C1 and PC10

Urocortin-induced vasodilatation of coronary artery: role of SOC entry and iPLA2

A. Domínguez-Rodríguez[1], A. Hmadcha[2], E. Calderon[1], A. Ordóñez[1] and T. Smani[1]

[1]Cirugia, Universidad de Sevilla, Sevilla, Spain and [2]Centro Andaluz de Biología Molecular & Medicina Regenerativa, Sevilla, Spain

Urocortin (Ucn) has been shown to produce vasodilatation in several arteries but the precise mechanism of its action is still poorly understood. Here we studied the role of store-operated Ca^{2+} (SOC) entry and Ca^{2+}-independent phospholipase A2 (iPLA2) in agonist-induced vasoconstriction of rat coronary artery and their possible modulation by Ucn. Ca^{2+} measurement and vessel contraction were studied in SMC and rat coronary arteries as described previously (1,2). We show that Ucn vasodilates phenylephrine hydrochloride (PE)-induced rat coronary artery contractions independently of the L-type Ca^{2+} channel pathway. Moreover, we demonstrate that SOC entry regulated by iPLA2 is involved in the vasoconstriction. We found that SOC channels inhibition by 2-aminoethoxydiphenyl borate (2APB), diethylstilbestrol (DES) and iPLA2 inhibition by bromoenol lactone (BEL) produces relaxation of PE, but not high K^+, -induced contraction. In addition, we found that Ucn inhibits SOC influx evoked by the emptying of the stores with thapsigargin in rat coronary smooth muscle cells. Ucn inhibits Ca^{2+} and Mn^{2+} influx induced by thapsigargin in primary cultured and freshly dispersed SMC. Furthermore, Ucn inhibits iPLA2 activity and decreases the expression of iPLA2 RNA messenger and proteins. The present study provides evidence to demonstrate a new mechanism of relaxation induced by Ucn that involves iPLA2 and SOC entry modulation in rat coronary artery.

Smani T, Zakharov SI, Csutora P, Leno E, Trepakova ES & Bolotina VM (2004). Nat Cell Biol 6, 113-120.

Del Valle-Rodriguez A, Calderon E, Ruiz M, Ordonez A, Lopez-Barneo J & Urena J (2006). Proc Natl Acad Sci U S A 103, 4316-21.

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

C2 and PC11

Role of receptor-operated (TRPC6) calcium entry in human pulmonary artery smooth muscle cells in response to hypoxia

F. Meng, W.K. To, P. Kumar and Y. Gu

Department of Physiology, University of Birmingham, Birmingham, UK

Hypoxic pulmonary vasoconstriction (HPV) is a physiological defence mechanism that, through contraction of pulmonary artery smooth muscle cells (PASMC), enables redistribution of pulmonary blood flow away from areas of lowered O_2 tension toward better-ventilated areas. Increases in $[Ca^{2+}]_i$ are primary events in the contraction of PASMC and the current, general concept is that this hypoxia-mediated increase is subsequent to voltage-gated Ca^{2+} entry following inhibition of K^+ channels (Ward *et al.* 2004; Archer *et al.* 2006).

Primary cultured, human pulmonary artery smooth muscle cells (hPASMC) were grown in smooth muscle growing medium (Promocell Ltd) under 5% CO_2 at 37ºC. Cells were used within 5 passages and $[Ca^{2+}]_i$ imaged using fura-2. Significance (taken as $P<0.05$) was determined using Student's t-test. Data shown as mean ± S.E.M.

