O10

Capacitative calcium entry in neonatal rat myometrium

Karen Noble and Susan Wray

The physiological laboratories, University of Liverpool, Liverpool L69 3BX, UK

Depletion of SR Ca^{2+} stores in many cell types activates Ca^{2+} permeable store-operated channels (SOCCs) on the plasma membrane, which replete the empty stores through a process known as capacitative calcium entry (CCE). This pathway may be present in smooth muscle cells (Gibson *et al.* 1998) and may regulate contraction, as well as refill the SR. We have recently shown that emptying the SR using the Ca^{2+}-ATPase inhibitor cyclopiazonic acid (CPA) produces a large increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) and force (Noble & Wray, 2002). The aim of the present study is therefore to investigate the mechanism whereby depleting SR Ca^{2+} produces increased $[Ca^{2+}]_i$ and force.

Simultaneous measurements of $[Ca^{2+}]_i$ and force were made on strips of uterus taken from 10-day-old neonatal rats, after humane killing. The uterus was spontaneously active at 37°C and data were normalised to spontaneous Ca^{2+} and force at the start of each experiment. Results are expressed as means ± S.E.M.; significance was tested using the appropriate Student's unpaired *t* test with significance taken at $P < 0.05$; *n* is number of animals. Experiments were first carried out in the presence (controls) and absence of extracellular Ca^{2+}.

In zero Ca^{2+} solutions the CPA-induced rise in basal Ca^{2+} was of significantly decreased amplitude (69 ± 11 % *vs.*175 ± 4 %) and duration than controls (3.8 ± 0.3 *vs.* 17 ± 3 min; $n = 4$). To further investigate the mechanism of CPA-induced external Ca^{2+} entry paired experiments were undertaken in the presence of (a) 0.1 μM nifedipine, a selective blocker of L-type voltage-gated Ca^{2+} channels (VOCC) or (b) 0.1 μM nifedipine and 50 μM SKF96365, which blocks SOCC and VOCC. In the presence of nifedipine, spontaneous contractions ceased but the CPA-induced rise in basal Ca^{2+} (220 ± 64 %) and force (302 ± 66 %; $n = 5$) were similar to controls. SKF96365 when added to the nifedipine solution significantly decreased the CPA-induced rise in basal Ca^{2+} to 38 ± 4 %; $n = 5$ of values obtained in nifedipine alone and, most strikingly, in all experiments SKF96365 abolished the CPA-induced force.

We have recently shown in neonatal uterus that carbachol both empties the SR Ca^{2+} store and induces a large prolonged Ca^{2+} transient, which is largely insensitive to nifedipine (Noble & Wray, 2002). These data suggests support the hypothesis that in neonatal rat uterus, which has a large functioning SR Ca^{2+} store, CCE may be the major pathway for external Ca^{2+} entry when SR Ca^{2+} is depleted.

Gibson A *et al.* (1998). *Trends Pharmacol Sci* **19**, 266–69.

Noble K & Wray S (2002). *J Physiol* (in the Press).

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All procedures accord with current UK legislation.

O11

Voltage-gated $InsP_3$-dependent Ca^{2+} release from internal stores in rat vascular smooth muscle cells

Alberto del Valle, José López-Barneo and Juan Ureña

Laboratorio de Investigaciones Biomédicas. Departamento de Fisiología y Hospital Virgen del Rocío, Universidad de Sevilla, Sevilla, Spain

Contractility of vascular smooth muscle cells (VSMCs) depends on a rise of cytosolic $[Ca^{2+}]$ resulting from two major processes: (i) Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCCs) of the plasma membrane or (ii) Ca^{2+} release from the sarcoplasmic reticulum (SR). Although it has been suggested that agonist-induced Ca^{2+} release from internal stores can be modulated by membrane voltage (e.g. Ganitkevich & Isenberg, 1993), the underlying mechanisms are unknown. We have investigated whether depolarization can induce Ca^{2+} release from internal stores in myocytes freshly dispersed from rat basilar artery using patch-clamp and microfluorimetry.

Animals were killed by I.P. injection of pentobarbital (50 mg kg^{-1}). Activation of VDCCs by either direct membrane depolarization in voltage-clamped myocytes or application of solutions with 70 mM K^+ in undialysed cells evoked sharp, transient $[Ca^{2+}]_i$ release signals in the absence of extracellular Ca^{2+} plus 2 mM EGTA. This response was suppressed after inhibition of the Ca^{2+}-ATPase by thapsigargin, although it was present when actin cytoskeleton desorganization was induced with calyculin A and jasplakinolide (Patterson *et al.* 1999). Inhibition of G protein, phospholipase C and $InsP_3$ receptor with GDPβS, U73122 and 2-amino-ethoxydiphenyl borate, respectively, abolished the increase in $[Ca^{2+}]_i$. These results suggested that depolarization-induced Ca^{2+} release (DICR) operates via G protein activation and $InsP_3$ production. In addition, several lines of evidence indicated that activation of G protein by membrane depolarization depends on VDCCs. (i) The DICR *vs.* voltage relationship was similar to the conductance–voltage curve for L-type VDCCs; (ii) Ca^{2+} release was drastically reduced when instead of applying depolarizing voltage steps, the membrane potential was changed slowly, leading to inactivation of the channels; and (iii) DICR was potentiated by agonists (Bay K 8644 and FPL 64176) and inhibited by antagonists (diltiazem) of VDCCs. These unprecedented observations suggest a novel role of VDCCs inVSMCs. Besides mediating transmembrane Ca^{2+} influx, Ca^{2+} channels act as voltage sensors that on cell depolarization induce $InsP_3$ production and Ca^{2+} release from SR to further increase cytosolic $[Ca^{2+}]$.

Ganitkevich VYa & Isenberg G (1993). *J Physiol* **470**, 35–44.

Patterson RL *et al.* (1999). *Cell* **98**, 487–499.

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All procedures accord with current National and local guidelines.

O12

Characterization of calcium-activated chloride currents in rat and human uterine smooth muscle

Karen Jones, Tony Shmygol, Sajeera Kupittayanant and Susan Wray

Physiology Department, University of Liverpool, Liverpool L69 3BX, UK

In myometrium control of electrical activity is essential for a successful pregnancy and parturition. Through understanding the mechanisms behind spontaneous activity we may better understand problems occurring in labour. Ca^{2+}-activated Cl^- currents ($I_{Cl\text{-}Ca}$) occur in smooth muscles where they play a role in electrical activity and spontaneous activity. These currents have been observed in rat myometrium (Arnaudeau, 1994) although they are not well characterized. The aim of this work is to characterize these currents in rat myometrium to investigate their functional significance and to correlate the results to human data.

Pregnant rats (18–21 days gestation) were killed humanely and longitudinal myometrium strips dissected. Human biopsies of non-labouring myometrium was obtained from elective caesareans at term, with local ethics committee approval and written informed consent. Strips were either loaded with the calcium-sensitive indicator Indo-1 and used to measure force/calcium or single cells produced via enzyme digestion. Whole-cell membrane currents were recorded using the patch clamp technique. The results are expressed as means ± S.E.M. where appropriate; significance was tested using the appropriate Student's paired t test with significance taken at $P < 0.05$, n = number of experiments.

