C23

Characterisation of spontaneous Ca^{2+}-transients in smooth muscle cells of intact rat retinal arterioles.

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Intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) is an important regulator of function in smooth muscle cells. When microvascular smooth muscle (MVSM) cells are imaged *in situ* in intact retinal arterioles, a variety of spontaneous $[Ca^{2+}]_i$ transients are seen (Curtis *et al.* 2003). These have now been characterised further. Sprague Dawley rats (200-300g) were anaesthetised and killed by cervical dislocation. Arterioles were mechanically dispersed from retinae, incubated with 10μM Fluo-4AM, and then superfused at 37ºC. They were linescanned using a confocal scanning laser microscope (Biorad, MR/A1, 500 scans s^{-1}, excitation=488nm, emission=530-560nm). Fluorescence (F) was normalized to the resting fluorescence (F_0). $[Ca^{2+}]_i$ transients were analysed in terms of their amplitude ($\Delta F/F_0$), their full duration at half maximal (FDHM), and their full width at half maximal (FWHM). Data were summarized as the mean±SEM. P-values were determined using Student's unpaired t-test. Under resting conditions, two main types of spontaneous $[Ca^{2+}]_i$ transients were observed in MVSM. Brief, spatially-localised events resembling Ca^{2+}-sparks were often seen near the cell membrane, as well as more prolonged Ca^{2+}-oscillations which spread across the full width of the cell. Sparks were observed arising from basal $[Ca^{2+}]_i$ levels with a frequency of 0.56±0.06 s^{-1} (60 cells, 102 events). They had a mean amplitude ($\Delta F/F_0$) of 0.81±0.04, a mean FDHM of 23.6±1.2 ms, and a mean FWHM of 1.25±0.05 μm. Ca^{2+}-oscillations occurred at an average frequency of 0.13±0.01 s^{-1} (35 cells, 162 events). Their amplitudes were similar to those of the sparks ($\Delta F/F_0$ = 0.93±0.04), but they were very much longer in duration (FDHM = 1992±69 ms; P<0.001 v. sparks). Many oscillations appeared to result from the summation of Ca^{2+}-sparks, and spark-like events were superimposed on the oscillations themselves. These appeared to originate from the same sites as basal-sparks in the same cells, but occurred with a much higher frequency (mean frequency=2.86±0.25 s^{-1}, 78 events; P<0.001 v. basal sparks). They had a smaller amplitude ($\Delta F/F_0$ = 0.69±0.04; P<0.05 v. basal sparks), a wider spread (FWHM = 1.67±0.07 μm; P<0.001 v. basal sparks), but were similar in duration to basal sparks (FDHM = 22.2±1.1 ms; N.S.). Thus, there were two distinct spontaneous spark populations arising from the same sites, presumably reflecting changes in localised Ca^{2+}-release at different levels of cytoplasmic- and sarcoplasmic reticulum-$[Ca^2]$. The link between these changes and the mechanisms responsible for generation of the Ca^{2+}-oscillations themselves remains to be determined.

Curtis, TM *et al.* (2003). *J Physiol* **555P**, C135

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C24

The cellular basis of spontaneous electrical activity in guinea-pig sub-urothelial myofibroblasts

