-4-loaded multicellular preparations or freshly isolated single cells, and wide-field fast confocal imaging were used. Results are expressed as mean values ± S.E.M.; *n* is the number of preparations and significance was tested using Student's paired *t* test.

In cells *in situ* localised, short-lasting Ca^{2+} sparks, potentiated by caffeine (1–2 mM) and inhibited by ryanodine (50 μM) and CPA (20 μM) were seen in 30–40 % of isolated cells (122 cells observed from 20 animals) and in 87 % of intact preparations (47 observed from 20 animals). The Ca^{2+} sparks originated at the cell membrane close to the middle of the cell, from one to four frequent discharging sites (FDSs). Following global Ca^{2+} transients evoked by electrical field stimulation (7 V, 200 ms), an increase in the number of sparking cells (5–6 times) and FDSs per cell (3–5 times) and the frequency (from 0.1–0.4 to 2.5 Hz) of Ca^{2+} sparks of active FDSs were seen. Stimulation of the ureter during this period of elevated Ca^{2+} spark activity (30–40 s) failed to induce a propagating global rise of Ca^{2+}, suggesting that the ureteric muscle was refractory to stimulation during this time. In whole cell voltage-clamp experiments, with K^+ in the pipette solution, voltage step from –60 mV to 0 mV produced initial inward current followed by small sustained outward current with spontaneous transient outward currents (STOCs) superimposed on it. STOCs appeared after about 100 msec delay following inward current. The temporal characteristics of the STOCs were close to those of Ca^{2+} sparks. The STOCs were inhibited by 1 mM TEA, 50 μM ryanodine or 20 μM CPA and potentiated by 1 mM caffeine. TEA significantly (paired *t* test $n = 7$) decreased the refractory period from 60 s to 10 s. Similar effects on the refractory period were also seen with ryanodine ($n = 4$) and CPA ($n = 5$). In contrast, caffeine, which enhanced Ca^{2+} spark frequency and STOCs, blocked electrical field stimulated propagating global Ca^{2+} transients ($n = 11$). The inhibitory action of caffeine on the global Ca^{2+} rise was completely reversed by TEA, ryanodine and CPA.

These data suggest that Ca^{2+} uptake by the SR during Ca^{2+} entry on the action potential plays a key role in controlling the refractory period, by stimulating Ca^{2+} spark activity, which by targeting BK channels affects the resting membrane potential. This is achieved through an increase in FDSs, the number of firing cells and the frequency of Ca^{2+} sparks.

Herrera G & Nelson MT (2002). *Novartis Found Symp* **246**, 221–227.

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All procedures accord with current UK legislation

PS C6

Calcium-dependent regulation of intracellular pathways associated with proliferation in developing and fully developed rat portal vein

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Proliferation of vascular smooth muscle (VSM) involves gene expression dependent upon the activation of extracellular signal-regulated kinase 1/2 (ERK1/2) and the transcription factor cAMP response element-binding protein (CREB). Growth factors stimulate these pathways and can also increase intracellular Ca^{2+} ($[Ca^{2+}]_i$) which may play an important regulatory role in this process. This study examines the Ca^{2+} dependency of platelet derived growth factor-BB (PDGF) and endothelin-1 (ET-1)-induced CREB and ERK1/2 activation in proliferating (Tasker *et al.* 1999), compared to fully differentiated VSM.

Portal veins (PV) from 2- to 4-day-old neonatal and 6-week-old Sprague-Dawley rats humanely killed by cervical dislocation were stimulated with PDGF (50 ng ml^{-1}) or ET-1 (50 nM). Following stimulation, homogenised tissues were subjected to SDS-PAGE and activation of ERK1/2 or CREB detected by immunoblotting with phospho-specific antibodies. PDGF- and ET-1-induced increases in $[Ca^{2+}]_i$ from dispersed PV cells loaded with Fura-2 were determined by fluorescence Ca^{2+} imaging.

Following a 15 min incubation, PDGF stimulated CREB phosphorylation (2 ± 0.3-fold increase compared to control; $n = 5$, $P < 0.05$, mean ± S.E.M., Student's unpaired t test) and ERK1/2 phosphorylation (2.3 ± 0.5-fold; $n = 4$, $P < 0.05$) in the neonatal PV. PDGF did not produce a significant increase in CREB activation in developed PV but significantly increased ERK1/2 activation (2.4 ± 0.8-fold; $n = 4$, $P < 0.05$). BAPTA (which buffers $[Ca^{2+}]_i$) and 2-APB (inhibits InsP_3-induced Ca^{2+} release) were used to examine the dependence of $ERK_{½}$ and PDGF activation on $[Ca^{2+}]_i$. BAPTA (30 μM) and 2-APB (10 μM) completely inhibited ERK1/2 activation induced by PDGF in developed PV ($n = 4$; $P < 0.05$). However, in neonatal PV ($n = 3$), BAPTA and 2-APB did not reduce ERK1/2 or CREB activation. PDGF produced an increase in $[Ca^{2+}]_i$ in myocytes isolated from adult PV ($n = 32$, $P < 0.01$) but not from neonates. These experiments were repeated following stimulation with ET-1. In both neonatal and developed PV, ET-1 produced an activation of CREB and ERK1/2 that was significantly inhibited by BAPTA or 2-APB. Stimulation with ET-1 increased the $[Ca^{2+}]_i$ in isolated myocytes from both neonatal ($n = 53$) and developed rats ($n = 19$).

In conclusion, PDGF stimulation of these proliferative pathways is Ca^{2+} dependent in developed, but not in proliferating, PV. This is, at least partly, agonist specific. Although the intracellular mechanism of this altered Ca^{2+} dependency in proliferating VSM is unknown, it may be directly correlated with a concurrent agonist-induced increase in $[Ca^{2+}]_i$.

Tasker PN *et al.* (1999). *Circ Res* **84**, 536–542.

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All procedures accord with current UK legislation

PS C7

Effects of rho-associated kinase inhibitors on agonist-dependent contractions of isolated human adult and fetal arteries

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Agonist-dependent smooth muscle contraction results in Ca^{2+} sensitisation of the myofilaments by activation of an intracellular signalling cascade involving rho-associated kinase (ROK). Hyperstimulation of this pathway has been implicated in the increased vascular tone of hypertension, and the development of arteriosclerosis, in animal models (e.g. Shimokawa *et al.* 2001). Enhanced ROK activation may, therefore, contribute to the increased vascular resistance associated with pregnancy-related disorders such as pre-eclampsia. However, the involvement of ROK activation in contractility of arteries from pregnant women or term fetuses is unknown. Therefore, in this study, we have examined the ability of ROK inhibitors, Y-27632 (Ueheta *et al.* 1997) or HA1077 (Sward *et al.* 2000), to alter agonist-dependent contractions of human maternal and fetal arteries.

Omental arteries (from term pregnant women undergoing elective Caesarean section) and placental chorionic plate arteries (from women undergoing normal vaginal delivery at term) were isolated from biopsies obtained following written informed consent according to local ethical committee guidelines. Vessels were normalised on a wire myograph (37°C, 95 % air–5 % CO_2) and constricted with ~EC_{70} dose of the thromboxane mimetic U46619 (100 nM) and exposed to incremental doses (0.1–10 μM) of Y-27632 or HA1077.