Depolarisation of single cells produced an initial inward Ca^{2+} current (I_{Ca}). Chloride current ($I_{Cl\text{-}Ca}$) was observed upon repolarisation. In the isolated rat cells I_{Ca} was manipulated using Ba^{2+}, which is a suitable charge carrier but is unable to activate calcium activated currents. Ba^{2+} abolished $I_{Cl\text{-}Ca}$ ($n = 4$), despite producing an initial inward current. Bay K8644 (2 μM), an agonist for I_{Ca} increased I_{Ca} and $I_{Cl\text{-}Ca}$ proportionally (2.2 ± 0.2-fold and 1.8 ± 0.1-fold, respectively, $P < 0.05$; $n = 13$). The $I_{Cl\text{-}Ca}$ reversal potential was altered using chloride substitution; with almost equal [Cl^-] inside the pipette and outside, the reversal potential was −3 ± 1.5 mV ($n = 3$); reducing extracellular [Cl^-] by 75 mM through replacement with glutamate gave a reversal potential of 16.7 ± 4.4 mV ($n = 3$). These values are consistent with calculated values. Niflumic acid (10 μM) was used to inhibit the chloride channel in single cells resulting in a loss of tail current ($n = 4$). In rat tissue strips, which were spontaneously active, application of niflumic acid resulted in a decreased frequency or abolition of the Ca^{2+} transients and contractions ($n = 5$ and $n = 4$ respectively). Experiments are being performed on human cells to compare the data obtained to that from rats.

Calcium activated chloride channels are present in rat myometrium and do have functional significance.

Arnaudeau S *et al.* (1994). *Am J Obstet Gynecol* **171**, 491–501.

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All procedures accord with current UK legislation.

O13

Role of PKC in control of force–Ca^{2+} relationship in phasic ureteric smooth muscle

S. Shabir and T. Burdyga

Physiological Laboratories, University of Liverpool, Crown Street, Liverpool L69 3BX, UK

Many agonists work through a PKC pathway, and so its modulation on the force–Ca^{2+} relationship in phasic ureter smooth muscle has been investigated.

Female Wistar rats and Duncan Hartley guinea-pigs were anaesthetised using CO_2, and killed by cervical dislocation. The ureters were loaded with indo-1-AM, and force and calcium measured. Data are given as means ± S.E.M., and n is the number of animals.

In the guinea-pig ureter both carbachol ($n = 3$) and a PKC activator (0.1 μM PdBu, $n = 4$) increased the amplitude of the phasic contractions evoked by electrical stimulations (carbachol, 60 ± 7 %; PdBu, 70 ± 29 %). This was associated mainly with an increase in the duration of the Ca^{2+} transient (carbachol, 22 % ± 2 %; PdBu, 38 ± 6 %). The selective inhibitor of PKC, 5 μM GF109203X reversed the stimulant action of PdBu, and partially reduced the stimulatory action of carbachol. The effects of carbachol and PdBu on the amplitude of the phasic contractions and duration of the calcium transient were not additive.

In rat ureter, carbachol ($n = 3$) and PdBu ($n = 3$) also increased the amplitude of phasic contractions (24 ± 8 % and 31 ± 5 %, respectively) but also markedly slowed the relaxation of the phasic contractions. The calcium transients however were not significantly affected by either agent suggesting force is being mainly affected by a change in sensitivity of the contractile machinery to calcium. The effects of PdBu on the amplitude and relaxation rate were completely reversed by the PKC inhibitor GF109203X, while that of carbachol was only partially reversed.

These data suggest that in ureter the effects of agonists on phasic contractions are species dependent. In the guinea-pig ureter agonists increase the amplitude of force, mainly by increasing the duration of the Ca^{2+} transient. We now show that part of this effect could be mediated by activation of PKC, which can increase the duration of the calcium transient via a prolongation of the action potential (Burdyga & Wray, 1999). In the rat ureter, agonists stimulate force mainly via sensitisation of the contractile machinery to Ca by activation of PKC and Rho/ROK pathways, as shown previously (Shabir & Burdyga, 2002).

Burdyga TV & Wray S (1999). *J Physiol* **520**, 867–883.

Shabir S & Burdyga T, (2002). *Pflügers Archiv* **443**, S157–S393 (abstract).

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All procedures accord with current UK legislation.

O14

Rapid spasmolytic effect of oestrogens on mouse duodenal muscle

C. Ramírez*, T. Gómez*, B. García†, R. Alonso‡ and M. Díaz*

*Departments of *Animal Biology and ‡Physiology, University of La Laguna, Tenerife and †Institute of Natural Products and Agrobiology, CSIC, Tenerife, Spain*

Recent evidence has suggested that gonadal steroid hormones have specific and rapid effects by acting at the plasma membrane of different tissues, including smooth muscle from blood vessels and reproductive organs (Valverde *et al.* 1999; Nadal *et al.* 2001). Until recently, the intestinal muscle has not been considered as a target for these rapid actions of oestrogen or antioestrogens, in spite of increasing evidence pointing to a direct effect of these compounds on crucial proteins involved in excitation–contraction coupling (Díaz, 2002). In the present study we have analysed the effects of different oestrogens and steroidal antioestrogens on the contractile activity of mouse duodenal muscle. Assays included spontaneous peristalsis, basal tone, response to calcium and KCl-induced depolarisation. 17β-Oestradiol causes a dose-dependent relaxation of spontaneous contractile activity, reduces basal tone and depresses the responses to $CaCl_2$ and KCl. Application of either 17β-E2 or the inactive estereoisomer 17α-E2, but not the steroidal antioestrogen ICI182, 780 (10 μM), relaxed duodenal muscle and abolished spontaneous activity within min after exposure. Dose–response analyses showed that 17β-E2 was more potent than 17α-E2, relaxing duodenal muscle and reducing $CaCl_2$- and KCl-induced contraction. The effects of oestrogens could be partially reversed by addition of Bay K 8644 (1 μM) to the bath, which suggests the involvement of L-type Ca^{2+} channels in the response to oestrogen. In addition, 17β-E2-relaxed tissues could be contracted by depolarisation with KCl (33 mM), TEA (5 mM) and charybdotoxin (38 nM), but not glibenclamide (10–50 μM), thereby indicating that oestrogen had also activated calcium-dependent K^+ channels (Maxi-K^+). The relaxing effects of oestradiol were mimicked by addition of 8-bromo-cyclicGMP (8-Br-cGMP, 100 μM) or forskolin (FSK, 20 μM). However, while the effect of 8-Br-cGMP could be reversed by KCl or TEA, the effect of FSK was not. The relaxing effects of 17β-E2 were not affected by preincubation of duodenal muscle with L-NAME (100–300 μM) nor KT5823 (0.3–1 μM), ruling out the possible participation of protein kinase G (PKG) in the final step of oestrogen-induced relaxation. We hypothesise that oestrogen rapidly activates Maxi-K^+ channels and inhibits L-type calcium channels to relax duodenal muscle.

Díaz M, (2002). *Eur J Pharm* **445**, 257–266.

Nadal A *et al.* (2001). *NIPS* **16**, 251–255.

Valverde *et al.* (1999). *Science* **285**, 1929–1931.

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All procedures accord with current National and local guidelines.

O15

Relative contributions of calcium influx and calcium stores to myogenic tone in the rat urethra

M. Shafei, K.D. Thornbury, N.G. McHale and M.A. Hollywood

Smooth Muscle Group, Department of Physiology, Queen's University, 97 Lisburn Road, Belfast, Northern Ireland, UK

The urethra is capable of generating tone which is at least partly myogenic in nature. In the rabbit (Sergeant *et al.* 2000) and human urethra (Hollywood *et al.* 2002) we have characterised a number of currents that could play an important role in generating urethral tone. The purpose of the present study was to assess the importance of calcium influx and calcium stores in the generation and modulation of myogenic tone in the rat urethra using a novel isolated urethra preparation.

Female Sprague-Dawley rats were anaesthetised by CO_2 inhalation and killed by cervical dislocation in accordance with Home Office guidelines. The urethra was cannulated at the bladder neck and placed in a horizontal organ bath (perfused with oxygenated Krebs solution at 37°C) and connected to a constant-pressure reservoir. Flow was estimated by measuring side-arm pressure near the inflow cannula. Resting flow through the urethra under control conditions was 1.7 ± 0.14 ml min^{-1} (mean ± S.E.M., $n = 23$) and was increased to 5.0 ± 0.2 ml min^{-1} by electrical field stimulation (0.5 Hz, 0.3 ms pulse width, 50 V) consistent with a reduction in urethral tone.