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The mechanism for the urinary bladder to sense fullness is unresolved. Evidence suggests that ATP release from urothelium and its eventual action on sub-urothelial sensory afferent purinoceptors may be involved, but the signal transduction process is far from clear (Ferguson et al 1997; Cockayne et al 2000). Recently we proposed a role for sub-urothelial myofibroblasts as an intermediate variable gain stage in bladder sensation and demonstrated that these cells possess many characteristics of excitable cells and respond to ATP (Wu et al 2003). One physiologically important feature of these cells was the presence of spontaneous spikes of membrane depolarisation. The objective of this study was to clarify, using guinea-pig preparations, the cellular basis of these spontaneous activities. Urinary bladders were obtained from guinea-pigs sacrificed by a schedule 1 method. Myofibroblasts were dissociated from the urothelium and identified by vimentin staining (Sui et al 2002). Experiments were performed in HCO_3^-/CO_2 - buffered superfusate at 37°C, pH 7.4. Electrophysiological recordings were made with patch electrodes filled with a high K^+ based intracellular medium. Intracellular Ca^{2+}, $[Ca^{2+}]_i$, was measured simultaneously by dialysing fluorochrome fura-2 via the patch pipette.Spontaneous inward currents were recorded in 26/58 cells held at a resting potential of −60mV. These inward currents were accompanied by a spontaneous rise of $[Ca^{2+}]_i$. Phase-loop analysis showed that the rise of $[Ca^{2+}]_i$ preceded the inward currents. Variation of membrane potential altered the magnitude and polarity of spontaneous currents whilst the $[Ca^{2+}]_i$ rise was little affected. The reversal potential for the current was -26±8 mV (SD, n=8), close to the equilibrium potential of Cl^- ions under the experimental conditions. Suppression of the spontaneous $[Ca^{2+}]_i$ rise by Ca deprivation abolished the inward current (n=3). Raising $[Ca^{2+}]_i$, either by thapsigargin (0.5μM, n=6) or ionomycin (10μM, n=3), evoked the membrane current. Spontaneous inward currents with associated Ca^{2+} rises could be triggered following application of ATP. These results show that spontaneous inward currents are present in sub-urothelial myofibroblasts to support spontaneous electrical activity. These currents are mainly Ca^{2+}-activated Cl^- currents and tightly regulated by spontaneous changes to $[Ca^{2+}]_i$. Existence of these active, Ca^{2+} responsive currents provides a distinct coupling mechanism between $[Ca^{2+}]_i$ and membrane electrical activity, subject to regulation by the putative sensory mediator ATP.

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C25

Periodic Ca^{2+} oscillations underpin prenatal airway peristalsis.

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As first observed in chick embryos (Lewis, 1924), prenatal airways exhibit spontaneous phasic contractility immediately *ex vivo* and *in vitro*. Agonists e.g. acetylcholine and K^+ depolarising solution augment contractions (Sparrow et al. 1994) whereas calcium antagonists e.g. nifedipine reduce or even abolish narrowing of the airways (McCray 1993). We therefore postulate Ca^{2+} influx underlies spontaneous contractility of prenatal airway smooth muscle. This study tests the above hypothesis. Embryonic lungs (n=10) were microdissected on day 13.5 of gestation (term = day 21) from time-mated Sprague-Dawley rats that were killed humanely. Lungs were cultured at 37°C in 5% CO_2 and passively loaded with two calcium-sensitive indicators after 54 and 78 hours. Indo-1 was utilised for photometric measurements of $[Ca^{2+}]_i$ and Fluo-4 for Confocal imaging. For each lung primordia Ca^{2+} transients were observed for at least 30 minutes at 22°C and 30°C. Relaxation half-times (T_{50}) of the calcium transient were determined from at least four representative spikes and are expressed as means ± SE.Ca^{2+} waves propagated at 220-420μm/sec longitudinally through the embryonic bronchi and consistently preceded airway contraction. Both techniques (photometric and confocal) yielded Ca^{2+} transients with similar temporal characteristics. Ca2+ transients feature an initial fast phase (amplitude = 70% of peak), a second slow phase (attainment of peak), followed by a plateau phase at peak. Only the slow and plateau phases were prolonged at 22°C compared to 30°C. Relaxation half-time (T_{50}) of the Ca^{2+} transient was 8.29 ± 1.89 seconds at 22°C and 4.40 ± 0.73 seconds at 30°C. Ca^{2+} oscillations occur every 60 seconds at room temperature, every 30-40 seconds at 30°C, and were potentiated by Ca^{2+} agonist Bay-K 8644 (1μM) and inhibited by nifedipine, (10μM). We have shown for the first time propagation of Ca^{2+} transients mediate embryonic airway contractility via L-type Ca^{2+} channel dependent calcium influx.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C26

IP_3 receptors are coupled to Ca^{2+}-activated Cl^- channels and ryanodine receptors to Ca^{2+}-activated K^+ channels in guinea pig portal vein.