Placental arterial constrictions to U46619 were reduced in a dose-dependent manner with 10 μM Y-27632 attenuating contractions to 53 ± 11 % (mean ± S.E.M., $P < 0.05$, Student's paired t test, $n = 5$) of control. However, HA1077 was without effect (contractions were 93 ± 6 % of control). Similarly, omental arterial constrictions were inhibited in a concentration-dependent fashion with 10 μM Y-27632 significantly reducing contractions to 19 ± 5 % of control ($n = 5$). Omental contractions in the presence of HA1077 were 60 ± 15 % ($n = 4$) of control. Y-27632 appeared to be a more potent inhibitor of agonist-dependent contractions in intact placental and omental arteries than HA1077 consistent with an increased specificity of Y-27632 for ROK over HA1077 (Ueheta *et al.* 1997; Sward *et al.*, 2000). Western blot analysis of placental and arterial tissue homogenates illustrated both ROK isoforms (α and β) to be present.

These data therefore suggest that ROK activation may play a role in agonist-mediated contraction of human adult and placental arteries.

Shimokawa H *et al.* (2001). *Cardiovasc Res* **51**, 169–177.

Sward K *et al.* (2000). *J Physiol* **522**, 33–49.

Ueheta M *et al.* (1997). *Nature* **389**, 990–994.

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All procedures accord with current local guidelines and the Declaration of Helsinki

PS C8

The diadenosine polyphosphates are selective vasoconstrictors in human coronary artery bypass grafts

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The use of arterial grafts, such as the radial artery (RA) and internal mammary artery (IMA), in coronary artery bypass grafting (CABG) is often preferred over saphenous vein (SV) because of the improved long-term patency. However, arterial grafts, particularly the RA, are prone to spasm. The rise in vasoconstrictor levels following CABG has been implicated in the pathogenesis of spasm (Downing & Edmunds, 1992), but whilst the properties of most platelet-derived vasoconstrictors have been extensively studied in human conduits, there is limited information on the diadenosine polyphosphates (DPPs; Ap_nA, where n is 3–7). Despite this, Ap_4A and Ap_5A have been proposed as hypotensive agents (Kikuta *et al.* 1999; van Ginneken *et al.* 2002). We therefore investigated the effect of DPPs on conduits used in CABG.

Sections of human RA, SV and IMA were obtained surplus to CABG, with ethical committee approval. Arterial rings in Krebs saline at 37°C, gassed with 95 % O_2–5 % CO_2, were set at a pretension equivalent to 100 mmHg for 1 h followed by 30 min at 20–30 mmHg. SV was pretensioned at 35 mN for 1 h followed by 30 min at 10 mN. Data are presented as means ± S.E.M. from n patients.

RA gave maximal responses to KCl (90 mM) of 68.3 ± 4.0 mN ($n = 8$) and IMA 25.5 ± 1.6 mN ($n = 4$). RA responded to the P2X receptor agonist α,β-methyleneATP with concentration-dependent increases in tension, giving EC_{50} values of 1.9 ± 0.3 μM ($n = 3$) and maximal responses 74.0 ± 8.1 % KCl. Ap_5A also increased tension in a concentration-dependent manner, giving EC_{50} values of 9.9 ± 2.7 μM and a maximal response 92.9 ± 3.0 % KCl. Prior application of 100 μM α,β-methylene-ATP to desensitize P2X receptors reduced the response to 10 μM Ap_5A to 16.2 ± 5.1 % of control values ($n = 5$). However, in the presence of 30 μM pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) the response to 10 μM Ap_5A was enhanced to 151.7 ± 11.8 % ($n = 4$) of controls. RA also responded to Ap_4A and Ap_6A giving EC_{50} values of 20.2 ± 7.9 μM ($n = 3$) for Ap_4A and 12.1 ± 2.1 μM ($n = 3$) for Ap_6A and maximal responses 79.1 ± 9.4 % and 88.6 ± 17.9 % KCl for Ap_4A and Ap_6A, respectively. In contrast, sections of IMA gave only weak responses to 100 μM α,β-methylene-ATP (10.0 ± 5.3 % KCl; $n = 3$) and negligible responses to 100 μM Ap_4A or 50 μM Ap_5A ($n = 3$). SV showed robust concentration-dependent contraction to Ap_4A, Ap_5A and Ap_6A ($n = 3$).

In conclusion, the DPPs are potential mediators of spasm in the RA but not the IMA and their use as hypotensive agents should be treated with caution.

Downing SW & Edmunds LH (1992). *Ann Thorac Surg* **54**, 1236–1243.

Kikuta Y *et al.* (1999). *Acta Anaesthesiol Scand* **43**, 82–86.

van Ginneken EEM *et al.* (2002). *Clin Pharmacol Ther* **71**, 448–456.

All procedures accord with current local guidelines and the Declaration of Helsinki

PS C9

Depletion of membrane cholesterol: effects on rat myometrial spontaneous force and calcium

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Lipid rafts and caveolae are cholesterol-rich membrane microdomains that play an important role in membrane signalling and smooth muscle contraction. These microdomains depend on cholesterol for stability and depletion of cholesterol results in disruption of caveolae and rafts. Dreja *et al.* (2002) demonstrated in rat tail artery that contractions elicited by 5-hydroxytryptamine were inhibited by depletion of cholesterol whereas contractions to high potassium were unaffected, highlighting the importance of cholesterol in membrane signalling. We have used methyl-β-cyclodextrin (MCD), cholesterol oxidase (CO) and cholesterol esterase (CE) to reduce membrane cholesterol and investigated their effect on force and $[Ca^{2+}]_i$ in the rat myometrium.

Pregnant Wistar rats or neonates (10–14 day) were humanely killed with CO_2 and longitudinal strips of myometrium loaded with the Ca^{2+}-sensitive indicator indo-1 (10μM for 3 h) to enable simultaneous measurement of calcium and force. MCD, CO and CE were added to the superfusion solution as required. All experiments were carried out at 35 °C; all values represent mean ± S.E.M.; n is the number of animals, and statistical differences were tested for by Student's paired t test ($P < 0.05$).

Strips from pregnant rats were spontaneously active and contacted at a rate of around 1 min^{-1}. Addition of 2 % MCD resulted in rapid inhibition of spontaneous contractions ($n = 4$), abolition of associated Ca^{2+} transients and a transient decrease in the basal $[Ca^{2+}]_i$ which then rose to above the resting level. Removal of MCD after 30 min produced a transient increase in force which returned towards the basal level. MCD also siginificantly decreased spontaneous contractions in neonatal strips but at a much slower rate than in the adult tissue ($n = 4$). In strips from pregnant rats, application of either CO (2 U ml^{-1}) or CE (0.5 U ml^{-1}) resulted in rapid abolition of spontaneous contractions and Ca^{2+} transients similar to that seen with MCD. Upon their removal the spontaneous contractions were restored.

We have shown that MCD, CO and CE inhibit spontaneous contractions in myometrial strips. In the case of CO and CE, removal resulted in rapid restoration of spontaneous activity whereas with MCD the inhibition was long lasting. We suggest that the inhibitory effects of these agents were produced via depletion of cholesterol from the cell membrane and the disruption of lipid rafts and caveolae. In turn these data indicate that aspects of Ca^{2+} signalling in myometrial cells is localized to membrane rafts.

Dreja K *et al.* (2002). *Arterioscler Thromb Vasc Biol* **22**, 1267–1272.

All procedures accord with current UK legislation

PS C10

Antioxidative enzyme expression and lipid peroxidation in human myometrium with parturition

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Intrauterine hypoxia, an important marker of potentially abnormal outcome in the neonatal period, may lead to perinatal asphyxia (Supnet *et al.* 1994). In addition, intrapartum acidosis is a common cause of fetal cerebral damage in preterm deliveries (Low *et al.* 1994). Evidence suggests that production of reactive oxygen species (ROS) and reduced antioxidant capacity accompanies these dysfunctional pregnancies. The aim of this study was to investigate expression levels of key antioxidative enzymes (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHpx)) and the levels of lipid peroxidation as a marker of oxidative stress in human myometrium with term and preterm parturition.