To assess the contribution of calcium influx through L- and T-type channels to tone generation, the effects of both nifedipine and Ni^{2+} were examined on flow. In the presence of 10 μM nifedipine, resting flow was increased from 1.8 ± 0.2 to 2.5 ± 0.3 ml min^{-1} ($P < 0.01$, paired t test, $n = 7$). When T-type calcium channels were blocked by 300 μM Ni^{2+} (in the presence of nifedipine) resting flow was further increased to 3.4 ± 0.5 ml min^{-1} ($P < 0.01$, $n = 6$).

We next examined the effects of CPA and 2APB on flow to assess the contribution of calcium stores to myogenic tone. Inhibition of the calcium-ATPase with CPA (30 μM) significantly increased flow from 1.7 ± 0.5 to 5.1 ± 1.0 ml min^{-1} ($P < 0.01$, $n = 5$). Prior to application of CPA, nerve stimulation (0.5 Hz, 1 min) increased flow to 5.2 ± 0.3 ml min^{-1}. However, in the presence of CPA nerve stimulation decreased urethral flow dramatically from 5.1 ± 1.0 to 0.3 ± 0.2 ml min^{-1}. Inhibition of calcium release from IP_3 dependent stores by 2APB (100 μM) increased mean flow from 1.2 ± 0.2 to 3.9 ± 0.2 ml min^{-1} ($P < 0.001$, $n = 6$) and nerve stimulation in the presence of 2APB further increased flow to 5.2 ± 0.2 ml min^{-1}.

These data support the idea that urethral tone in the rat is dependent on the influx of calcium through T and L channels and the release of calcium from intracellular stores.

Hollywood MA *et al.* (2002). *J Physiol* **543.P**, 76*P*.

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

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All procedures accord with current UK legislation.

O16

Spontaneous calcium transients in isolated interstitial cells from the rabbit urethra

M.A. Hollywood, L. Johnston, N.G. McHale and K.D. Thornbury

Smooth Muscle Group, Department of Physiology, Queen's University, 97 Lisburn Road, Belfast, Northern Ireland, UK

Recently we have demonstrated that a specialised population of cells in the urethra (Sergeant *et al.* 2000) share characteristics typical of interstitial cells described in the gastrointestinal tract. These urethral interstitial cells are non-contractile, possess abundant calcium-activated chloride current and show regular spontaneous transient inward currents when recorded under voltage clamp. The purpose of the present study was to use confocal microscopy to examine the mechanisms underlying this spontaneous activity in freshly dispersed interstitial cells from the rabbit urethra.

Rabbits were humanely killed with pentobarbitone (I.V.) and their urethras removed. Cells were isolated as previously described (Sergeant *et al.* 2000) and incubated for 15 min at 37 °C with the fluorescent Ca^{2+}-sensitive indicator Fluo-4AM (10 μM). Cells were then plated onto glass-bottomed petri dishes for 30 min before being perfused with Hanks' solution. Single cell imaging was performed at 5 frames s^{-1} using a Nipkow spinning disc laser confocal microscope. When cells were maintained at 37 °C either calcium sparks, or more frequently, calcium waves were apparent and usually initiated in the perinuclear region. Wave amplitudes were calculated as a ratio (F/F_0) by dividing the mean intensity of the region of interest (ROI) during the peak of the wave by the mean intensity during quiescent periods. The waves occurred at a mean frequency of 3.1 ± 0.2 min^{-1}, had peak amplitudes of 1.8 ± 0.21 (F/F_0) and a mean ± S.E.M. duration of 14.3 ± 1.6 s ($n = 6$ cells). To investigate the contribution of calcium release from intracellular stores we examined the effects of ryanodine, tetracaine, caffeine and 2-aminoethoxydiphenylborane (2APB) on the calcium waves. Application of either ryanodine (30 μM) or tetracaine (100 μM, $n = 3$) abolished spontaneous activity. In addition, basal fluorescence was reduced to 0.93 ± 0.02 (F/F_0) in the presence of tetracaine. Application of caffeine (10 mM), transiently increased F/F_0 to 1.97 ± 0.2 and reversibly abolished spontaneous waves ($n = 5$). When release of calcium from IP_3-sensitive stores was inhibited by 2APB (100 μM), basal fluorescence increased and the amplitude of the waves gradually diminished but frequency was not reduced ($n = 3$). Similar effects were found with 30 μM 2APB ($n = 3$).

When external calcium was removed from the bathing solution (Mg^{2+} substituted Hanks', 5 mM EGTA), F/F_0 decreased to 0.87 ± 0.04 and spontaneous activity was immediately abolished ($n = 5$). To test if the inhibition of spontaneous activity was due to depletion of the intracellular stores, caffeine was reapplied in the absence of external calcium. In four experiments, caffeine application produced a transient increase in F/F_0 to 2.0 ± 0.3 in normal Hanks' solution. When caffeine was reapplied approximately 1 min after removal of external calcium, spontaneous activity was abolished but F/F_0 increased to 1.8 ± 0.3, suggested that the caffeine-sensitive stores were not significantly depleted.

The abolition of calcium waves in the absence of external calcium suggested that the influx of calcium is an essential step for initiating spontaneous calcium waves in urethral interstitial cells. To test if this influx was through L-type calcium channels we examined the effects of 10 μM nifedipine. In three cells, nifedipine failed to alter the amplitude or frequency of calcium waves.

These data suggest that spontaneous calcium waves in rabbit urethral interstitial cells are dependent on the release of calcium from intracellular stores which is triggered by calcium influx through a nifedipine-insensitive pathway.

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

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All procedures accord with current UK legislation.

O17

Evidence for a calcium-activated chloride current in freshly dispersed cells from rabbit corpus cavernosum

M. Craven, M.A. Hollywood, N.G. McHale and K.D. Thornbury

Smooth Muscle Group, Department of Physiology, Queen's University, 97 Lisburn Road, Belfast, BT9 7BL, N. Ireland, UK

The object of this study was to characterise membrane currents in corpus cavernosum cells.

Male rabbits were 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. Cells were held at −60 mV and stepped to potentials of −80 mV to +50 mV.

Transient inward current was observed at the beginning of the test steps, typical of L-type calcium current. There was also a slowly developing current that was inward at negative potentials, reaching a maximum of -127 ± 19 pA (mean ± S.E.M., $n = 19$) at −20 mV. This current reversed between −10 and 0 mV, which was close to the chloride equilibrium potential of 0 mV, and became a large outward current at positive potentials (911 ± 85 pA at +50 mV, $n = 19$). In eight cells the chloride channel antagonist anthracene-9-carboxylic acid (9AC; 1 mM) reduced the slow inward current at −20 mV from -180 ± 57 to -41 ± 12 pA ($P < 0.02$, Student's paired t test) and reduced the outward current at +50 mV from 871 ± 151 to 227 ± 41 pA ($P < 0.01$). Similarly, niflumic acid (100 μM) reduced the maximal inward and outward currents from -161 ± 44 to -86 ± 64 pA ($P < 0.05$, $n = 6$) and from 1141 ± 45 to 500 ± 82 pA ($P < 0.01$, $n = 6$), respectively. The slow inward current at −20 mV was also greatly reduced by nifedipine (10 μM) from -254 ± 98 to -19 ± 12 pA ($P < 0.05$, $n = 4$), as was the outward current at +50 mV from 1364 ± 354 to 135 ± 27 pA ($P < 0.05$, $n = 4$). These results suggest that the slow current was a Ca^{2+}-activated Cl^- current, similar to those previously described in the urethra (Cotton *et al.* 1997; Sergeant *et al.* 2000). More than 80 % of the corpus cells also generated spontaneous transient inward current (STICs) when held constant at −60 mV. The STICs were reduced in amplitude by 1 mM 9AC (from 146 ± 73 to 28 ± 15 pA, $P < 0.01$, $n = 5$) and by 100 μM niflumic acid (from 215 ± 85 to 108 ± 38 pA, $P < 0.05$, $n = 5$). The reversal potential of the STICs was measured by applying 200 ms ramp potentials from −50 to +50 mV. With 135 mM Cl^- in the pipette and bath, the reversal potential was -1 ± 1 mV. This shifted to 20 ± 1 mV when external Cl^- was reduced to 49 mM by replacement with glutamate (giving a calculated Cl^- equilibrium potential of +27 mV) and to -19 ± 4 mV when all of the external Cl^- was replaced with I^-, suggesting that STICs were mediated by Cl^- channels.