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Activation of sarcolemmal Ca^{2+}-activated channels was investigated in single, voltage-clamped myocytes from the portal veins of guinea pigs (~ 500 g, humanely killed by stunning then immediate exsanguination). Increases in cytosolic Ca^{2+} ($[Ca^{2+}]_c$) were produced by photolysed caged IP_3 (25 μM) or by pressure ejection of caffeine (10 mM). At −70 mV, IP_3-evoked Ca^{2+} release induced an inward current with properties similar to a Ca^{2+}-activated Cl^- current. These included activation by an increase in $[Ca^{2+}]_c$, a reversal potential (−31 ± 4 mV, n=8, mean ± sem) close to the predicted equilibrium potential for Cl^- (−29 mV) and inhibition of the current by niflumic acid (100 μM; at −70 mV control − 565 ± 94 pA, n=9 and in niflumic acid −48 ± 39 pA, n=5, $p<0.01$, statistical significance was determined by unpaired Student's t-test, where $p<0.05$ was considered significant). In the absence of extracellular Ca^{2+}, IP_3 evoked neither a rise in $[Ca^{2+}]_c$ nor an inward current, confirming that the current did not arise from a direct action of IP_3 on the channel. In contrast to IP_3, $[Ca^{2+}]_c$ increases evoked by RyR activation with caffeine did not activate a Ca^{2+}-activated Cl^- current. Activation of Ca^{2+}-activated K^+ channels occurred at membrane potentials positive to ~−25 mV, as monitored by spontaneous transient outward currents (STOCS). Caffeine and IP_3 each caused large outward Ca^{2+}-activated K^+ currents and subsequently inhibited STOCS suggesting that their actions were mediated by depleting SR Ca^{2+} stores. Guinea pig portal vein cells have two separate intracellular Ca^{2+} stores, one with both IP_3 receptors (IP_3R) and ryanodine receptors (RyR), and the other with RyR alone. The common store (IP_3R and RyR) requires extracellular Ca^{2+} for refilling; the store with RyR alone can refill in the absence of extracellular Ca^{2+}. Removal of extracellular Ca^{2+} abolished STOCS but responses to caffeine were maintained suggesting that the store with RyR alone contained Ca^{2+} but did not contribute to the generation of STOCS. The selective activation of sarcolemmal Ca^{2+}-activated channels by Ca^{2+} release from the common store (IP_3R and RyR) suggests this store is located peripherally, whereas the solely RyR store may be deeper in the myoplasm.

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C27

FK506-binding protein (FKBP12) and calcineurin regulate ryanodine and IP_3 receptor-evoked Ca^{2+} release in guinea-pig smooth muscle

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Ca^{2+} release from the intracellular store, the sarcoplasmic reticulum, is largely mediated by two receptor channel complexes, the ryanodine receptor (RYR) and the IP_3 receptor (IP_3R). A family of FK506-binding proteins (FKBPs) and a Ca^{2+}-dependent phosphatase, calcineurin, may each interact with both RYR and IP_3R to modulate receptor-mediated Ca^{2+} release. The physiological effects of FKBPs and calcineurin on RYR and IP_3-mediated Ca^{2+} release were each investigated in single voltage-clamped smooth muscle cells which were isolated from the colon of guinea-pigs (humanely killed by cervical dislocation and immediate exsanguination). $[Ca^{2+}]_c$ was measured as fluorescence using the membrane-impermeable dye fluo 3 (penta-ammonium salt) introduced into the cell via the patch pipette. IP_3 (25 μM) was released from its caged compound by flash photolysis and caffeine (10 mM) was applied by hydrostatic pressure ejection. The FK506-binding protein 12 kDa (FKBP12) and calcineurin were confirmed to be associated with RYR2 and IP_3R by co-immunoprecipitation. The immunosuppressant FK506 (20 μM) did not affect the extent of the association between calcineurin and IP_3R. FK506 (10 μM, which dissociates FKBPs and inhibits calcineurin) increased the $[Ca^{2+}]_c$ rise each evoked by the RYR activator caffeine and by IP_3R activation following photolysed caged IP_3. Another immunosuppressant rapamycin (10 μM, which dissociates FKBPs but does not inhibit calcineurin) also increased the amplitude of the caffeine-evoked but reduced that of the IP_3-evoked $[Ca^{2+}]_c$ transient. The calcineurin inhibitors, cypermethrin (10 μM) and okadaic acid (5 μM) each increased the IP_3-evoked $[Ca^{2+}]_c$ transient. Cypermethrin also increased the $[Ca^{2+}]_c$ rise evoked by caffeine. Following inhibition of calcineurin by cypermethrin, FK506 reduced the IP_3-evoked $[Ca^{2+}]_c$ transient in contrast to the increase occurring in the absence of cypermethrin. Together, these results indicate that FKBP12 binds to RYR2 and reduces its activity in smooth muscle but potentiates IP_3R activity. Calcineurin regulates both RYR and IP_3R by reducing channel activity.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C28