The study was approved by the Southern Derbyshire Ethics Committee and written informed consent was obtained from all women taking part in the study. Myometrial biopsies were obtained from women undergoing either elective (not in labour) or emergency (in labour) sections at both term and preterm (< 36 weeks) gestations. The patients were divided as follows: term not in labour TNL ($n = 11$), preterm non-labour PTNL ($n = 9$), term in labour TL ($n = 10$) and preterm in labour PTL ($n = 5$). Tissues were homogenized and centrifuged to give cytosolic and membrane fractions with only the cytosolic component being used for Western blotting. Proteins were resolved by SDS-PAGE and blots probed with antisera to SOD, CAT and GSHpx followed by chemiluminescent detection of enzyme expression. Densitometric analysis was carried out and enzyme expression compared amongst the four groups. The membrane fraction was used to colorimetrically determine the level of lipid peroxidative damage. Malonialdehyde (MDA), a product of lipid peroxidation present in biological samples, reacts with thiobarbituric acid (TBA) under acidic conditions at 95°C with an absorbance maximum at 532 nm. Statistical analysis was carried out by one-way analysis of variance (ANOVA) with Scheffé correction to determine differences between groups. A probability level (P) of < 0.05 was considered statistically significant.

This study demonstrated that SOD and CAT enzyme levels in human myometrium of non-labouring or labouring women do not change with term or preterm gestations. However, GSHpx levels were found to be significantly ($P < 0.05$) lower in the TL and PTL compared with TNL and PTNL groups. This finding was also mirrored by a significant ($P < 0.05$) increase in lipid peroxidation levels in both the TL and PTL groups when compared with the TNL and PTNL groups.

Our results indicate that increased lipid peroxidation observed with the onset of labour may arise as a result of reduced antioxidative GSHpx expression. Further studies are necessary to investigate whether lipid peroxidation and/or altered antioxidative enzyme expression are consequences or causes of normal and dysfunctional labour.

Low JA *et al.* (1994). *Am J Obstet Gynecol* **170**, 1081–1087.

Supnet MC *et al.* (1994). *Pediatr Res* **36**, 283–287.

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All procedures accord with current local guidelines and the Declaration of Helsinki

PS P50

Noradrenaline evokes a cation current in single smooth muscle cells of guinea-pig ileum

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It is well known that opening of cation channels is one of the major ways of depolarizing and contracting smooth muscle. Activation of adrenoreceptors generally causes hyperpolarization of guinea-pig intestinal smooth muscle (Horinouchi *et al.* 2003) but if the potassium conductance is blocked often depolarization is produced (Vladimirova & Shuba 1980).

In these experiments on single cells isolated by enzyme treatment from the longitudinal layer of guinea-pig ileum, tight-seal voltage-clamp recordings (in whole-cell configuration) were made using internal (pipette) and bathing solutions containing 124 mM Cs^+. A 10 mM BAPTA–4.6 mM $CaCl_2$ mixture was used to clamp $[Ca^{2+}]_i$ at 10^{-7} M and calcium and magnesium were omitted from the external solution.

Guinea-pigs were humanely killed by cervical dislocation followed by exsanguination. Noradrenaline (10 μM) evoked an inward cation current (nI_{cat}) of 56 ± 28 pA (mean ± S.E.M., $n = 20$) at a holding potential of −40 mV which had a U-shaped current–voltage relationship. However, 10 μM carbachol evoked a cationic current of more that 400 pA amplitude (Zholos & Bolton, 1997). The reversal potential for nI_{ca} was 1 ± 4 mV ($n = 8$) close to E_{Cs} (which was 0 mV) and the involvement of Cl^- ions was excluded by replacing 100 mM CsCl with caesium methane sulphonate in the pipette solution. The noradrenaline-evoked conductance curve could be described by a Bolzmann relationship with $V_{½} = -74.8$ mV, $k = 3.9$ mV and $G_{max} = 3.57$ nS.

Although carbachol (10 μM) evoked a cation current of more than 400 pA, its current–voltage relationship and reversal potential were very similar to those of nI_{cat}. Application of 10 μM carbachol prior to 10 μM noradrenaline increased the noradrenaline-evoked cation current by 20–30 %. It appears that both muscarinic and noradrenaline receptors are able to activate the same cation current and that interaction between their signal pathways can occur.

Horinouchi T *et al.* (2003). *Naunyn Schmiedebergs Arch Pharmacol* **367**, 193–203.

Vladimirova IA & Shuba MF (1980). *Fiziol Zh* **26**, 547–52.

Zholos AV & Bolton TB (1997). *Br J Pharmacol* **122**, 885–893.

All procedures accord with current local guidelines

PS P51

P2 receptor-mediated Ca^{2+} transients in rat cerebral artery smooth muscle cells

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It has been reported that significant Ca^{2+} release was evoked by the activation of L-type Ca^{2+} current in rat superior cerebral artery smooth muscle cells (Kamishima & McCarron, 1997). Here we examined whether Ca^{2+} influx through P2X receptors also triggers Ca^{2+} release in this preparation.

Male Sprague-Dawley rats (200–300 g) were rendered unconscious by exposure to a rising concentration of CO_2 and killed by exsanguination. Single smooth muscle cells were dissociated, and membrane current and Ca^{2+} transient were simultaneously determined as previously described (Kamishima & McCarron, 1997).

Application of P2X agonists evoked membrane currents and concomitant Ca^{2+} transients in whole-cell voltage-clamped single cells. The expected increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was calculated from the time-integrated P2X current by assuming Ca^{2+} is the only permeant ion. The measured increase in $[Ca^{2+}]_i$ was plotted as a function of expected increase in $[Ca^{2+}]_i$, and Ca^{2+} buffering power was obtained as a reciprocal of the linear fit to this relationship. Ca^{2+} buffering power of the control cells was 4752 ± 459 (mean ± S.E.M., $n = 11$). In the presence of a blocker of Ca^{2+}-induced Ca^{2+} release, ryanodine, Ca^{2+} buffering power was 4947 ± 845 ($n = 6$). In the presence of a putative activator of Ca^{2+}-induced Ca^{2+} release, cyclic ADP ribose, Ca^{2+} buffering power was 3996 ± 303 ($n = 5$). No significant difference was detected among these values by one-way ANOVA, suggesting that Ca^{2+} influx through P2X receptors does not trigger significant Ca^{2+} release in this preparation. Comparison of Ca^{2+} buffering power for L-type Ca^{2+} channels and P2X receptors suggested that about 5 % of the P2X current, corresponding to the fractional unitary current of about 25 fA at −60 mV, is carried by Ca^{2+}. To test the hypothesis that P2X response may influence the subsequent P2Y response, we induced a P2Y-mediated Ca^{2+} transient with and without depolarization. The maximum rate of Ca^{2+} increase, obtained as the steepest slope of the linear regression to the rising phase of the P2Y receptor-mediated Ca^{2+} transient, was 1526 ± 446 nM s^{-1} ($n = 5$) without depolarization. The maximum rate of Ca^{2+} increase with depolarization was 5446 ± 1224 nM s^{-1} ($n = 4$). Student's unpaired t test detected a significant difference in these values ($P < 0.05$).

Thus, membrane depolarization caused by P2X current may influence the subsequent P2Y-mediated elevation in $[Ca^{2+}]_i$ by modulating second messenger signalling cascade.

Kamishima T & McCarron JG (1997). *J Physiol* **501**, 497–508.