In conclusion, we have identified a calcium-activated chloride current in corpus cavernosum cells. Since it is likely to have a depolarizing effect under physiological conditions, we speculate that this current causes tone in these cells and therefore contributes to the detumescent state.

Cotton KD *et al.* (1997). *J Physiol* **505**, 121–131.
Sergeant GP *et al.* (2000). *J Physiol* **526**, 359–366.

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All procedures accord with current UK legislation.

O19

Comparison of the effects of hypoxia and acidification on human myometrium: a common mechanism of action?

S.J. Pierce, E. Monir-Bishty and Susan Wray

Physiological Laboratory, University of Liverpool, Crown Street, Liverpool L69 3BX, UK

Inefficient uterine activity leads to prolongation of labour and causes increased morbidity and mortality to both mother and child. During each contraction the reduction in blood flow produces localised hypoxia and stimulation of anaerobic metabolism, resulting in alteration of metabolites such as ATP and P_i and a decrease in pH. Hypoxia and acidification reduce the spontaneous contractile activity of rat myometrium but there is only very limited data on their effects on human myometrium, e.g. changes in $[Ca^{2+}]_i$, the main determinant of uterine force, are unknown. We have therefore investigated the effects of both hypoxia and acidification on contractions and calcium in human myometrium.

Human, non-labouring myometrium, obtained with ethical approval and informed consent, from elective cesarean sections at term, was dissected into strips and loaded with indo-1. Hypoxic conditions were simulated with cyanide (2 mM) and changes in pH_i produced by iso-osmotic substitution (40 mM) of NaCl with weak acid, sodium butyrate. Statistical analysis was performed on paired data using Student's *t* test (95 % confidence). Values represent means ± S.E.M., and *n* is the number of samples.

Spontaneous intracellular calcium transients and contractions were significantly reduced and ultimately abolished, by both hypoxia ($n = 7$) and acidification ($n = 4$), although both conditions caused basal calcium to rise significantly (hypoxia, 133 % ± 11; acidification, 112 % ± 1).

Oxytocin was used to assess whether agonist-induced contractions are more resistant to the effects of acidification ($n = 6$) or hypoxia ($n = 8$); the data were similar to those found during spontaneous activity, i.e. force and Ca^{2+} transients were abolished. Basal Ca^{2+} was increased significantly in all cases (hypoxia, 187 ± 29 %; acidification, 108.7 ± 0.67 % compared to control (100 %)).

Clinically, oxytocin is used to augment labour, so we investigated whether the application of oxytocin to hypoxic or acidic myometrium could restore tone and $[Ca^{2+}]_i$. No significant effect was seen with hypoxia or acidification although 1/7 under acidic conditions showed some recovery.

Both hypoxia and acidification are found to be potent inhibitors of human myometrial contraction. Although experimentally acidification was faster acting, both were very similar in their effects. The reduction in the Ca^{2+} transient also suggests a shared mechanism of action, i.e. inhibition of L-type Ca^{2+} entry. The basal calcium rise may be due to release from intracellular organelles. Oxytocin is unable to augment contractions in tissues with underlying hypoxaemia or acidosis. Possibly, the anaerobic metabolism occurring during prolonged labours, underlies the clinical finding that oxytocin is not always efficacious in slow labours.

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

P58

Relaxation induced by milrinone in human coronary artery bypass conduits

P. Medina, G. Segarra, M.D. Mauricio, B. Torondel, F. Prieto and J.M. Vila

Departamento de Fisiología, Facultad de Medicina y Odontología, Universidad de Valencia, Avda. Blasco Ibanez 17, 46010 Valencia, Spain

We studied the effects of milrinone, an inhibitor of type 3 phosphodiesterase on vascular segments of coronary artery bypass grafts (approved by Ethics Committee of our Institution). Segments of internal thoracic artery and saphenous vein were taken from 12 patients undergoing coronary artery bypass surgery and segments of left coronary and radial arteries were obtained from five multiorgan donors. Vascular rings (3 mm in length) were suspended in organ bath chambers and isometric tension was recorded at 37°C. Milrinone (10^{-9}–10^{-5} M) caused concentration-dependent relaxation in precontracted vascular rings that was of greater magnitude in saphenous vein (median effective concentration, $EC_{50} = 1.8 \times 10^{-8}$ M) and radial artery ($EC_{50} = 5.7 \times 10^{-8}$ M) than in coronary ($EC_{50} = 2.1 \times 10^{-7}$ M) and internal thoracic ($EC_{50} = 3.0 \times 10^{-7}$ M) arteries. Compared with other vasodilators, the order of potencies in terms of EC_{50} was sodium nitroprusside ⩽ milrinone ⩽ papaverine. The results indicate that milrinone causes dilatation of human vascular segments used in the coronary bypass surgery and in human coronary artery. The data suggest the possible use of milrinone in the treatment of hypoperfusion caused by graft spasm.

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

P59

Effects of type 5 phosphodiesterase inhibitors on human penile dorsal and cavernous artery

B. Torondel, M. Peiró, F. Prieto, M. Guaita, M.D. Mauricio and J.M. Vila

Departamento de Fisiología, Facultad de Medicina y Odontología, Universidad de Valencia, Avda. Blasco Ibanez 17, 46010 Valencia, Spain

This study was designed to investigate the effects of sildenafil and zaprinast, two inhibitors of type 5 cyclic guanosine monophosphate phosphodiesterase (PDE5), on human penile dorsal and cavernous arteries (approved by Ethics Committee of our Institution). Artery rings obtained from eighteen multiorgan donors were suspended in organ bath chambers for isometric recording of tension at 37°C. Sildenafil (10^{-9}–3×10^{-5} M) caused concentration-dependent relaxation in precontracted arteries that was of greater magnitude in dorsal (median effective concentration, $EC_{50} = 4.7 \times 10^{-8}$ M) than in cavernous artery ($EC_{50} = 2.3 \times 10^{-7}$ M). Compared with sildenafil, zaprinast was

12 times less potent ($EC_{50} = 3.8 \times 10^{-7}$ M) in the dorsal artery and seven times ($EC_{50} = 3.5 \times 10^{-6}$ M) in the cavernous artery. Sildenafil (10^{-7} M) and zaprinast (10^{-6} M) potentiated the relaxation induced by sodium nitroprusside (10^{-10}–10^{-5} M) in the dorsal artery. In the cavernous artery the potentiating effect was small. However, the response to sodium nitroprusside was greater in the cavernous artery ($EC_{50} = 6.1 \times 10^{-9}$ M) than in the dorsal artery ($EC_{50} = 6.0 \times 10^{-8}$ M). The results demonstrate that PDE5 inhibitors sildenafil and zaprinast have a modest relaxant effect on human cavernous artery, probably due to a low PDE5 activity in this artery.

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

P60

Characterization of intracellular Ca^{2+} stores in gallbladder smooth muscle

S. Morales, P.J. Camello, G.M. Mawe* and M.J. Pozo

*Dept of Physiology, University of Extremadura, Cáceres, Spain and *Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT, USA*

The existence of functionally distinct intracellular Ca^{2+} stores has been proposed in some types of smooth muscle. Ca^{2+} release from these stores can induce both contraction and relaxation or regulate non-contractile events such as gene expression. To date, characterization of intracellular Ca^{2+} stores in the gallbladder smooth muscle is lacking. In this study, we sought to examine Ca^{2+} stores in the gallbladder by measuring $[Ca^{2+}]_i$ in fura-2 loaded isolated myocytes, and by recording membrane potential and isometric contractions in whole mounted preparations.