Depolarisation of cells with hypoxia solution (P_{O2} *ca.* 20mmHg) evoked a transient $[Ca^{2+}]_i$ elevation that was reduced by 68.8±4.4% by removal of extracellular calcium (+EGTA). Nifedipine (20mM) and verapamil (20mM) attenuated the intracellular $[Ca^{2+}]_i$ elevation induced by hypoxia by around 30% suggesting the presence of alternate Ca^{2+} entry pathways. Expression of the Na^+-Ca^{2+} exchanger, TRPC1 and 6 in hPASMC but not TRPC3, 4, 5 and 7 was found by RT-PCR and confirmed by Western blot. The antagonist of the Na^+-Ca^{2+} exchanger, KBR9743 (10μM), enhanced, slightly, the $[Ca^{2+}]_i$ response to hypoxia, whilst the general antagonists for TRPC, 2APB (100μM) and SKF (40μM), significantly reduced the response to 41.5 ±8.2% and 37.5±3.3% respectively. TRPC6 in hPASMC was knocked out by stealth siRNA (Invitrogen). After 48 hours, the protein levels for TRPC6 were greatly reduced and the hypoxia response was reduced to 21% of the control, suggesting that TRPC6 might mediate the initial response to hypoxia. U73122 (10 μM), an antagonist of PLC-PI and D609 (10 μM), an antagonist of PLC-PK, inhibited 18.2±3.3% and 11.8±3.0% of the $[Ca^{2+}]_i$ response to hypoxia, respectively. Considering the proportion of calcium response mediating by TRPC channel, the relatively small effect of PLC suggests other mechanisms must be present to activate TRP channels. AICAR (1mM), the agonist of AMP-activated kinase (AMPK), induced a gradual calcium elevation. Compound C (40μM), the antagonist of AMPK, almost abolished the hypoxia response, suggesting that AMPK may activate TRPC6. Co-immunoprecipitation revealed that AMPK was not co-localised with TRPC6.

Taken together, our data support a role for TRPC6 in mediating the $[Ca^{2+}]_i$ elevation in response to hypoxia in PASMC and suggest that this response may be linked to cellular energy status via an elevation in AMPK.

Archer SL *et al.* (2006). *Novartis Found Symp.* **272**, 157-171

Ward JP *et al.* (2004). *Cell Calcium* **36**, 209-220

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C3 and PC12

Isolated endothelial cells from rat mesenteric artery display spontaneous outward currents

V.S. Telezhkin, P.L. Yarova, C.J. Garland and K.A. Dora

Pharmacy and Pharmacology, University of Bath, Bath, UK

Spontaneous outward currents have been recorded in freshly isolated single vascular endothelial cells from the aorta. The currents reflect the activation of K^+ channels, and are reminiscent of equivalent and well-characterized events in vascular smooth muscle. The latter serve to suppress muscle contraction, of particular importance in arteries developing spontaneous (myogenic) tone. Endothelial cells exert a significant predominantly

inhibitory influence on vascular smooth muscle tone, which in small resistance arteries importantly involves a spread of hyperpolarization to the muscle. As the characteristics of vascular cells are known to change considerably as artery size decreases, we investigated whether endothelial cells in resistance-size arteries develop spontaneous outward currents.

Endothelial cell sheets were isolated as previously described (McSherry I et al. 2005). Throughout all recording periods, the cells were continually superfused and kept at room temperature (21-23°C). For measurement of membrane currents, the conventional whole-cell patch-clamp technique was used. In most experiments membrane voltage was clamped at -20 mV, which is close to the resting membrane potential of these cells. In separate experiments, to measure changes in endothelial cell $[Ca^{2+}]_i$, cells were incubated with the Ca^{2+} indicator Oregon Green 488 BAPTA-1 AM (10 μM) for at least 40 min. Fluorescence intensity (F) was recorded using a laser scanning confocal system (Olympus FV500) with Tiempo software at 0.5-1 Hz. Data were only used if the cell responded to 1 μM acetylcholine, and either evoked an outward current of at least 20 pA, or a clear increase in fluorescence intensity (F/F_0 greater than 0.3). Values are means ± S.E.M.

Spontaneous outward currents were recorded from 10.3% of cells (n=58 sheets of cells). At a holding potential of -20 mV, outward current developed with a frequency of 0.09 ± 0.01 Hz (range 0.03-0.15 Hz) and amplitude of 107 ± 29 pA (n=6 sheets of cells). The amplitude of spontaneous currents decreased progressively as voltage was stepped from -20 mV to -70 mV (n=3 sheets of cells). Spontaneous oscillations in $[Ca^{2+}]_i$ were observed in 10.5% of cells (4 cells of 38 cells in 4 sheets of cells). The amplitude of spontaneous oscillations in $[Ca^{2+}]_i$ reached 69 ± 12% of the response to 1μM acetylcholine, and the frequency of $[Ca^{2+}]_i$ oscillations was 0.04 ± 0.02 Hz (n=4 cells).