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All procedures accord with current UK legislation

PS P52

Characterization of outward currents in interstitial cells isolated from the guinea-pig bladder

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Interstitial cells of Cajal (ICC) have recently been reported to exist in the guinea-pig bladder (McCloskey & Gurney, 2002). To date, little is known of their physiological properties, although they are likely to be involved in controlling the activity of bladder smooth muscle. In the present study, the patch clamp technique was used to investigate outward currents in ICC from the guinea-pig detrusor.

Bladders were removed from guinea-pigs humanely killed by cervical dislocation. A heterogeneous population of cells was isolated as described previously (McCloskey & Gurney, 2002),

the majority of which had a smooth muscle-like morphology. A subpopulation of branched cells were typical of the kit-positive ICC reported previously. These cells were selected for patch-clamp studies using K^+-based pipette solution.

Application of depolarising pulses to cells voltage clamped at –80 mV resulted in the generation of outward currents. Penitrem A (100 nM) significantly inhibited the outward currents across the range of voltages tested; mean control current at +50 mV was reduced from 1922 ± 535 pA (mean ± S.E.M.) to 427 ± 70 pA ($P = 0.038$, $n = 5$, Student's paired t test). The penitrem A-sensitive current was large and noisy and activated positive to –40 mV typical of large-conductance Ca^{2+}-activated K^+ (BK) currents. This current was also reduced by iberiotoxin (100 nM, $n = 3$). Nifedipine (1 μM) inhibited the current at +40 mV, from 1666 ± 463 pA to 1040 ± 274 pA ($n = 5$, $P = 0.034$) thereby demonstrating its Ca^{2+} dependence. When the BK current was blocked by penitrem A and nifedipine, a voltage-dependent current was unmasked which activated at potentials positive to –50 mV and displayed voltage-dependent inactivation (half-maximal inactivation, –71 ± 4 mV, $n = 8$). Examination of the shift in reversal potential of tail currents from –65 ± 2 mV ($n = 6$, actual E_K –83 mV) in 5.8 mM $[K^+]_o$, to –27 ± 3 mV in 60 mM $[K^+]_o$, ($n = 3$, actual E_K –21 mV) suggested that this current was largely carried by K^+ ions. Tetraethylammonium ions inhibited the current in a concentration-dependent fashion (EC_{50} 4.1 ± 0.5 mM, $n = 5$) but 4-AP (10 mM), charybdotoxin (100 nM) and apamin (50 nM) had no effect suggesting that SK, I_K, Kv1.2 and Kv1.3 channels are unlikely to be components.

In conclusion, bladder ICC have at least two components of outward K^+ current. A major portion of the total outward K^+ current was BK and a delayed rectifier current made up a significant component of the remainder. These may be involved in repolarization of the action potential and might contribute to the resting membrane potential or hyperpolarization of the cells.

McCloskey KD & Gurney AM (2002). *J Urol* **168**, 832–836.

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All procedures accord with current UK legislation

PS P53

Human myometrial TrpC isoform expression is regulated during pregnancy

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There is increasing functional evidence for store-operated calcium entry (SOCE) in human myometrium. SOCE appears to be enhanced in human myometrium at labour onset (Tribe *et al.* 2000) and IL-1β, a cytokine implicated in labour, induces calcium oscillations and SOCE in human myometrial smooth muscle cells (Tribe *et al.* 2003). TrpC1, 3, 4 and 6 proteins, which are associated with SOCE in other cells, are expressed in pregnant human myometrial tissue (Dalrymple *et al.* 2002). In this study we determined whether TrpC1, 3, 4, 6 and 7 mRNA and proteins change with pregnancy and labour.

Human myometrium was obtained at Caesarean section (with written consent from women without underlying disease) at term not in labour (TNL, $n = 13$, 38–41 weeks), term active labour (TAL, $n = 13$, 39–42 weeks). Non-pregnant myometrium was collected at hysterectomy (NP, $n = 6$). mRNA and total proteins were isolated from myometrial tissue and TrpC expression determined using RT-PCR and Western blotting (Dalrymple *et al.* 2002). RT-PCR and Western blots were analysed using Bio-Rad scanning software. Values are expressed in arbitrary absorbance units as means ± S.E.M. Significant difference ($P < 0.05$) was determined using ANOVA with Tukey-Kramer's multiple comparison test.

TrpC1 protein expression was not detected in NP myometrium but significantly increased in TAL and TNL samples (NP, 0.000 ± 0.000; TAL, 0.076 ± 0.020; TNL, 0.074 ± 0.014; $P < 0.05$). There was no significant difference in TrpC1 protein expression between TNL and TAL samples. TrpC3 protein expression was minimal in the NP and TNL myometrium, but expression was significantly increased in TAL (NP, 0.003 ± 0.003; TAL, 0.062 ± 0.009; TNL, 0.022 ± 0.007; $P < 0.01$). TrpC4 protein expression was not detected in NP myometrium, was minimally expressed in TNL and significantly increased in TAL samples (NP, 0.000 ± 0.000; TAL, 0.118 ± 0.039; TNL, 0.018 ± 0.007; $P < 0.05$). TrpC6 protein expression was not detected in the NP myometrium, minimally expressed in TNL but was significantly increased in TAL myometrial samples (NP, 0.000 ± 0.000 *vs.* TAL, 0.072 ± 0.012, $P < 0.01$; TAL *vs.* TNL 0.036 ± 0.007, $P < 0.05$). RT-PCR data mirrored protein data for all genes except TrpC4, where mRNA expression was similar between NP, TNL and TAL samples. Pregnancy is associated with an increase in myometrial expression of TrpC isoforms and there is a further increase in TrpC3, 4, and 6 with labour. The functional impact of these data warrants further study.

Dalrymple A *et al.* (2002). *Mol Hum Reprod* **8**, 946–951.

Tribe RM *et al.* (2000). *Biol Reprod* **63**, 748–55.

Tribe RM *et al.* (2003). *Biol Reprod* **68**, 1842–9.

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All procedures accord with current local guidelines and the Declaration of Helsinki

PS P54

Molecular analysis of subtype-selective inhibition of cloned K_{ATP} channels by PNU-37883A

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PNU-37883A inhibits vascular K_{ATP} channels (Wellman *et al.* 1999). It is selective for Kir6.1 over Kir6.2, and Kir6.1 may therefore form the pore of arterial K_{ATP} channels (Surah-Narwal *et al.* 1999; Kovalev *et al.* 2001). However, in some systems PNU-37883A cannot distinguish between different K_{ATP} channel subtypes (Cui *et al*. 2003). In this study, we have used Kir6.1/Kir6.2 chimeric proteins and current recordings to further investigate the basis of PNU-37883A inhibition of cloned K_{ATP} channels.

Chimeras between rat Kir6.1 and Kir6.2 were made by site-directed mutagenesis and subcloning. Channels were expressed following RNA injection into *Xenopus* oocytes. Membrane current was recorded by two-microelectrode voltage clamp of whole oocytes in a 98 mM K^+ extracellular solution and at a membrane potential of –60 mV. Kir6.*x* constructs were co-expressed with either SUR1 or SUR2B, and currents were

induced by 100 μM pinacidil (for SUR2B) or 100 μM diazoxide and 10 μM carbonyl cyanide *m*-chlorophenyl-hydrazone (for SUR1). Solutions and drugs were delivered by chamber perfusion (flow rate, 2 ml min^{-1}; chamber vol., 200 μl). Kir6.1, Kir6.2, and chimeras between Kir6.1 and Kir6.2 were expressed with SUR2B, and fractional inhibition by 10 μM PNU-37883A reported.