Ca gradients in voltage clamped uterine myocytes: role of SERCA and ryanodine receptors distribution.

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In many types of smooth muscle cells, the sarcoplasmic reticulum (SR) has both peripheral and central components. Presumably, peripheral SR hinders calcium diffusion into cytosol during depolarisation induced calcium entry and contributes to calcium removal from cytosol (superficial buffer barrier, see reference for review). The superficial buffer barrier hypothesis implies that there should be gradients of cytosolic calcium concentration ($[Ca^{2+}]_i$) between the cell periphery and the bulk of cytosol during depolarisation induced Ca entry. In this study we have investigated the spatio-temporal profile of depolarisation induced $[Ca^{2+}]_i$ transients in isolated uterine myocytes and its relation to the distribution of the SR within the cell. Late pregnant (19-21 days) Sprague-Dawley rats were killed by cervical dislocation after CO_2 anaesthesia, in accordance with Schedule 1 of the UK Home Office. Single cells were isolated from the longitudinal layer of the myometrium and superfused with Krebs solution. Patch clamp technique combined with Ca imaging was used to measure Ca current and $[Ca^{2+}]_i$ in different parts of the cell. Cells were loaded with the Ca sensitive dye Oregon Green 488 BAPTA (OG) via patch electrode. Images were acquired at video rate. Distribution of SERCA pump and ryanodine receptors in uterine smooth muscle cells were examined on a confocal microscope using fluorescently labelled thapsigargin and ryanodine. The surface membrane was visualised using membrane-associated dyes di-8-ANEPPS and C18-Calcium Green. Depolarisation of the cell membrane from a holding potential of -60 mV to 0 mV elicited inward calcium current (540±72 pA, n=7) accompanied by $[Ca^{2+}]_i$ transient. $[Ca^{2+}]_i$ rose synchronously throughout the entire cell. The amplitude of $[Ca^{2+}]_i$ transients measured as normalised OG signal was higher in the middle of the cell compared to the cell periphery. Confocal microscopy of the di-8-ANEPPS or C18-Calcium Green stained cells revealed multiple invaginations of the surface membrane similar to the T-tubules in striated myocytes, although not as regular. SR was distributed both centrally and peripherally showing close apposition to the surface membrane invaginations preferentially on the cell periphery. Apparent synchronicity of the $[Ca^{2+}]_i$ transients and differences in their amplitude may be explained if L-type calcium channels are located within the membrane invaginations at higher density than on the surface of the cell.

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C29

Mitochondrial Involvement in Calcium Release Via the IP_3 Receptor in Colonic Smooth Muscle Cells

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Changes in the cytosolic calcium concentration ($[Ca^{2+}]_c$) control a diverse array of cellular functions. $[Ca^{2+}]_c$ can be elevated either by influx of extracellular Ca^{2+}, for example following activation of voltage-gated Ca^{2+} channels, or by release of Ca^{2+} from intracellular stores. The major Ca^{2+} store in smooth muscle cells