These data indicate that endothelial cells from small resistance arteries develop spontaneous outward currents, probably reflecting the activation of Ca^{2+}-activated K^+ channels. This suggests that spontaneous hyperpolarization originating in the endothelium may contribute to the regulation of vascular tone.

Rusko J et al. (1992). J Physiol 455, 601-621.

Hoyer J et al. (1998). FASEB J 12, 359-66.

McSherry I et al. (2005). Cell Calcium 38, 23-33.

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C4 and PC13

Intracellular Ca^{2+} stores play a major role in Ca^{2+} signalling in endothelial and smooth muscle cells of pre-capillary arterioles and are resistant to removal of external Ca^{2+}

L. Borisova[1], D. Eisner[2], S. Wray[1] and T. Burdyga[1]

[1]Department of Physiology, University of Liverpool, Liverpool, UK and [2]Unit of Cardiac Physiology, University of Manchester, Manchester, UK

Elevations in cytosolic Ca^{2+} to chemical or hormonal transmitters and/or changes in physical parameters can control multiple cellular processes in endothelial cells and vasomotion in smooth muscle cells of the terminal branches of pre-capillary arterioles.The patterns, mechanism of initiation, and sources of Ca^{2+} involved in control of Ca^{2+} signalling induced by these factors in pre-capillary vessels are poorly understood. We have therefore investigated Ca^{2+} signalling in *in situ* rat pre-capillary arterioles, using Fluo-4 and wide-field, confocal imaging to monitor changes in $[Ca^{2+}]_i$. Endothelial cells showed spontaneous Ca^{2+} events, ranging from Ca^{2+} puffs to Ca^{2+} waves which were strongly potentiated by carbachol (50nM). Time to peak, half-time of relaxation, duration at 50% of peak in the presence of 50nM carbachol were increased from 0.19±0.02 to 0.48±0.04s, 0.29±0.02.19 to 0.59±0.03s, 0.17±0.02 to 0.55±0.06s (n=18), respectively and the frequency of Ca^{2+} puffs in the presence of carbachol was also significantly increased from 0.40±0.02 to 1.02±0.08Hz (n=18). Remarkably, carbachol-induced Ca^{2+} oscillations in endothelial cells could be observed after long (40-60min) exposures to Ca^{2+}-free solutions (n=5) but were reversibly blocked by cyclopiazonic acid (SERCA blocker, n=9), 2-APB (IP_3Rs antagonist, n=5) or U73122 (phospholipase C inhibitor, n=6). In the same preparations and conditions, phenylephrine (10μM) and endothelin-1 (1-10nM) produced asynchronous Ca^{2+} waves in vascular myocytes which were independent of extracellular Ca^{2+} and could be observed after long (60-90 min) exposures to Ca^{2+}-free solutions containing 2mM EGTA (n=9), little affected by ryanodine (n=11), but blocked by 2-APB (n=7) and U73122 (n=6). This was in marked contrast to rat mesenteric artery which under identical experimental conditions showed elevations of cytosolic Ca^{2+} in response to agonists which required both Ca^{2+} release and Ca^{2+} entry and cooperation of both RyRs and IP_3Rs channels.Carbachol activation of the endothelium had no effect on agonist-induced vasomotion in the pre-capillary arterioles but inhibited Ca^{2+} entry and vasoconstriction in rat mesenteric arteries. Inhibition of gap junctions by 18-β-glycyrrhetinic acid (50μM) inhibited communication between endothelial cells and myocytes in mesenteric arteries but had no effect in the pre-capillary arterioles.

We suggest that, unlike larger diameter arteries, in pre-capillary arterioles, vasomotion evoked by agonists is insensitive to the endothelium and produced exclusively by intracellular Ca^{2+} waves in the form of asynchronous Ca^{2+} oscillations, mediated by IP_3Rs.

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C5 and PC14

Differential mechanisms underlying potassium- and EDHF-mediated uterine vasodilatation

N. Gokina, T. Goeks and O. Kuzina

Department of Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA

Normal pregnancy is characterized by a dramatic increase in uterine blood flow, in part, due to augmented uterine vasodilatation. EDHF plays an important role in endothelium-mediated dilatation of uterine arteries; however, the nature of EDHF

and its regulation by pregnancy remains largely unknown. The purpose of this study was to: (1) explore the role of small (SK) and intermediate (IK) conductance K^+ channels in EDHF-dependent vasodilatation; (2) study vasodilator effects of K^+ ions in uterine arteries of non-pregnant (NP) and late pregnant (LP) rats; (3) evaluate the role of K^+ ions as EDHF.