Channels containing Kir6.1 were more sensitive to inhibition than those containing Kir6.2 (fractional inhibition: means ± S.E.M., 0.70 ± 0.05, $n = 10$, cf. 0.07 ± 0.03, $n = 5$). A chimeric channel with the Kir6.1 pore and the Kir6.2 intracellular N and C terminal domains was PNU-37883A insensitive (0.06 ± 0.07, $n = 5$). A chimera with the Kir6.1 C terminus and Kir6.2 N terminus and pore was inhibited (0.48 ± 0.04, $n = 5$). These results, and those obtained with other chimeras, suggest the C terminus is an important determinant of PNU-37883A inhibition by Kir6.1. Similar results were seen when constructs were co-expressed with SUR1. Further chimeric constructs localised PNU-37883A sensitivity to an 87 amino acid residue-long section in the Kir6.1C terminus.

Our data show structural differences between Kir6.1 and Kir6.2 are important in determining sensitivity to PNU-37883A. This compound may prove useful in probing structural and functional differences between the two channel subtypes.

Cui Y *et al.* (2003). *Br J Pharmacol* **139**, 122–128.

Kovalev H *et al.* (2001). *J Physiol* **531P**, S173.

Surah-Narwal S *et al.* (1999). *Br J Pharmacol* **128**, 667–672.

Wellman GC *et al.* (1999). *Br J Pharmacol* **128**, 909–916.

Helen Kuhlman was a British Heart Foundation PhD student (FS/98047).

All procedures accord with current UK legislation

PS P55

Calcium-activated potassium channels do not modulate spontaneous contractions in pregnant rat myometrium

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Calcium-activated potassium channels (BK_{Ca}) are thought to contribute to the suppression of myometrial contractility during pregnancy prior to labour onset (Khan *et al.* 2001). There is a paucity of data, however, concerning the precise effect of BK_{Ca} channel blockade on contractile activity in pregnant myometrium. The aim of this study was to determine the effect of three BK_{Ca} channel inhibitors on spontaneous contractile activity *in vitro* in myometrium from the non-pregnant and pregnant rat.

Myometrial tissue was obtained from humanely killed Sprague-Dawley rats: virgin ($n = 18$), early pregnant (day 7, $n = 12$), mid-pregnant (day 14, $n = 12$) and late pregnant (day 21, $n = 12$). Tissue was dissected longitudinally and mounted in four organ baths attached to a tension transducer and a MacLab system. Spontaneous contractile activity developed during 1 h equilibration period. Spontaneous contractions were then recorded for 15 min (control period) followed by the cumulative addition of (i) paxilline (1 or 10 μM), (ii) iberiotoxin (1, 10, 50 or 100 nM) or (iii) penitrim A (1, 10, 50, 100 or 500 nM). Vehicle controls were also performed. Contraction interval, integral, duration, and mean integral tension were measured for each drug addition and expressed as a percentage of the control period. Means (± S.E.M.) were compared to vehicle controls using ANOVA with Bonferroni's correction for multiple comparisons, a value of $P < 0.05$ considered significant.

None of the three inhibitors of BK_{Ca} channels significantly augmented spontaneous contractile activity in myometrium from non-pregnant or pregnant rats when compared to vehicle controls. It was expected that inhibition of BK_{Ca} channels would significantly enhance spontaneous myometrial contractility. However the inability of the three inhibitors tested to modulate contractility in myometrium from non-pregnant and pregnant rats suggest that BK_{Ca} channels do not play a major role in mediating uterine quiescence in the pregnant rat. It remains to be elucidated whether BK_{Ca} channels contribute more to the control of myometrial contractility when tissues are co-incubated with fetal membranes or stimulated with agonists.

Khan RN *et al.* (2001). *Exp Physiol* **86**, 255–264.

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All procedures accord with current UK legislation

PS P56

Expression of mRNA encoding the two-pore domain K^+ channels TASK and TREK in human myometrium

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Understanding the control of uterine smooth muscle contractility has important therapeutic implications for the treatment of preterm or dysfunctional labours. *In vitro* hypoxia (or metabolic inhibition) rapidly reduces contractility in a variety of smooth muscle preparations, including myometrium (Taggart & Wray, 1998), suggesting that hypoxia is likely to influence cellular excitability. TASK (TWIK-related acid-sensitive K^+ channel) and TREK (TWIK-related K^+ channel) are members of the mammalian two-pore domain K^+ channel family that play an essential role in determining resting membrane potential and action potential duration in many electrically excitable cells (Patel & Honore, 2001). Functionally, TASK 1 and 3 are known to be oxygen sensitive, as is the mechano-sensitive member TREK 1, but the expression of these channels in human myometrium is unknown. As human myometrium contracts phasically during labour and is likely to experience periods of hypoxia/reoxygenation, characterisation of these K^+ channels may elucidate mechanisms regulating human uterine excitability and contractility. We have therefore examined the mRNA expression of TASK and TREK in human myometrium.

Myometrial tissue was obtained from uterine biopsies ($n = 12$) of women undergoing hysterectomy or Caesarean section at term (not in labour and in active labour). Biopsies were taken with informed written consent and according to local ethical committees' regulations. Total RNA was extracted and quantified from each sample using standard techniques. RT-PCR was performed using gene-specific primers for TASK 1 to 5 and TREK 1 and 2 that amplified products of correct sizes (relative to a molecular ladder) when validated with control tissues (commercial human brain or placental total RNA; Becton

Dickinson Bioscience). TASK 1, 4, 5 and TREK 1 mRNA was detected in all myometrial tissues, whereas expression of TASK 2 and 3 was variable. TREK 2 was not detected in human myometrium.

These data demonstrate that mRNA encoding certain TASK and TREK members of the two-pore domain K^+ channel family are expressed in human myometrium.

Taggart MJ & Wray S (1998). *J Physiol* **509**, 315–325.
Patel AJ & Honore E (2001). *Trends Neurosci* **24**, 339–346.

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All procedures accord with current local guidelines and the Declaration of Helsinki

PS P57

Effects of L-nitrosocysteine and the soluble guanylate cyclase activator YC-1 on calcium-activated chloride currents in freshly dispersed cells from rabbit corpus cavernosum

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Penile erection results from the relaxation of the corpus cavernosum smooth muscle. A wealth of work has established the role of the nitric oxide (NO)/cyclic GMP (cGMP) pathway in mediating the relaxation (Andersson, 2001). Despite the success of this line of research, the mechanisms by which NO/cGMP causes relaxation of the corpus cavernosum are incompletely understood and the intracellular targets of cGMP only partly known (Andersson, 2001). Two recent studies have described spontaneous Ca^{2+}-activated Cl^- currents (STICs) in the corpus cavernosum and suggested that these may be involved in the generation of tone in the detumescent state (Karkanis *et al.* 2003; Craven *et al.* 2003). The purpose of the present study, therefore, was to examine the effect of the rapidly degrading NO donor L-nitrosocysteine and the soluble guanylate cyclase activator YC-1 (3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole) on STICs.

Male rabbits were humanely killed with pentobarbitone (I.V.) and cells were freshly dispersed from the corpus cavernosum for study using the amphotericin B perforated patch technique. Patch pipettes contained caesium to block potassium currents. More than 80% of the corpus cells generated spontaneous transient inward current (STICs) when held at −60 mV.

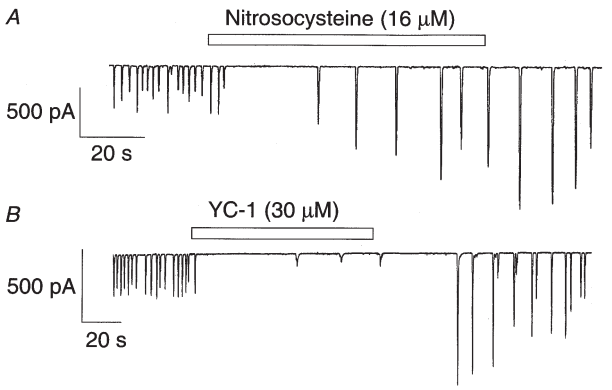

Figure 1. *A*, effect of nitrosocysteine (16 μM) on STICs in a cell held at −60 mV. *B*, effect of YC-1 (30 μM) in a different cell.