Tissue was obtained from adult guinea pigs killed by rapid cervical dislocation. Application of caffeine (10 mM) to single myocytes induced a rapid, spike-like elevation in $[Ca^{2+}]_i$ that reached a peak within 6–9 s after the onset of elevation and had a mean amplitude of 0.60 ± 0.09 (expressed as a ratio of the fluorescence at 380 and 340 nm, F_{80}/F_{340}, $n = 10$). When cholecystokinin (CCK, 10 nM) was used to induce IP_3-dependent Ca^{2+} release, the mean amplitude of the peak was 0.22 ± 0.03 ($n = 14$). These data support the idea that ryanodine receptors (RyRs) could release a substantial amount of Ca^{2+} in these cells. When caffeine was applied in Ca^{2+}-free solution, the increases in $[Ca^{2+}]_i$ were smaller, indicating a possible leakage of Ca^{2+} in these stores. The refilling of caffeine-sensitive stores involved SERCA activation, as it was inhibited in the presence of 1 μM thapsigargin. Similarly, IP_3-sensitive stores had a leak of Ca^{2+} and were depleted by thapsigargin. The moderate Ca^{2+} elevation caused by CCK induced a contraction of 13.1 ± 1.3 mN ($n = 8$), but caffeine-induced Ca^{2+} elevation was unable to induce gallbladder contraction. Thus, addition of caffeine (100 μM–10 mM) to the organ bath did not alter resting tone. Similarly, no changes were recorded in response to ryanodine application. Nevertheless, caffeine caused a concentration-dependent relaxation in gallbladder strips precontracted with 10 nM CCK or 60 mM KCl, which could be related to the hyperpolarization (6.6 ± 0.9 mV, mean ± S.E.M., $n = 5$) that this drug caused in gallbladder smooth muscle or to the inhibition of IP_3 receptors. The inability of caffeine to trigger contraction could be related to accumulation of c-AMP as the result of phosphodiesterase (PDE) inhibition. However, in the presence of IBMX, a general inhibitor of PDE, the relaxing effects of caffeine persisted.

All together our results suggest that there is no evidence for multiple, pharmacologically distinct Ca^{2+} pools but the location of IP_3 and ryanodine receptors must be different, with the latter facing the subsarcolemmal space.

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All procedures accord with current National guidelines.

P61

TASK-1 like properties of the resting K^+ conductance in rabbit pulmonary artery myocytes

A.M. Gurney*, O.N. Osipenko†, K.M. McFarlane† and F.E.J. Kempsill‡

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G4 0NR, †Quintiles Scotland Ltd, Heriot-Watt University Research Park, Edinburgh EH14 4AP and ‡Inverclyde Royal Hospital, Greenock PA16 OXN, UK

The resting potential of pulmonary artery myocytes depends upon a non-inactivating K^+ conductance (I_{KN}) with low sensitivity to block by 4-aminopyridine and quinine and insensitivity to tetraethylammonium ions (Evans *et al.* 1996). Both acute (Osipenko *et al.* 1997) and chronic (Osipenko *et al.* 1998) hypoxia inhibit I_{KN} and cause depolarisation. The two-pore domain K channel, TASK-1 (KCNK3), has similar pharmacology and contributes to the resting potential in neuronal cells. It has been implicated in the hypoxia-induced depolarisation of cerebellar granule neurons (Plant *et al.* 2002) and carotid body cells (Buckler *et al.* 2000).

We investigated the potential role of TASK-1 channels in mediating I_{KN} and the resting potential in pulmonary artery myocytes, isolated from rabbits humanely killed with pentobarbitone (80 mg kg^{-1}, I.V.). The resting membrane potential and amplitude of I_{KN} at a holding potential of 0 mV were measured in rabbit pulmonary artery smooth muscle cells, using the whole-cell patch-clamp technique. Drugs were applied in the extracellular solution. The composition of the pipette solution was varied to alter the intracellular concentrations of Ca^{2+} or ATP.

Reducing extracellular pH from 7.3 to 6.3 reduced the amplitude of I_{KN} by ~70 %, while increasing pH to 8.3 increased current amplitude by ~10 %. These effects were accompanied by changes in membrane potential from around −45 mV (pH7.3) to −25 mV (pH 6.3) or −50 mV (pH 8.3). Halothane (1–2 mM) had a variable effect, but usually produced a transient increase in current amplitude (of 32 ± 16 %, $n = 8$; mean ± S.E.M.) lasting ~30 s, followed by a 43 ± 10 % decrease in amplitude. It had a biphasic effect on membrane potential, brief hyperpolarisation being followed by depolarisation. At 100 μM, Zn^{2+} inhibited I_{KN} by 56 ± 10 % ($n = 4$) and almost abolished the resting potential. Removing extracellular Ca^{2+}, applying the Ca^{2+} ionophore A23187 (10 μM), or varying the intracellular EGTA concentration (0.05–5 mM) had no significant effect on I_{KN} amplitude. Varying the intracellular ATP concentration (0–5 mM) also failed to affect I_{KN}.

These results are consistent with a major role for TASK-1 channels in mediating the resting K^+ conductance and membrane potential of pulmonary artery smooth muscle cells.

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Evans AM *et al.* (1996). *J Physiol* **496**, 407–420.
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Plant LD *et al.* (2002). *Stroke* **33**, 2324–8.

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All procedures accord with current UK legislation.

P62

Molecular and immunohistochemical identification of TASK-1 in rabbit and mouse pulmonary artery myocytes

E.R.M. Hunter*, D. MacMillan† and A.M. Gurney*

**Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G4 0NR and †Neuroscience and Biomedical Systems, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK*

A non-inactivating potassium conductance determines the resting membrane potential of pulmonary artery smooth muscle cells (Evans *et al.* 1996). The two-pore domain potassium channel TASK-1 may encode this conductance, as it is similar in pharmacology and oxygen sensitivity (Gurney, 2002). In the present study RT-PCR was used to identify the presence of TASK-1 in rabbit pulmonary arterial myocytes.

Animals were humanely killed with pentobarbitone (80 mg kg $^{-1}$, I.V.) and the main, branch and intrapulmonary arteries were excised. Smooth muscle cells were dissociated as previously described. A general primer pair which recognises multiple TASK channel isoforms identified transcripts in suspensions of pulmonary arteries ($n = 3$). TASK-1 transcripts were identified using eight different primer pairs ($n = 3$ each), recognising different parts of the sequence. PCR products were sequenced and confirmed to be TASK-1.

Immunohistochemistry was used to verify the presence of TASK-1 in mouse lung. Lungs were removed from mice which had been humanely killed by cervical dislocation, fixed in ice-cold 4% paraformaldehyde in phosphate buffered saline for 15 min, dehydrated in sucrose under vacuum, and frozen in liquid N_2. Antibodies raised against the C-terminus (Alomone Labs, $n = 5$) and N-terminus (Autogen Bioclear UK Ltd, $n = 4$) of human TASK-1 were used. Examination of cryosections by both epi-fluorescence and confocal microscopy revealed strong TASK-1 immunoreactivity in smooth muscle cells of pulmonary arteries. Immunoreactivity was also seen in alveolar cells and the epithelium of the bronchioles. Staining patterns were comparable with both antibodies. The antibody raised against the C-terminus of human TASK-1 also revealed TASK-1 immunoreactivity in smooth muscle cells dispersed from rabbit pulmonary artery.

In conclusion, we have demonstrated that smooth muscle cells from the pulmonary artery stain positively for TASK-1 protein and that they express transcripts for TASK, consistent with the proposed role for TASK-1 in regulating the membrane potential of pulmonary artery myocytes.