is the sarcoplasmic reticulum, from which Ca^{2+} release occurs via either inositol 1,4,5-trisphosphate (IP_3) receptors (IP_3R) or ryanodine receptors (RyR). Although thought of primarily as the site of ATP generation, mitochondria also take up Ca^{2+}, modulating Ca^{2+} influx or release via IP_3R or RyR. Indeed recent evidence has suggested that some mitochondria may be closely apposed to IP_3R, such that mitochondrial Ca^{2+} handling may have very localised effects on nearby IP_3R. Hence the contribution of mitochondria to IP_3-mediated Ca^{2+} signals was examined in single smooth muscle cells, freshly dissociated from guinea pig colon (humanely killed by stunning and exsanguination) and patch-clamped in the whole-cell configuration.The IP_3-generating agonist carbachol (CCh) or IP_3 release by UV flash photolysis of the caged compound introduced via the patch pipette evoked large $[Ca^{2+}]_c$ transients. Depolarisation of the mitochondrial membrane potential ($\Delta\psi_m$) with CCCP plus oligomycin, or rotenone plus oligomycin inhibited IP_3-induced $[Ca^{2+}]_c$ transients (peak declined to 47.1 ± 8.86 % of control values in CCCP plus oligomycin, or 49.3 ± 9.86 % in rotenone plus oligomycin, n=11 for each; $p<0.01$ by unpaired Students t-test, data expressed as mean ± SEM) or CCh-induced $[Ca^{2+}]_c$ transients (peak declined to 8.32 ± 6.45 % in CCCP plus oligomycin, or 61.78 ± 9.27 % in rotenone plus oligomycin, n=3 for each; $p<0.01$). This indicates that mitochondrial Ca^{2+} uptake did occur following IP_3- or CCh-induced $[Ca^{2+}]_c$ elevation. Simultaneous imaging of $[Ca^{2+}]_c$ and $\Delta\psi_m$ in single myocytes co-loaded with the Ca^{2+} indicator fluo-4 AM and the membrane potential sensitive dye TMRE indicated that there was no detectable alteration in $\Delta\psi_m$ during IP_3- or CCh-induced $[Ca^{2+}]_c$ elevation. Inhibition of the mitochondrial Ca^{2+} efflux pathway, the mitochondrial Na^+/Ca^{2+} exchanger, with CGP-37157 increased mitochondrial Ca^{2+} content and both the time to maximum $[Ca^{2+}]_c$ and the time for $[Ca^{2+}]_c$ to fall back to starting values following IP_3 release. Subsequently there was also an IP_3-release dependant elevation of the baseline $[Ca^{2+}]_c$ and a decline in the maximum IP_3-evoked $[Ca^{2+}]_c$.These results suggest that mitochondrial Ca^{2+} uptake modulates the IP_3-mediated Ca^{2+} signal. Mitochondrial Ca^{2+} uptake occurs without significant mitochondrial depolarisation and Ca^{2+} export via the mitochondrial Na^+/Ca^{2+} exchanger is essential for maintenance of mitochondrial regulation of IP_3-mediated Ca^{2+} signals.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C30

Synergism between IP_3 and Ca^{2+} in opening of muscarinic cationic channels.