The effect of L-nitrosocysteine (initial concentration in superfusate was 16 μM; dead space time 15 s) is shown in Fig 1*A*. This inhibited STICs for approximately 25 s, after which they returned with greater amplitude and slower frequency before the drug was washed out. These effects were confirmed in five cells, where STICs in the control period had a mean frequency of $14.4 \pm 3.2\ s^{-1}$, and amplitude of -175 ± 77 pA (data are given as means ± S.E.M.). These values both fell to zero for at least 20 s in the presence of L-nitrosocysteine ($P < 0.05$, Student's paired t test). YC-1 (30 μM) also inhibited STICs and on washout they returned with reduced frequency and increased amplitude (Fig. 1*B*). Similar results were obtained in 5 cells, when the frequency and amplitude of the STICs were reduced from 10 ± 4.3 to $3.2 \pm 1.6\ s^{-1}$ and from -292 ± 84 to 81 ± 17 pA, respectively ($P < 0.05$).

These results suggest that nitric oxide, acting through cGMP can inhibit STICs in corpus cavernosum cells. This may provide one mechanism whereby this pathway causes relaxation of these cells.

Andersson, K-E (2001). *Pharm Rev* **53**, 417–450.
Craven M *et al.* (2003). *J Physiol* **548.P**, 4*P*.
Karkanis T *et al.* (2003). *J Appl Physiol* **94**, 301–313.

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All procedures accord with current UK legislation

PS P58

BK_{Ca} channels in smooth muscle cells from human conduit arteries

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Radial arteries (RAs) and internal mammary arteries (IMAs) are commonly used in revascularisation surgery. Long term patency and survival of IMA grafts is good, but RAs are prone to vasospasm, which limits their use. The use of calcium channel blockers and nitrates to prevent RA spasm has been suggested to improve results (Chanda *et al.* 2000; Kalus *et al.* 2001) and a recent study suggests that K^+ channel openers may also be effective (Hamilton *et al.* 2002). In order to better target and treat the vasospasm, a clearer understanding of the mechanisms that control tone in these vessels is needed.

In this study we compared the K^+ channels expressed in smooth muscle cells (SMCs) from human RAs and IMAs, using the whole-cell patch-clamp technique, with the aim of identifying differences that could account for the RA-specific vasospasm. Vessels were obtained from consenting patients undergoing coronary artery bypass surgery. Results are reported as means ± S.E.M. Data were compared using Student's paired t test and the level of significance set at $P < 0.05$.

SMCs isolated from RAs and IMAs had similar input resistances (IMA, 7 ± 3 GΩ, $n = 30$; RA, 10 ± 3 GΩ, $n = 42$), membrane capacitance (IMA, 31 ± 3 pA pF^{-1}, $n = 30$; RA, 28 ± 2 pA pF^{-1}, $n = 42$) and resting membrane potentials (IMA, -41 ± 5 mV, $n = 12$; RA, -48 ± 3 mV, $n = 35$). Macroscopic K^+ currents were activated upon stepping to test potentials $\geqslant -30$ mV and in cells from both vessels the currents displayed similar kinetics and peak amplitudes (at 40 mV IMA, 39 ± 9 pA pF^{-1}, $n = 26$; RA,

58 ± 9 pA pF^{-1}, $n = 35$). At concentrations up to 10 mM, 4-aminopyridine had no consistent effect on the current recorded from IMA cells ($n = 7$), but at 10 mM it caused significant inhibition of the current in RA cells (37 ± 10 % at 40 mV, $P < 0.05$, $n = 7$). The effect on RA cells was, however, variable, with no effect in one cell and inhibition ranging from 13 to 81 % in six others. The effects of a number of inhibitors of Ca^{2+}-activated K^+ channels were also tested: iberiotoxin (100 nM), charybdotoxin (100 nM), apamin (50 nM) and clotrimazole (1–10 μM). Each drug was tested on cells from at least three RAs and three IMAs. Only charybdotoxin caused consistent inhibition of the current in either vessel. Its effects were concentration dependent and of similar magnitude in each vessel type. At 40 mV, it reduced current amplitude by 64 % in IMA cells (from 22 ± 6 to 8 ± 1 pA pF^{-1}; $n = 5$, $P < 0.05$) and 67 % in RA cells (from 13 ± 2 to 4 ± 1 pA pF^{-1}; $n = 6$, $P < 0.05$). The currents were consistently noisy and single-channel recordings in excised membrane patches showed that the predominant K^+ channel had a large conductance (198 ± 9 pS, $n = 11$; RAs) and was acutely sensitive to the intracellular [Ca^{2+}]. These properties suggest that large-conductance BK_{Ca} channels were the main mediators of the K^+ current. This is despite the finding that iberiotoxin failed to inhibit it.

Overall, the results show that an iberiotoxin-insensitive, large-conductance Ca^{2+}-activated K^+ channel mediates most of the K^+ current in smooth muscle cells from human IMAs and RAs. The only difference observed between cells from RAs and IMAs was the variable expression in RAs of a 4-aminopyridine-sensitive, voltage-gated K^+ current.

Chanda J *et al.* (2000). *Ann Thorac Surg* **70**, 2070–2074.
Hamilton CA *et al.* (2002). *Atherosclerosis* **160**, 345–353.
Kalus JS *et al.* (2001). *Ann Pharmacother* **35**, 631–635.

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All procedures accord with current local guidelines and the Declaration of Helsinki

PS P59

Myogenic regulation during sustained elevations in extravascular pressure in the normal coronary artery from the rat.

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We have previously demonstrated that changes in both intravascular pressure (IvP) and extravascular pressure (EvP) will modify arterial diameter (Azzawi & Austin, 2002) of isolated rat coronary arteries. Here, we specifically examine, using novel methodology, whether isolated rat coronary arteries can actively regulate their diameter during more sustained elevations of EvP.

Male Wistar rats were humanely killed by stunning and cervical dislocation. Septal coronary arteries were dissected out and mounted on a modified pressure myograph, superfused with physiological salt solution (PSS, pH 7.4, 37 °C, 95 % air–5 % CO_2). A secure lid over the myograph chamber allowed EvP to be elevated (via a 95 % air–5 % CO_2 source) over sustained periods. The internal vessel diameter was determined using a video dimension analyser. EvP was elevated in 20 mmHg increments over the range 20–100 mmHg, and stable diameter measurements recorded. Evidence of active diameter regulation was also noted at each step. To determine the passive characteristics of arteries this was repeated in Ca^{2+}-free PSS. In some arteries, endothelium denudation was achieved by introduction of four to five air bubbles through the lumen. This abolished the endothelial-dependant dilator response to acetylcholine (10 μM). Data given as means ± S.E.M.