Evans AM *et al.* (1996). *J Physiol* **496**, 407–20.
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All procedures accord with current UK legislation.

P63

The role of A-type K^+ channels in neonatal rats uterus

Yatong Li and Susan Wray

The Physiological Laboratories, The University of Liverpool, Crown Street, Liverpool L69 3BX, UK

We have previously reported that there are three distinct K^+ channels in the neonatal uterus (Li *et al.* 2002), and that the predominant K^+ current was A type. A-type currents are rapidly activating and inactivating K^+ currents, and it is considered that they can regulate the firing frequency of action potentials. The aim of this work was to characterize the A-type current present in these cells, and to determine their functional role. Therefore, we report here electrophysiological, force and imaging data.

Female rats, 13–15 days old, were humanely killed by cervical dislocation following CO_2 anaesthesia. The whole-cell patch clamp technique was used to measure outward K^+ currents in enzymatically isolated uterine single cells. Force measurements in intact tissue were made to study their physiological significance and blockers of A-type channels were applied externally. Ca^{2+} wave activity was examined in whole uterine horns using confocal microscopy in fluo-4 AM loaded preparations. The data are given as means ± S.E.M., and n refers to the number of cells or animals.

Overall K^+ currents were evoked by using depolarising steps varied from −40 mV to +70 mV in 10 mV increments at a holding potential of −80 mV. A-type currents were obtained by using digital subtraction from −90 mV conditioning pulse to −40 mV, and were present in 90% of cells ($n = 50$). At +70 mV, the time to peak was 4.2 ± 1.5 ms ($n = 12$) and peak current densities averaged 0.06 nA pF^{-1} ($n = 12$). A-type K^+ currents were activated at −60 ± 5 mV and the half-inactivation potential was −65 ± 15 mV ($n = 12$). At +70 mV, the time constant of A-type current inactivation was best fitted by two exponentials and revealed two types of cells, one with slow inactivation time constants of 24 ± 1.9 ms and 3.3 ± 0.5 ms ($n = 7$), another with fast inactivation constants of 14.1 ± 2.2 ms and 1.1 ± 0.2 ms ($n = 7$). As reported previously, the A-type currents were sensitive to 4-aminopyridine (4-AP) in a dose-dependent manner. Force measurements of intact tissue from neonatal and adult uteri showed that the spontaneous contractions of neonate were more frequent than adult (8 ± 1 *vs.* 3 ± 1 (5 min)$^{-1}$, $n = 5$). Application of 0.5 mM 4-AP increased the amplitude and frequency of spontaneous contraction in neonates 15 ± 5% and 7 ± 2 (5 min)$^{-1}$ ($n = 5$), respectively, but not in adults. Intracellular Ca^{2+} waves were observed in the neonatal tissue. They were asynchronous in the muscle bundles and not associated with contractions ($n = 6$).

In conclusion, these data confirm and characterize the presence of heterogeneous A-type K^+ channels in neonatal uterus. These are functionally important as their inhibition produced an increase in force amplitude and frequency, suggesting they act to reduce excitability. It is suggested that they may play a role in keeping the spontaneous Ca^{2+} waves asynchronous and thereby not producing contraction.

Li Y *et al.* (2002). *Pflugers Arch* **443**, S157–S393.

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All procedures accord with current UK legislation.

P64

An investigation into the role of the sarcoplasmic reticulum and Ca^{2+}-activated ion channels in the mouse myometrium

A. Matthew and S. Wray

Department of Physiology, University of Liverpool, Crown Street, Liverpool L15 3BX, UK

In vitro studies of animal and human myometrium have greatly increased our understanding of uterine physiology and, in particular, of the mechanisms governing excitation–contraction coupling. It is still the case, however, that much remains to be elucidated concerning how uterine contractions are controlled, especially in pre-term labours. Knock-out mice are adding to our knowledge. However, there is little background information about mouse myometrium, especially concerning contractility, ion channels and the sarcoplasmic reticulum (SR). Thus, the aim of this work was to investigate these aspects of excitation–contraction coupling.

CD-1 mice were killed humanely under CO_2 anaesthesia, and the uterus removed. Small strips were loaded with the fluorescent Ca^{2+} indicator, indo-1. The SR was inhibited using cyclopiazonic acid (CPA, 10 μM).

Mouse myometrium produced regular, phasic contractions preceded by intracellular Ca^{2+} transients ($n = 20$). There was no spontaneous activity in the absence of extracellular Ca^{2+}, and baseline Ca^{2+} decreased ($n = 16$). Oxytocin, carbachol and high-K^+ depolarising solution all increased the Ca^{2+} signal and contractions ($n = 8$, 4 and 6 respectively). In zero Ca^{2+}, oxytocin produced a small increase in Ca^{2+} and force ($n = 6$). Inhibition of the SR by CPA significantly increased the frequency of contractions ($n = 8$). Baseline calcium was also increased. To investigate the mechanism whereby depletion of the SR Ca^{2+} increases force, we inhibited the Ca^{2+}-activated ion channels. Application of the K^+ channel inhibitors tetraethylammonium or iberiotoxin significantly increased the frequency and amplitude of contractions ($n = 3$). Niflumic acid, a Cl^- channel blocker, abolished spontaneous contractions ($n = 5$).

We concluded that these data suggest the presence of both Ca^{2+}-activated K^+ and Cl^- channels and therefore Ca^{2+} released from the SR may activate them. Given the finding of increased contractility when the SR was emptied, the current data suggest that K^+ plays the dominant role. The SR also has an IP_3-releasable store, but its contribution to agonist-induced force production appears to be limited.

All procedures accord with current UK legislation.

P65

Actin filaments regulate the stretch sensitivity of large conductance Ca^{2+}-activated K^+ channels in rabbit coronary arterial smooth muscle cells

Lin Piao, Wonkyung Ho and Yung E. Earm

National Research Laboratory for Cellular Signalling and Department of Physiology, Seoul National University College of Medicine, Yonkeun-Dong 28, Chongno-Ku, Seoul, 110-799, Korea

The stretch sensitivity of large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) in vascular smooth muscle has been considered a negative feedback in vasoconstriction. The activity of BK_{Ca} was shown to be modulated by the cytoskeleton, but whether the cytoskeleton regulates stretch sensitivity of BK_{Ca} is unknown. In the present study, the function of cytoskeletons in the regulation of BK_{Ca} activity and its stretch sensitivity was investigated.

Rabbits were anaesthetized with pentobarbital sodium (50 mg kg^{-1}). Using inside-out patch clamp technique, the single channel currents were recorded from enzymatically dispersed coronary arterial smooth muscle cells. Both the pCa 6.5 bath solutions and the pipette solution contained 150 mM KCl. BK_{Ca} was identified by the large unitary conductance (about 300 pS), by the voltage and calcium dependence, and by 100 nM iberiotoxin, a specific blocker of BK_{Ca}.

Disruption of actin filaments after brief treatment with cytochalasin D (1 μM), an actin filament disrupter, increased the open probability (NP_o) from 0.15 ± 0.03 to 0.26 ± 0.06 (mean ± S.E.M., $n = 4$, $P < 0.01$, Student's paired t test). The increase in NP_o by cytochalasin D was largely reversed by phalloidin (1 μM), an actin filament stabilizer, to 0.03 ± 0.01 ($n = 4$, $P < 0.01$). A brief treatment with colchicine (10 μM), a microtubules disrupter, increased NP_o from 0.14 ± 0.03 to 0.31 ± 0.07 ($n = 4$, $P < 0.01$). A microtubules stabilizer, taxol (1 μM), markedly decreased NP_o to 0.04 ± 0.05 ($n = 4$, $P < 0.01$).

Applying −30 cmH_2O negative pressure to the pipette, NP_o of BK_{Ca} increased from 0.11 ± 0.03 to 0.54 ± 0.06 ($n = 4$, $P < 0.01$). With the stepwise increase (from −10 to −50 cmH_2O) of negative pressure, the activity of BK_{Ca} gradually increased without changing the unitary conductance.