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Activation of muscarinic receptors, the major excitatory receptors involved in the parasympathetic control of smooth muscle function, produces membrane depolarization through the cationic channel opening. Ca^{2+} mobilized from intracellular stores in response to the activation of M_3 receptors potentiates muscarinic cationic current (mI_{cat}). We therefore related the dynamics of carbachol (CCh)-induced $[Ca^{2+}]_i$ changes to the kinetics of mI_{cat} and evaluated the effect of Ca^{2+} release through ryanodine receptors (RyRs) and IP_3 receptors (IP_3Rs) on mI_{cat} by combining confocal imaging of $[Ca^{2+}]_i$ with simultaneous patch-clamp recording of mI_{cat}. The experiments were carried out on myocytes freshly isolated from the guinea-pig ileum. The animals were killed by decapitation after cervical dislocation as approved under Schedule 1 of the UK Animals (Scientific Procedures) Act 1986. Fast x-y confocal imaging of the myocytes loaded with Ca^{2+}-sensitive indicator fluo-3 revealed that CCh (10 μM)-induced Ca^{2+} waves propagated from the cell ends towards the myocyte centre at 45.9±8.8 μm s^{-1} (mean±S.E.M., n=13) and that Ca^{2+} wave initiation preceded any measurable mI_{cat} by 229±55 ms (n=7). CCh-induced $[Ca^{2+}]_i$ transient peaked 1.22±0.11 s (n=17) before mI_{cat} reached its maximum. At −50 mV, spontaneous release of Ca^{2+} through RyRs resulting in Ca^{2+} sparks had no effect on CCh-induced mI_{cat} but activated BK channels leading to spontaneous transient outward currents. Ca^{2+} release through RyRs induced by brief application of 5 mM caffeine was initiated at the cell centre but did not augment mI_{cat} (n=14). The latter was due neither to an inhibition of the cationic channels by caffeine (since application of 5 mM caffeine did not inhibit mI_{cat}, when $[Ca^{2+}]_i$ was clamped with Ca^{2+}/BAPTA buffer) nor to an effect of caffeine on other mechanisms of the cationic channel Ca^{2+}-sensitivity (since in the presence of 5 mM caffeine, intracellular photorelease of Ca^{2+} potentiated mI_{cat} in the same way as in control). Intracellular photorelease of IP_3 augmented mI_{cat} (n=15) at lower $[Ca^{2+}]_i$ than required for potentiation of mI_{cat} by Ca^{2+} alone (n=10). Intracellular calcium store visualised with a low-affinity Ca^{2+} indicator fluo-3FF (n=35) consisted of the superficial sarcoplasmic reticulum (SR) network and some perinuclear formation interconnected with the superficial SR. Immunostaining of the myocytes with antibodies to IP_3Rs (n=40) and to RyRs (n=18) revealed that type1 IP_3Rs are predominant in the superficial SR while RyRs are confined to the central region of the cell. These results suggest that IP_3R-mediated Ca^{2+} release plays a central role in the modulation of mI_{cat} in the guinea-pig ileum and that IP_3 may sensitise to Ca^{2+} the regulatory mechanisms of the muscarinic cationic channels opening.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C31

The effect of knocking-out isoform 4 of the plasma membrane Ca-ATPase (PMCA) on force, in mouse myometrium

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Ca influx and removal mechanisms are vitally important to Ca homeostasis and thus the correct functioning of the myometrium. Both Na/Ca exchange and PMCA have been implicated in Ca extrusion following uterine stimulation. PMCA is encoded by four genes (PMCA 1-4), with PMCA 1 and 4 isoforms found in most tissues. The aim of this work was to investigate the functional effects of knocking out PMCA 4.Wild type, heterozygous, and

homozygous knockout PMCA 4 black 6129 mice were killed humanely under CO2 anaesthesia, and the uterus removed. Small strips of myometrium were dissected and attached to a force transducer. After establishing contraction to high K depolarisation (120 mM), the tissues were then placed in either 0-Ca (1mM EGTA) control solution, or 0-Ca 0-Na (1mM EGTA) solution, to impair Na/Ca exchange, and the half time of force decline calculated. The tissues were then stimulated with carbachol (100 μM) for 20s, again in either 0-Ca or 0-Ca 0-Na solution, and the amount of force produced by SR Ca release compared. The experiments were performed and analysed blindly. Western blotting was used to test for possible compensatory upregulation of the Na/Ca exchanger. Mean ± Standard error are given, and significance was tested using the paired t-test. Control mouse myometrium produced regular, phasic contractions proceeded by intracellular Ca2+ transients (n=20), and carbachol and high K increased the Ca2+ signals and contractions. There were no significant differences in the rate of fall of force in any group in 0-Ca solution (n=15). However upon removal of extracellular Ca and Na, the rate of fall was significantly reduced in the homozygous KO mice (37.73±7.8% of KCL contraction) and the heterozygous mice (14.19±4.4%) compared to the wild type (79.45±3.7%). There was no statistical difference observed between the force produced by the release of SR Ca2+ in the three genotypes. There was no observed difference in the expression of the Na/Ca exchanger in the PMCA 4 knock out mice when compared to the wild type (n=2). These data suggest that both Na/Ca exchange and PMCA are responsible for lowering Ca and force in the myometrium following stimulation. If PMCA activity is impaired, as in the KO mice, then increased Na/Ca exchange activity can compensate, despite no observed increase in the expression of the protein. The unchanged response to carbachol in the KO mice suggests that the SR has taken up no more Ca than in control mice, and is therefore functionally unaffected by the changes in plasmalemmal Ca extrusion. If the system is challenged in the homozygous KO mice, e.g. by reducing external Na, then extrusion and relaxation of force are greatly impaired.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C32