Coronary arteries had a mean diameter of 229 ± 16 μm ($n = 6$) when pressurised to an IvP of 60 mmHg (at zero elevated EvP, i.e. atmospheric pressure). All arteries developed myogenic tone (mean active diameter = 142 ± 15 μm, tone = 99 ± 12 μm). Elevation of EvP produced an immediate decrease in diameter in all arteries. Evidence of active regulation was observed in all arteries, such that maintained active diameter values were essentially unchanged over an EvP range of 20–60 mmHg (138 ± 15 μm at 20 mmHg; 136 ± 16 μm at 40 mmHg; 136 ± 15 μm at 60 mmHg). Stable diameters were achieved within 1–9 min. Active diameters were significantly lower than passive diameters at 20, 40, 60 (Student's paired t test, $P < 0.01$) and 100 mmHg ($P < 0.05$) EvP. The effect of endothelial denudation on the autoregulatory response was then examined (mean diameter 249 ± 13 μm, $n = 5$). Myogenic tone was increased in all vessels (mean increase in tone = 43 ± 16μm). The active regulation of diameter at an EvP of 40 mmHg achieved in endothelium-intact vessels was lost subsequent to denudation.

Thus we demonstrate that coronary vessels show active regulation of coronary artery diameter in response to a sustained elevation of EvP. In addition, we show that such regulation is modulated by endothelium-derived mediators.

Azzawi M & Austin C (2002). *J Physiol* **543.P**, 73*P*.

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All procedures accord with current UK legislation

PS P60

Detection of spontaneous calcium release at rapid frame rates by a novel back illuminated electron multiplying charge coupled device

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Although Ca^{2+} sparks have been recorded in a variety of cell types including smooth muscle cells, few confocal imaging systems are capable of recording sparks from the entire cell at frame rates in excess of 30 frames s^{-1} (f.p.s.), particularly whilst maintaining good resolution in terms of signal/noise and spatial definition. In the present study we have developed, manufactured and evaluated a novel back-illuminated electron multiplying charge coupled device (BI-EMCCD) camera (Coates *et al.* 2003), which allows us to detect Ca^{2+} sparks at frame rates in excess of 100 f.p.s.

The EMCCD used contains a 512 × 512 pixel Marconi E2V chip (pixel size 16 μm) which achieves signal amplification through a unique electron multiplying structure built into the silicon, rather than the incorporation of an image intensifier tube. In contrast to intensified CCDs which typically possess quantum efficiencies (QE) of < 50 %, the EMCCD has a QE in excess of 90 % across a wider range of wavelengths and this makes it much better at detecting weak signals. For these experiments, guinea-pigs were humanely killed with pentobarbitone and freshly dispersed bladder smooth muscle cells were loaded with Fluo-4 AM (10 μM). An inverted Nikon TE300 Microscope with × 40

and × 100 objectives was combined with a spinning Nipkow disk arrangement (Visitech, UK). Using the EMCCD camera, Ca^{2+} sparks could be clearly resolved above background noise at speeds up to 31 f.p.s. (512 × 512 pixels). However when subregions of the CCD were selected, it was possible to reliably measure sparks at rates of 60 f.p.s. (512 × 200 pixels; 83 μm × 32 μm) and 120 f.p.s. (512 × 100 pixels; 83 μm × 16 μm). Figure 1 shows typical recordings from a 2.7 μm region of interest placed over the centre of a spark site and recorded at (*A*) 60 f.p.s. and (*C*) 113 f.p.s.. As panels *B* and *D* suggest, the differences in rise time and decay of individual sparks could be clearly resolved and support the idea that EMCCD cameras are suitable for detecting spontaneous calcium release across a large proportion of a smooth muscle cell with both high spatial and temporal resolution.

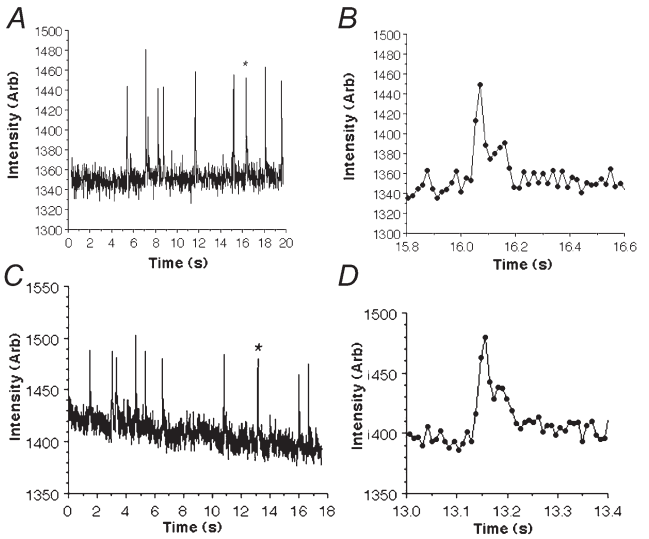

Figure 1. Sparks recorded from a guinea-pig bladder smooth muscle cell using an EMCCD at 60 f.p.s. (*A*) and 113 f.p.s. (*C*). *B* and *D* show expanded plots of sparks recorded (*) in *A* and *C*.

Coates CG *et al.* (2003). *SPIE Photonics West, Biomedical Optics* (in press).

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All procedures accord with current UK legislation

PS P61

Endothelial-mediated relaxations in human omental arteries: effects of 18-α glycyrrhetinic acid

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Endothelium-dependent relaxation of arteries is mediated by nitric oxide, prostacyclin and endothelium-dependent hyperpolarizing factor (EDHF). Direct cell-to-cell electrical and/or chemical coupling via gap junctions may be involved in the EDHF response (Edwards *et al.* 1999). The functional characteristics of gap junctions are dependent upon the expression patterns of integral connexin proteins. Therefore, in human resistance arteries, we have investigated the effects of a putative gap junctional uncoupler, 18-α glycerrhetinic acid (18-α GA), on EDHF-mediated relaxations together with assessing mRNA expression encoding several connexins.

Resistance vessels were dissected from omental biopsies taken, following written informed consent (Central Manchester Ethics Committee CEN/00/203), at elective Caesarean section from women with uncomplicated pregnancies. Vessels were mounted on a wire myograph, normalised in physiological salt solution (37 °C, bubbled with 95 % air–5 % CO_2) and constricted with U46619 (10^{-10}–10^{-6} M, $n = 11$). Upon a sustained constriction, incremental doses of bradykinin were added (10^{-9}–10^{-6} M). This was repeated in the presence of L-NNA (100 μM) and indomethacin (10 μM), to assess EDHF-dependent relaxation. The vessels were further incubated with 18-αGA (100 μM, 20 min) and responses of preconstricted vessels to bradykinin repeated.

Residual constriction to U46619 following the highest dose of bradykinin was 28 ± 4.0 % (mean ± S.E.M.) of control. After L-NNA/indomethacin incubation the residual constriction to U46619 was 47 ± 8.0 % ($P < 0.05$, Student's unpaired *t* test). This EDHF-attributable relaxation was further attenuated following incubation with 18-α GA: residual constrictions to U46619 were 73 ± 6.3 % of control. RNA was extracted from omental arteries ($n = 6$) and RT-PCR performed with gene-specific nucleotide primers to β-actin and connexins 43, 40 and 37. mRNA encoding connexins 43 and 40 was observed in 5 of 6 patient samples and connexin 37 in 4 of 6 samples.

Thus, in omental arteries from pregnant women pre-constricted with U46619, EDHF-mediated relaxation is significantly blunted by the gap junctional uncoupler 18 α GA. Additionally, mRNA encoding the common vascular connexins 43, 40 and 37 is often observed. These data support the concept that EDHF-mediated vasodilatation in human resistance vessels may, at least partly, be mediated via connexin-containing gap junctions.

Edwards G *et al.* (1999). *Br J Pharmacol* **128**,1788–1794.

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All procedures accord with current local guidelines and the Declaration of Helsinki

PS P62

Regional differences in contractility in pregnant rat uterus

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Preterm and difficult labours remain major health issues. With a better understanding of the mechanisms involved in phasic myometrial contractions, it may be possible to better control and alter uterine function and thus help to prevent such labour. Previous work has indicated several regional differences in uterine cellular characteristics (Smith *et al.* 1998) and thus the aim of this work was to determine if there were any regional differences in contraction during spontaneous activity and if agonist/antagonist application had a regional effect upon contraction.