In the presence of phalloidin, negative pressure hardly affected NP_o and NP_o at −30 and −40 cmH_2O was 0.07 ± 0.01 and 0.10 ± 0.01, respectively ($n = 4$). Taxol did not block the effect of negative pressure (at −30 cmH_2O, $NP_o = 0.25 \pm 0.04$; at −40 cmH_2O, $NP_o = 0.38 \pm 0.05$; $n = 4$).

So we could conclude that membrane stretch activates BK_{Ca} in coronary arterial smooth muscle cell. Actin filaments and microtubules modulate the activity of BK_{Ca}, but only actin filaments regulate the stretch sensitivity of BK_{Ca}.

All procedures accord with current National guidelines.

P66

Characterization of a cation-selective mechanosensitive ion channel in pulmonary arterial smooth muscle cells

Kyoung-Sun Park, Yangmi Kim, Won-Kyung Ho and Yung E. Earm

National Research Laboratory for Cellular Signalling and Department of Physiology, Seoul National University College of Medicine, Yonkeun-Dong 28, Chongno-Ku, Seoul, 110-799, Korea

Active force development by vascular smooth muscle in response to elevation of luminal pressure, or stretch, is termed the myogenic response, and is independent of neural, metabolic, hormonal and endothelial factors. The myogenic response is important for the local regulation of blood flow and for the generation of basal vascular tone. Mechanosensitive ion channels, especially non-selective cation channels (NSC) or chloride channels have been considered as possible candidates for transducing mechanical events into the contractile response of the cell. The aims of the present study are to confirm the existence of mechanosensitive ion channels in vascular smooth muscle cells at the single channel level and to investigate their characteristics.

The rabbits were terminally anaesthetized with pentobarbital sodium (50 mg kg^{-1}). We recorded single-channel currents from enzymatically dispersed pulmonary arterial smooth muscle cells (PASMCs) using the patch clamp technique. With 140 mM CsCl in the pipette solution, no channel opening was observed before applying negative pressure to the pipettes. Application of

negative pressure to the pipettes induced channel opening in 100 of 2000 patches, indicating the presence of mechanosensitive ion channels. Open probability (NP_o) of the channels measured at −40 mV was increased from 0.12 ± 0.03 to 0.85 ± 0.08 when the negative pressure was increased from −10 cmH_2O to −30 cmH_2O (mean ± S.E.M., $n = 5$, $P < 0.01$, Student's paired t test).

The single channel conductance was examined in excised inside-out patches under symmetrical ionic composition with 140 mM CsCl solutions both in pipettes and in the bath. The current–voltage relationship was linear between −80 mV and +80 mV, and the mean single channel conductance obtained from 15 patches was 34 ± 2.8 pS. When Cs^+ in the pipette solution was replaced with a large impermeant cation such as *N*-methyl-D-glucamine (NMDG), inward current was abolished, whereas outward currents remained active. Replacing Cs^+ with K^+ or Na^+ did not significantly affect the channel activity, except that the amplitude of inward current was slightly reduced in the presence of Na^+. These results indicate that major mechanosensitive channels present in PASMCs are NSC channels. Pharmacological characteristics were tested. Gadolinium (30 μM), a blocker of NSC channels, blocked this channel ($n = 5$). Interestingly, DIDS (300 μM), a frequently used blocker of Cl^- channels, also blocked this channel ($n = 5$).

In conclusion, mechanosensitive ion channels present in PASMCs are NSC channels which can be blocked both by gadolinium and DIDS.

Bae *et al.* (1999). *J Physiol* **514**, 747–754.

All procedures accord with current local guidelines.

P67

The recovery mechanisms from alkalosis in mesenteric arteriole of rat

Chae Hun Leem, Hyun Suk Cho, Jeong Mi Ha, Han Seok Choe, Yeon Jin Jang and Chun Sik Park

Department of Physiology, University of Ulsan College of Medicine, Seoul, Korea

All cells have the recovery mechanisms from the shift of intracellular pH (pH_i). Many mechanisms were found and characterized. In cardiac myocytes, two Cl^--dependent mechanisms were responsible for the recovery from alkalosis, such as Cl^-–OH^- exchange and Cl^-–HCO_3^- exchange (Leem & Vaughan-Jones, 1998). In mesenteric arteriole, it is still not unclear which mechanisms are responsible for the alkaline recovery (Aalkjaer & Hughes, 1991). In this report, we would like to characterize the alkaline recovery mechanisms in vascular smooth muscle.

We humanely killed rats with ketamine (100 mg kg^{-1}) and removed mesenteric vascular beds. We isolated the 4th branch mesenteric arteriole (< 150 μm) and loaded carboxy SNARF-1 to measure pH_i change (Leem & Vaughan-Jones, 1998). To induce alkalosis, we used the acetate pre-pulse technique or CO_2-removal technique. In HCO_3^--free Hepes-buffered conditions or CO_2/HCO_3^--buffered conditions, the pH_i recovered from induced alkalosis. The calculated proton flux in the CO_2/HCO_3^--buffered conditions was larger than that in HCO_3^--free Hepes-buffered conditions. This recovery was completely inhibited by the removal of extracellular Cl^- (Cl_o^-) which was replaced by glucuronic acid. 4,4′-Diiisothiocyanatostilbene-2,2′-disulfonic acid (DIDS, 500 μM), a classical blocker of the Cl^-–HCO_3^- exchanger, did not inhibit the alkaline recovery in HCO_3^--free Hepes-buffered conditions or CO_2/HCO_3^--buffered conditions. The other stilbene drugs such as 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS) or dibenzamido-stilbene-disulphonic acid (DBDS) also had no effect on the recovery. In CO_2/HCO_3-buffered conditions, the removal of extracellular Na^+ (Na_o^+), which was replaced by *N*-methyl-D-glucamine (NMDG), greatly accelerated the recovery (at $pH_i = 7.15$, −0.007 ± 0.001 pH min^{-1} *vs.* −0.051 ± 0.008 pH min^{-1}, mean ± S.E.M., $n = 3$, $P < 0.05$, Student's paired t test). When K^+ or Cs^+ were substituted for Na_o^+, the recovery was slightly accelerated but was greatly attenuated compared to NMDG substitution (K^+ substitution, at $pH_i = 7.15$, −0.015 ± 0.001 pH min^{-1}, mean ± S.E.M., $n = 3$).

These results suggest that in arteriolar smooth muscle, a novel Cl^--dependent and HCO_3^--dependent or -independent mechanism was responsible for the recovery from alkalosis. This mechanism was not sensitive to stilbene derivatives and affected by monovalent cations such as Na^+, K^+ or Cs^+ in the presence of HCO_3^-. Still we do not know the exact stoichiometry of this mechanism and it is necessary to perform further studies to identify the characteristics.

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P68

The effects of hypoxia on $[Ca^{2+}]_i$ signalling in phenotypically distinct myocytes from the rat pulmonary vein

S.F. Cruickshank and R.M. Drummond

Department of Physiology and Pharmacology, University of Strathclyde, 27 Taylor Street, Glasgow G4 ONR, UK

In the pulmonary vein (PV), two phenotypically distinct types of myocytes have been reported. Specifically, bundles of cardiac-like myocytes are found around typical spindle shaped smooth muscle cells, and are thought to be involved in modulating PV tone (Michelakis *et al.* 2001). Intrapulmonary veins contribute significantly to the total pulmonary vascular resistance and are known to constrict during hypoxia (Zhao *et al.* 1993). Despite this, the effect of hypoxia on $[Ca^{2+}]_i$ in the pulmonary vein has received little attention. Thus, we have examined $[Ca^{2+}]_i$ signalling in these two distinct myocyte phenotypes during hypoxia.