Rats were anesthetized with intraperitoneal injection of Nembutal and killed by decapitation. Segments of uterine radial arteries were cannulated and pressurized to 50 mmHg. Smooth muscle cells (SMCs) were loaded with fura 2 (5 μM); fura 2 fluorescence was measured using a photo-multiplier system. ACh- or K^+-induced changes in arterial diameter and SMC Ca^{2+} ($[Ca^{2+}]_i$) were studied in arteries pre-constricted with phenylephrine (PE) in the presence of 200 μM L-NNA and 10 μM indomethacin.

ACh (0.03-10 μM) induced a dose-dependent dilatation that was greater in arteries from LP rats compared to NP rats (26 ± 6, n = 22 vs. 5 ± 2%, n = 21 at 0.03 μM ACh, $P<0.01$, ANOVA) and was associated with a more marked reduction in $[Ca^{2+}]_i$ (42 ± 12%, n = 3 vs. 11 ± 4%, n = 6, $P<0.01$). Combined treatment of NP or LP arteries with 100 nM apamin and 30 nM charybdotoxin (or 10 μM TRAM 34) abolished the ACh-induced reduction in $[Ca^{2+}]_i$ and vasodilatation. 5 mM K^+ reduced $[Ca^{2+}]_i$ and dilated PE-pre-constricted NP arteries by 97 ± 2% (n = 5) and 92 ± 5% (n = 5) and LP arteries by 58 ± 10% (n = 5) and 60 ± 9%, (n = 8), respectively. K^+-evoked changes in $[Ca^{2+}]_i$ and diameters were significantly smaller in vessels from LP rats compared to NP controls ($P<0.05$, unpaired t-test). K^+-induced responses were incompletely inhibited with 50-100 μM $BaCl_2$ but were abolished by a combination of $BaCl_2$ and 10 μM ouabain. However, ACh-induced $[Ca^{2+}]_i$ reduction and vasodilatation were not affected by $BaCl_2$ and ouabain.

These findings demonstrate that pregnancy augments EDHF-dependent dilatation of uterine arteries in response to ACh. Endothelial SK and IK channels are critically involved in EDHF-mediated uterine vasodilation. K^+-induced vasodilatation is due to activation of both inward rectifier K^+ channels and Na^+-K^+ pump. Although K^+ ions can effectively dilate uterine arteries, they do not contribute significantly to the EDHF-mediated vasodilatation induced by ACh. These data suggested that electrotonic spreading of hyperpolarization from ECs to SMCs may be the underlying mechanism of EDHF in the maternal uterine circulation.

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C6 and PC15

Reduced expression of SKCa and IKCa channel proteins in rat small mesenteric arteries during angiotensin II-induced hypertension

R.H. Hilgers[1,2], J.G. De Mey[1] and R.C. Webb[2]

[1]Pharmacology & Toxicology, University of Maastricht, Maastricht, Netherlands and [2]Physiology, Medical College of Georgia, Augusta, GA, USA

Ca^{2+}-activated K^+-channels (KCa), in particular the small- and intermediate (SKCa and IKCa) channels are key players in endothelium-derived hyperpolarizing factor (EDHF)-mediated relaxation in small arteries. Hypertension is characterized by an endothelial dysfunction possibly via reduced EDHF release and/or function. We hypothesize that during angiotensin II (14-days)-induced hypertension (Ang II-14d), the contribution of SKCa and IKCa channels in ACh-induced relaxations is reduced, due to decreased expression of SKCa and IKCa channel proteins in rat small mesenteric arteries.

Nitric oxide- and prostacyclin-independent vasorelaxation to ACh was similar in small mesenteric arteries (MA) of sham-operated (untreated, SHAM) and Ang II-14d rats. Catalase had no inhibitory effects on these relaxations. The highly selective SKCa channel blocker UCL 1684 almost completely blocked these responses in MA of SHAM, but partially in MA of Ang II-14d rats. These changes were pressure-dependent since UCL 1684 caused a greater inhibition in MA of 1-day Ang II-treated normotensive rats compared to Ang II-14d rats. Expression levels of both mRNA and protein SK3 were significantly reduced in MA of Ang II-14d. The IKCa channel blocker TRAM-34 resulted in comparable reductions in the relaxation responses to ACh in MA of SHAM and Ang II-14d. Relative mRNA expression levels of IK1 were significantly reduced in MA of Ang II-14d, whereas protein levels of IK1 were not, but tended to be lower in MA of AngII-14d.