Hypertension-induced alterations in NO-cGMP pathway and lumen reduction in SHR middle cerebral arteries

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Introduction. Middle cerebral arteries (MCA) from adult spontaneously hypertensive rats (SHR) show a reduction in internal diameter (ID), which can jeopardize cerebral blood flow. Aims.To determine if ID reduction in MCA from adult SHR: 1)is due to a structural modification, an increased smooth muscle cell (SMC) contractility, or a defect in basal endothelial vasodilators and 2)if this ID reduction precedes or develops with hypertension.Methods.30-day old and 6-month old Wistar Kyoto rats (WKY) and SHR were used. Systolic blood pressure (SBP) was measured via the iliac artery in anaesthetised rats (sodium pentobarbital 50mg/kg i.p). Thereafter the rats were killed with pentobarbital. The investigation conformed with EU guidelines. MCA IDs were measured with pressure myography with and without Ca2+ (20-120 mmHg). Intrinsic tone and responses to 75 mM KCl, 10-4 M L-NAME (NOS inhibitor), 3x10-7M indomethacine (COX inhibitor), 10-8-10-5M sodium nitroprusside (SNP, NO donor) were studied at 70 mmHg. To determine NO and O2- availability, intact MCA segments were incubated with the fluorescent indicators diaminofluoresceine (10-5M, Em 550nm) and dihidroethidium (10-6M, EM 610nm) respectively, visualized with confocal microscopy and quantified by image analysis. Data were analyzed by 1 or 2-way ANOVA and are shown as mean ± SEM.Results.In 30-day old rats SBP was similar in WKY (89±4 mm of Hg n= 19) and SHR (100±4 n= 21, p=0.6) and it was significantly larger in adult SHR (183±10 n=17 p<0,05), when compared to age-matched WKY (127±10 n=12). In young SHR and WKY rats MCA ID was similar at all pressures tested in the presence or absence of Ca2+. MCA ID was significantly smaller in adult SHR the presence of Ca2+ at every pressure tested (at 70 mm Hg IDSHR=108±11, IDWKY=154±8, p<0,01) This difference was abolished by 0Ca2. Intrinsic tone was significantly larger in SHR MCA.In adult rats KCl response was similar between strains, suggesting no changes in SMC contractility. Indomethacine had no effect on ID, discarding a prostacyclin defect. L-NAME significantly reduced ID in basal conditions and abolished the differences in ID between strains (at 70 mm Hg IDSHR=186±4, IDWKY=200±10, p=0.22) suggesting a defect in NO pathway. In the presence of L-NAME, to exclude endogenous NO, SNP relaxation was significantly smaller in adult, but not in 30 day-old, SHR rats. MCAs from adult SHR showed an increase in NO and a decrease in O2- availability. Conclusions.In MCA from adult SHR: 1) an increased intrinsic tone due to a defect in NO-cGMP pathway is responsible for the reduction in ID, 2) an increased NO release may act as a mechanism to compensate for the reduced cGMP-mediated responses, and 3) these alterations are due to the effect of hypertension. Supported by MCyT (BFI 2001-0638).

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C33

The effect of farnesyl protein transferase inhibition on human vascular smooth muscle cell proliferation

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Vascular smooth muscle cell (VSMC) proliferation is a predominant component of primary atherosclerotic plaque formation and restenosis following balloon angioplasty. Angioplasty-dependent activation of a p21ras-dependent response following balloon angioplasty is now well established in models of arterial restenosis. Farnesylation of p21ras is an essential step in this cellular signal transduction pathway. We have previously shown inhibition of farnesyl transferase with FTP III inhibits restenosis following balloon angioplasty (Work et al 2001). As part of our present work on balloon injury of human atherosclerotic arteries in organ culture and culture of non-atherosclerotic veins we studied the effect of FTP III on VSMC proliferation. FTP III concentration-dependently (1-50μM) inhibited smooth muscle