Pregnant rats (18–21 days gestation) were killed humanely with CO_2 and myometrium dissected into three regions (cervical, middle and ovarian), and in some experiments loaded with Indo-1 for Ca^{2+} measurements. Force and frequency of spontaneous

and agonist-induced contractions were measured. Analysis was performed using Student's *t* test for unpaired data. Significance was taken as $P < 0.05$; n = number of animals.

The main finding of the study was that the ovarian end of the uterus contracted with a significantly greater frequency than the other two regions, though with less force ($n = 40$). The Ca^{2+} responses mirrored those of force ($n = 4$). In terms of responses to stimulation with oxytocin (100 nM, $n = 7$) and carbachol (100 μM, $n = 6$) there were no significant differences in responses, as was also the case with the agonist Bay K 8644 (2 μM, $n = 4$), and the antagonists nifedipine (500 nM, $n = 6$), an inhibitor of calcium entry, and caffeine (10 mM, $n = 6$), an inhibitor of SR calcium release. However, in the case of niflumic acid, a blocker of Ca^{2+}-activated Cl^- channels, it was found that contractility in the ovarian region was more sensitive than the other two regions in terms of decrease of contractility produced ($n = 6$).

From these data we conclude that there is little or no regional difference in term myometrium in its responses to stimulation and Ca^{2+} entry. There is evidence that the ovarian end may be involved in pacemaker activity as it contracts more frequently, and we suggest that Ca^{2+}-activated Cl^- channels may be involved in this process and be more abundant there. This part of the uterus has also been shown to be more spontaneously active in a study of rats in oestrous (Crane & Martin, 1991).

Crane LH & Martin L (1991). *Reprod Fertil Dev* **3**, 519–527.
Smith GC *et al.* (1998). *Am J Obstet Gynecol* **179**, 1545–1552.

All procedures accord with current UK legislation

PS P64

Effects of methyl-β-cyclodextrin on spontaneous and oxytocin-induced contractility of isolated human uterine smooth muscle

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Myometrial contractility involves the precise regulation of many signalling events to achieve efficient delivery of the fetus at term. Numerous signalling molecules have been suggested to cluster in cholesterol-rich plasmalemmal invaginations, termed caveolae. These include G-protein coupled-receptors and, in uterine myocytes, down-stream effectors such as rhoA and rho-associated kinase (ROK), where interaction with caveolar structural proteins, caveolins, may occur (Gimpl *et al.* 2000; Lee *et al.* 2001). Caveolae have also been implicated in the homeostasis of subcellular Ca^{2+} dynamics (Isshiki *et al.* 1998; Lohn *et al.* 2000). Thus, we studied the effects of a cholesterol-depleting agent, methyl-β-cyclodextrin (MβC), upon myometrial contractility.

Myometria were obtained, following written informed consent according to local ethics committee guidelines, from non-labouring term women undergoing elective Caesarean section (gestation 37–41 weeks). Small myometrial strips were dissected and mounted in standard organ baths in HCO_3^--buffered physiological salt solution (37 °C, 95 % air–5 % CO_2). The experiments were performed with parallel time controls.

Following stabilisation of spontaneous phasic contractions, a 5 min application of 10 nM oxytocin induced a 23 ± 5 % rise in peak phasic tone ($P < 0.05$, Wilcoxon's non-parametric test; mean ± S.E.M.; $n = 7$). Spontaneous contractions were allowed to recover. Subsequently, a 30 min incubation in 20 mM MβC resulted in an increased frequency of phasic contractile events, and a 42 ± 16 % increase in basal tone, with no change in peak phasic tone. After a 60–120 min incubation, phasic contractions were abolished and basal tone was 59 ± 31 % of the control. The ensuing application of 10 nM oxytocin, in the presence of 20 mM MβC, produced no further elevation of tone.

This study indicates that a cholesterol-depleting agent reduces spontaneous and oxytocin-induced contractions in human myometrium, with an increase in basal tone. Further studies are required to determine the role of caveolae in these alterations.

Gimpl G *et al.* (2000). *Exp Physiol* **85**, 41S–49S.
Isshiki M *et al.* (1998). *Proc Natl Acad Sci U S A* **95**, 5009–5014.
Lee Y-H *et al.* (2001). *Exp Physiol* **86**, 283–288.
Lohn M *et al.* (2000). *Circ Res* **87**, 1034–1039.

All procedures accord with current local guidelines and the Declaration of Helsinki

PS P65

Alterations in the expression profile of Na^+,K^+-ATPase isoforms during the progression of pregnancy in the rat uterus

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The activity of the sodium pump depends on the catalytic properties of its subunit constituents. The Na^+,K^+-ATPase consists of three types of subunit, α, β and γ, each of which is expressed as multiple isoforms (4 α, 4 β and at least 2 γ). We have previously shown that the α1 and 2 isoforms are expressed in uterine smooth muscle of the rat (Floyd *et al.* 2003). Contraction of uterine smooth muscle is dependent on the electrogenic action of the Na^+,K^+-ATPase, which creates cation gradients across the cell membrane to facilitate propagation of contraction and return of the membrane to resting potential. Previous molecular studies identified changes in the expression of α subunit mRNAs throughout pregnancy but little is known of the specific changes in protein expression during this period. Furthermore, there are no published reports of Na^+,K^+-ATPase isoform expression and cellular localisation in the uterus. The aim of this study was to determine whether there are any changes in levels of expression of Na^+,K^+-ATPase isoforms throughout pregnancy in the rat and to document any alterations in isoform protein expression.

Western blots were used to study the levels of α1, α2, α3 and β1, β2, β3 isoforms in tissues from non-pregnant and pregnant rats at days 10, 16 and 21 of pregnancy. We also studied the regional distribution of all isoforms in non-pregnant and term-pregnant (day 21) rats.

Non-pregnant female Wistar rats and animals at days 10, 16 and 21 of pregnancy were humanely killed by cervical dislocation under CO_2 anaesthesia. Three uterine horns per group were snap frozen in liquid nitrogen for Western blot analysis. Uterine horns from non-pregnant and 21-day pregnant rats were fixed in neutral buffered formalin before paraffin embedding and sectioning (6 μm thickness). Tissue sections were subjected to antigen retrieval in the presence of sodium dodecyl sulphate. Immunohistochemical analysis was performed after non-specific binding was blocked with 10 % normal goat serum (NGS). Total protein was extracted from snap frozen samples and run on SDS-PAGE gels. Western blotting was performed using isoform specific antibodies. Rat brain and kidney homogenates were used

as positive controls for α2/α3 and α1 isoform expression respectively.

Immunohistochemistry using a pan a antibody demonstrated high Na^+,K^+-ATPase expression in the outer longitudinal and inner circular smooth muscle layers and the epithelial lining of the endometrium. Western blot results confirmed that the major α isoform expressed in rat uterus was α1. However, lower levels of the α2 isoform were also present. The α3 isoform was not detectable by Western blotting. The expression level of the α2 isoform appeared to diminish with progression of pregnancy whereas the α1 isoform levels were unaffected.

These results suggest that there may be a shift in the Na^+,K^+-ATPase isoforms during pregnancy which may affect intracellular $[Na^+]_i$ and hence $[Ca^{2+}]_i$. This may explain the increase in uterine smooth muscle contraction during the latter stages of pregnancy.

Floyd R et al. (2003). *Ann N Y Acad Sci* **986**, 614-616.

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All procedures accord with current UK legislation