The findings demonstrate that EDHF-like responses are not compromised in a situation of reduced functional activity and expression of SK3 channels in small mesenteric arteries of Ang II-induced hypertensive rats. The role of IK1 channels is less clear, but might compensate for reduced SK3 activity.

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C7 and PC16

A shared pathway for hydrogen peroxide and 4-aminopyridine inhibition of whole-cell K^+ currents in isolated human uterine smooth muscle cells

G.K. Fyfe[1], G. Bru-Mercier[2], A.M. Blanks[2], A. Shmygol[2], S. Thornton[2], R.N. Khan[3] and M.J. Taggart[1]

[1]Maternal and Fetal Health Research Centre, The University of Manchester, Manchester, UK, [2]Clinical Sciences Research Institute, University of Warwick, Coventry, UK and [3]Institute for Clinical Research, University of Nottingham, Derby, UK

Ischaemic-reperfusion cycles, and concomitant hypoxia-reoxygenation periods, are likely to generate reactive oxygen species (ROS). In human myometrium, such cycles likely occur during labour. An additional source of ROS may be the invasion of leukocytes into the human myometrium at the time of spontaneous labour (1,2). These raise the possibility that ROS generation within the myometrium is a mechanism governing labour-associated contractility. In many cell types, ROS have been associated with alterations in cellular excitability via effects on K^+ channels (3). As myometrial excitability, and therefore contractility, is likely to be governed by K^+ channels we examined the effect of hydrogen peroxide (H_2O_2) on whole-cell currents

of uterine myocytes enzymatically isolated from biopsies collected from women at Caesarean section (following written informed consent).
Whole-cell voltage clamp (using physiological K^+ solutions) was used to assess the effects of H_2O_2 (10 or 100 μM) on transmembrane currents. Cells were held at -50mV and pulsed from -60 to +50mV in 10mV steps each of 200msec duration. All data were normalised to control current values and expressed as mean±SEM. Statistical comparisons of steady-state current levels (140 ms into a 200 ms sweep) between treatments were made at +40 mV, using arcsine transformation of individual data points followed by a paired t test.
Peak outward current was 1.34±0.38 nA (n=16). Treatment of uterine myocytes with 10 μM or 100 μM H_2O_2 resulted in inhibition of outward current at all potentials positive to 0mV. The slopes of log transformed current-voltage plots were similar in control and H_2O_2 conditions. 10 μM and 100 μM H_2O_2 inhibited current at +40mV by 19.9±7.6% and 32.1±11.9% respectively (n=4; $p<0.01$ for both). Separate application of 4-aminopyridine (4AP, 5 mM), a blocker of voltage-dependent K^+ currents, also resulted in reduction of outward current at positive voltages. At +40mV outward current was significantly inhibited by 36.6±7.9% (n=8, $p<0.05$). In a separate series of experiments, treatment with 4AP alone followed by 4AP and 100 μM H_2O_2 together gave current inhibitions of 25.9±5.7 % (n=5) and 23.6±5.7 % (n=5, $p>0.05$ compared to 4AP alone) respectively. We conclude that H_2O_2 inhibits K^+ channel current sensitive to 4AP in human uterine myocytes. A likely uterine target, therefore, for H_2O_2, and potentially other ROS, is voltage-gated K^+ channels.

Thomson AJ *et al.* (1999). *Human Reprod* **14**, 229-236.

Kobayashi T *et al.* (2001). *Redox Rep* **6**, 27-36.

Liu Y & Gutterman DD (2002). *Vascul Pharmacol* **38**, 43-49.