cell proliferation as measured by [3H] thymidine incorporation (IC50, 10±1mM. To mimic in-vivo short term delivery we exposed cells to FTP III (25μM) in a time-dependent manner (15mins-120mins). Time-dependent exposure to FTP III resulted in 45 ± 5% reduction in smooth muscle cell proliferation. This component of our study of the processes involved in the proliferation of VSMCs derived from human arteries and veins demonstrates a likely role for p21ras-dependent mechanisms. Moreover, a 15min exposure to FTP III had profound effects on VSMC proliferation. This finding may have important implications in the clinical setting as short-term local delivery of a drug may have the potential to limit adverse drug complications.

Work et al (2001) Circ. 104:1538-1543

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Adrenergic and serotonergic synergism in the mouse thoracic aorta

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The vasoactive nature of 5-hydroxytryptamine (5-HT) and its involvement in synergy is well-studied. Stupecky *et al.* (1986) discussed synergistic interactions of α_1-adrenergic and serotonergic contractions in the rabbit aorta by using single equi-effective concentrations of agonists producing a response of 0.1g Force, passing the contractile 'threshold stimulus'. Synergism was demonstrated where combinations of these agonists concentrations produced responses of 0.5g to 2.7g Force. Agonist synergy is also indicated from the sensitivity of the concentration response curve (CRC). We have investigated synergy between 5-HT and α_1-AR mediated contractions in the mouse aorta. 4 month old male (30-40g) 129/Sv/C57BL/6J mice were euthanased by CO_2 and their aortae isolated. 2mm rings were mounted on wire myographs in Krebs at 37°C. After an initial challenge to 125mM KCl, the endothelium was tested with 30μM ACh (10μM phenylephrine/PE precontraction). Cumulative CRCs (1nM-300μM) were constructed to PE or 5-HT in the absence or presence of 10 or 30nM 5-HT or PE respectively. Maximum responses and logEC_{50} values were compared using a one-way ANOVA with a Bonferroni post-test.The maximum response to PE (Mean ± S.E.M., 0.97 ± 0.06g) was unaffected by 10nM (1.00 ± 0.12g) or 30nM 5-HT (1.01 ± 0.07g). Similarly, the maximum 5-HT response (1.33 ± 0.10g) was unaffected by 10nM (1.53 ± 0.18g) or 30nM PE (1.12 ± 0.09g). 10nM 5-HT or PE had no effect on PE or 5-HT sensitivity respectively. However 30nM 5-HT caused a 6-fold increase in sensitivity to PE whilst 30nM PE resulted in 5-HT sensitivity being increased 3-fold (Table 1).In our preparation 10nM PE or 5-HT was a sub-threshold concentration for contraction, whilst 30nM PE or 5-HT was sufficient to cause a contraction (i.e. over the threshold stimulus). Addition of the agonist on top of this supra-threshold concentration resulted in an enhanced response. We have demonstrated there needs to be an increased tone as a result of the PE or 5-HT treatment before synergy can be observed. Thus our results agree with Stupecky *et al.* (1986). Furthermore, the small 6-fold and 3-fold increases in sensitivity are likely to be a result of "mutual effect amplification" described by Leff's (1987) mathematical model of synergy. In conclusion, serotonergic and α_1-adrenergic synergy has now been demonstrated in the mouse thoracic aorta

Table 1. log EC_{50} values of PE in the absence and presence of 10nM & 30nM 5-HT and 5-HT in the absence and presence of 10nM & 30nM PE (Mean ± S.E.M., *'n'*, $^*p<0.05$)

	PE	5-HT
Control	-6.47 ± 0.09, *12*	-6.84 ± 0.02, *12*
+ 10nM	-6.44 ± 0.15, *6*	-6.86 ± 0.06, *6*
+ 30nM	-7.29 ± 0.14*, *7*	-7.34 ± 0.20*, *7*

Leff P (1987) *J. Pharmacol. Exp. Ther.* **243** 1035-1042
Stupecky G *et al.* (1986) *J. Pharmacol. Exp. Ther.* **238** 802-808

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