Male Sprague-Dawley rats (200–300g) were killed by cervical dislocation and the heart and lungs removed *en bloc*. Myocytes were then isolated from intrapulmonary veins (> 400 μm) using methods similar to those used for the pulmonary artery, as described by Drummond & Tuft (1999) and incubated with 5 μM fura-2 AM. Hypoxic solutions were achieved by bubbling extracellular bath solution with N_2. All experiments were carried out at room temperature. Where appropriate mean data ± S.E.M. are given and n is the number of cells studied. Statistical differences were tested for using Student's paired t test. $P < 0.05$ was considered to be significant.

In cardiac-like myocytes isolated from the vein, spontaneous oscillations in $[Ca^{2+}]_i$ were observed. Under normoxic conditions (P_{O_2} ~145 mmHg), these oscillations had a frequency of 0.6 ± 0.2 Hz. During the oscillations, $[Ca^{2+}]_i$ increased from a basal level of 113 ± 17 nM to 333 ± 26 nM. Hypoxia (P_{O_2}

~20 mmHg) increased oscillation frequency to 1.1 ± 0.1 Hz ($P < 0.05$), and also increased basal $[Ca^{2+}]_i$ to 178 ± 14 nM ($P < 0.01$), in five cells. During hypoxia, the peak amplitude of the $[Ca^{2+}]_i$ oscillations increased to 553 ± 33 nM ($P < 0.05$). These effects were all reversible on return to normoxic conditions. Typical spindle-shaped smooth muscle cells did not show spontaneous oscillations. Hypoxia (P_{O_2} ~20 mm Hg) increased basal $[Ca^{2+}]_i$ from 105 ± 4 nM to 156 ± 3 nM in 3 of 10 cells. However, hypoxia increased the peak amplitude of caffeine-induced Ca^{2+} transients in these cells from 706 ± 35 nM during normoxia to 1630 ± 51 nM during hypoxia ($n = 10$, $P < 0.01$).

These results show that hypoxia affects $[Ca^{2+}]_i$ signalling in the two distinct phenotypes of myocytes found in the pulmonary vein.

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All procedures accord with current UK legislation.

P69

Intracellular recording of spontaneous electrical activity in the isolated rabbit urethra

Ursula A. Anderson, M.A. Hollywood, K.D. Thornbury and N.G. McHale

Smooth Muscle Group, Queen's University, Belfast BT9 7BL, UK

The smooth muscle of the urethra is capable of playing an important role in continence by generating sufficient tone to prevent voiding of urine from the bladder (Bridgewater *et al.* 1993). This tone appears to be myogenic, at least in part, but the mechanism of its generation and maintenance are poorly understood. We have learned a lot in recent years, using the patch-clamp technique, about the conductances underlying excitation in isolated smooth muscle cells dispersed from sheep and rabbit urethra (Cotton *et al.* 1997; Hollywood *et al.* 2000) but this technique does not allow us to assess the relevance of any given conductance to the function of the syncitium. In the present study we have used intracellular microelectrodes to record the effects of some ion channel blockers on spontaneous action potentials in segments of rabbit urethra.

The bladder and urethra were removed from both male and female rabbits immediately after they had been killed by lethal injection of pentobarbitone. The most proximal 3 cm of the urethra was removed and placed in Krebs solution. This was then opened up, the urothelium removed and the preparation pinned out on a silicon rubber base and superfused with Krebs solution at 35–37 °C. Smooth muscle cells were impaled with glass microelectrodes filled with 3 M KCl (80–120 MΩ). Resting membrane potential varied from −40 to −65 mV (mean ± S.E.M., 49.3 ± 2.28, $n = 29$) Approximately 90 % of the preparations showed spontaneous electrical activity. Three different types of spontaneous activity were observed: (1) slow waves consisting of a small initial spike followed by a large plateau (of duration at half-amplitude 0.99 ± 0.16 s^{-1}, $n = 4$); (2) spike complexes consisting of between 1 and 12 (mean 4.48 ± 0.68, $n = 19$) rapid spikes superimposed on a small plateau (15–20 mV, mean 12.5 ± 1.54 mV); and (3) continuous firing of single rapid spikes.

The effects of Penitrem A (a specific blocker of the BK_{Ca} channel) on the above activity were examined. Penitrem increased the frequency of firing of spike complexes (from 4.7 ± 3.47 to 9.9 ± 5.9 min^{-1}), increased the maximum spike amplitude (from 34.2 ± 3.8 to 55.9 ± 6.15 mV) and decreased the duration at half amplitude of the plateau (from 1.5 ± 0.34 to 0.48 ± 0.087 s^{-1}). The other obvious effect of Penitrem A was the blockade of a rapid hyperpolarisation following each individual spike.

We conclude that calcium-activated potassium current in rabbit urethra plays an important role in repolarisation of the action potential and that its activation is sufficiently rapid to limit the spike amplitude under normal conditions.

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All procedures accord with current UK legislation.

P70

Characterisation of T-type calcium current and its role in spontaneous activity in rabbit urethral smooth muscle

J.E. Bradley, U.A. Anderson, S.M. Woolsey, N.G. McHale, K.D. Thornbury and M.A. Hollywood

Smooth Muscle Group, Department of Physiology, Queen's University, 97 Lisburn Road, Belfast, Northern Ireland, UK

Recently we have described the presence of T-type calcium current in smooth muscle isolated from the human proximal urethra (Hollywood *et al.* 2002) and preliminary data suggested that this current is present in rabbit urethral smooth muscle. The aim of the present study was to characterise the T current in isolated rabbit urethral myocytes using the patch clamp technique and examine the effects of blocking this current on spontaneous activity recorded from strips of urethra with sharp microelectrodes.

Rabbits were humanely killed with pentobarbitone (I.V.) and their urethras removed. Cells were isolated as previously described (Sergeant *et al.* 2000). These were perfused with Hanks' solution at 37 °C and studied using the amphotericin B perforated patch technique with Cs^+-rich pipette solutions. When cells were held at −100 mV and depolarised in 10 mV steps for 500 ms, inward currents were evoked at potentials positive to −70 mV. When the current voltage (*I–V*) relationship was plotted, it consisted of two peaks – one at ~−40 mV and the other at 0 mV. To dissect these two components, currents were evoked from holding potentials of −100 mV and −60 mV. When the *I–V* curve obtained at a holding potential of −60 mV was plotted, the negatively activating component of inward current was abolished and little inward current was observed at potentials negative to −40 mV. Subtraction of the currents obtained at these two holding potentials revealed a current that activated at potentials positive to −70 mV, peaked at −30 mV and reversed at approximately 30 mV. To examine the voltage dependence of inactivation of both currents, cells were stepped to either −40 or 0 mV after a series of 2 s preconditioning potentials from −100 mV through to 0 mV. Under these conditions the current evoked by a step to −40 mV half-maximally inactivated at −76 ± 2 mV (mean ± S.E.M., $n = 5$) compared with −41 ± 3 mV ($n = 5$) for the current evoked by a step to 0 mV. We next examined the effects of Ni^{2+} on both components of inward current. Ni^{2+} (100 mM) reduced currents evoked at −40 mV from −66 ± 26 to −11 ± 9 pA but only reduced the current at 0 mV from −659 ± 102 to −559 ± 100 pA ($n = 3$).

To examine the contribution of T current to spontaneous electrical activity, we investigated the effects of Ni^{2+} on spike complexes in strips of urethra using sharp microelectrodes.

Application of Ni^{2+} at concentrations of 10 mM, 30 mM and 100 mM decreased the frequency of spike complexes from 4.2 ± 0.5 min^{-1} to 2.4 ± 0.5, 2.0 ± 0.4 and 0.3 ± 0.3 min^{-1}, respectively ($n = 5$ cells, 3 animals). These data suggest that the negatively activating T current may play an important role in modulating action potential frequency in the rabbit urethra.

Hollywood MA *et al.* (2002). *J Physiol* **543.P**, 76*P*.

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

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All procedures accord with current UK legislation.