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C8 and PC17

Pericytes can regulate capillary diameter in rat retina and cerebellum

C. Howarth, C.M. Peppiatt-Wildman, P. Mobbs and D. Attwell

Physiology, UCL, London, UK

Neural activity increases local blood flow in the CNS, and this increase is the basis of both BOLD and PET functional imaging techniques. It is usually assumed that blood flow is controlled by smooth muscle surrounding pre-capillary arterioles, which is innervated by noradrenaline-releasing neurons. However, 65% of vascular noradrenergic innervation in the brain is of capillaries rather than arterioles (Cohen *et al.* 1997), suggesting that blood flow may also be regulated at the capillary level. Capillaries lack smooth muscle but in places are surrounded by contractile cells called pericytes, which express both muscle and non-muscle actin and myosin. In culture or on isolated blood vessels, pericytes have been shown to contract and dilate in response to various neurotransmitters, resulting in a local change in capillary diameter. Here we show a role for pericytes in controlling capillary diameter in situ in whole retina and in cerebellar slices.
Pericytes were labelled by an antibody directed at the membrane chondroitin sulfate proteoglycan NG2, and were found to be separated by a distance of 34.2±3.6 μm (mean±s.e.m., n=24) along retinal capillaries.
Electrical stimulation of retinal pericytes evoked a localised constriction of capillaries, which propagated along the capillary at about 2 μm/s to evoke constriction of distant pericytes. Superfused ATP or UTP also caused localised constriction of capillaries at points close to pericytes, suggesting a possible role for purinergic P2 receptors in the control of capillary diameter. Pericyte constriction led to a reduction in capillary diameter by up to 77%, implying a flow reduction of 99% by Poiseuille's law for laminar flow, and a larger reduction for flow involving red blood cell movement through narrow capillaries. In the first few minutes of simulated ischaemia, retinal pericytes constricted capillaries at localised points. In cerebellar slices, noradrenaline constricted pericytes on capillaries in the molecular layer. This constriction could be reversed by applying glutamate to mimic glutamate release by neuronal activity.
Electrical stimulation near the retinal inner plexiform layer evoked a pericyte constriction which was blocked by TTX. Puffing GABA receptor blockers also evoked a constriction. Thus, neuronal activity can regulate retinal capillary diameter via pericytes.
These data identify pericytes as possible contributors to the vascular response to changes in neural activity. Pericytes potentially have a role in CNS vascular disease and therapy.

Cohen Z *et al.* (1997). *J Cereb Blood Flow Metab* **17**, 894-904.

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C9 and PC18

Magnesium and lithium ions modulate the single-channel conductance of the large conductance potassium (BKCa) channel expressed in the HEK 293 cell line

F. Saleem, I.C. Rowe and M.J. Shipston

Membrane Biology Group, Centre for Integrative Physiology, College of Medicine & Veterinary Medicine, Hugh Robson Building, University of Edinburgh, Edinburgh, EH8 9XD, UK

Large conductance voltage- and calcium-activated potassium (BKCa) channels link electrical excitability with cellular signalling. Multiple conserved sites on the c-terminus of the α-subunit confer regulation by divalent cations such as magnesium, which can alter both single-channel conductance and open probability (Po) (Yang et al. 2006). However, alternative splicing of the α-subunit can alter BKCa channel properties including sensitivity to divalent cations. Here we investigated the ability of magnesium ions to modulate the single-channel properties of an alternatively spliced BKCa channel variant, the stress regulated exon (STREX). In addition, as the monovalent lithium ion shares many physicochemical properties, and interacts in a competitive manner, with magnesium (Mota de Freitas et al. 2006) the effects of lithium were also investigated.
Single-channel patch clamp recording was used to determine the activity of the STREX BKCa channel variant expressed in HEK

293 cells. Excised inside-out patch recordings were made in symmetrical 140mM KCl, using pipettes with resistances of 5-15MΩ. The intracellular free calcium ion concentration was buffered using BAPTA to 0.1 or 1.0 μM.
In excised inside out patches, when the concentration of free magnesium or free lithium at the intracellular surface of the membrane was raised from 2mM to 6mM the STREX variant single-channel amplitude was reduced by 30% and 25% respectively. For example, at 40mV the channel amplitude fell from 10.5 ± 0.1 pA (n=13) to 7.5 ± 0.1 pA in magnesium (n=4) and 7.7 ± 0.4 pA in lithium (n=5) (data are mean ± s.e.m., $p<0.0001$ ANOVA plus Tukey post-hoc). This was accompanied by a significant increase in Po from 0.21 ± 0.01 (n=13) to 0.43 ± 0.03 for magnesium (n=4) at 40mV ($p<0.0001$ Student's paired t-test). In contrast, lithium had no significant effect on STREX variant Po. The effects of both ions were voltage dependent in that the decrease in channel amplitude was only observed at depolarized potentials (+20mV to +80mV), and were reversible upon washout. Control experiments for lithium in which the monovalent ion sodium was increased from 2mM to 6mM did not alter either the Po or single-channel conductance of the STREX variant. The data indicate that the STREX BKCa channel variant is sensitive to modulation by both magnesium and lithium ions. However, the ions display a difference in their ability to modulate conductance and Po, which may be indicative of separate sites of action. Further work, including site directed mutagenesis will hopefully resolve these sites.

Mota de Freitas D, Castro MM & Geraldes CF (2006). Acc Chem Res 39, 283-291.

Yang H, Hu L, Shi J & Cui J (2006). Biophys J 91, 2892-2900.

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C10 and PC19

Role of vascular smooth muscle Kv channels in a mouse model of essential hypertension

P. Cidad, A. Moreno-Domínguez, E. Alonso, J.R. López-López and M.T. Pérez-García

Department of Physiology, Universidad de Valladolid, Valladolid, Spain

Essential hypertension involves a gradual and sustained increase in total peripheral resistance, reflecting an increased vascular tone. This increased vascular tone is associated with a depolarization of vascular smooth muscle cells (VSMCs) and relies on a change in the expression profile of voltage-dependent ion channels (mainly Ca^{2+} and K^+ channels) that promotes arterial contraction. Voltage-dependent K^+ channels (Kv channels) are principal contributors to determine the resting membrane potential of VSMCs. However, the changes in their expression and/or modulation during hypertension are poorly defined, mainly due to their large molecular diversity and to the variations in their expression in the different vascular beds. Here we have undertaken the study of the molecular and functional characterization of Kv channels in VSMCs (both at the mRNA and protein level) and their regulation in essential hypertension by using VSMCs from resistance (mesenteric) or conduit (aortic) arteries obtained from a hypertensive inbred mice strain, BPH, and the corresponding normotensive strain, BPN. Mice were killed by decapitation after isofluorane anaesthesia and arteries were cleaned of connective and endothelial tissues. Real-time PCR using low-density taqman® arrays reveals a differential distribution of Kv channel subunits mRNA in the different vascular beds as well as arterial bed-specific changes under hypertensive conditions. In resistance arteries, the hypertensive phenotype associates with an increase in the expression of Kcng3 and a decrease in Kcnd1 mRNA. To study the functional relevance of these changes, we characterize the electrophysiological properties of freshly dissociated VSMCs from BPN and BPH mice by using the patch-clamp technique. VSMCs from BPH mesenteric arteries were more depolarized than BPN ones, and showed significantly larger capacitance values (23.43±2.1 versus 13.46± 0.8 pF respectively). We found that Kv channels current density was significantly reduced in BPH mesenteric VSMCs at all voltages. Application of 50 nM stromatoxin inhibited 34% of the Kv current at +40 mV in BPN cells, but only 21% in BPH cells, suggesting that the reduction in the functional expression of Kv2 channels in BPH cells contributes to the decreased Kv current in these cells. These data indicate that impaired Kv2 channel activity, mediated by changes in the expression level of the accessory gamma subunit Kcng3, could contribute to the changes in excitability of VSMCs from resistance arteries during essential hypertenison.

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PC1

Flufenamic acid (FFA) stimulates novel calcium channel activity in human conditionally immortalised podocytes (hCIPs) in a nephrin-dependent manner

R.R. Foster[1], G.I. Welsh[1], S.C. Satchell[1], D.O. Bates[2], P.W. Mathieson[1] and M.A. Saleem[1]

[1]Clinical Science @North Bristol, University of Bristol, Bristol, UK and [2]Department of Physiology, University of Bristol, Bristol, UK

Recent studies have linked mutations in TRPC6 (a non-selective cation channel) with progression of renal disease (1,2). These studies showed that the podocyte, within the glomerulus is the target cell for abnormal calcium signalling. Here we demonstrate a difference in calcium channel activity between the two cell types that form the ultrafiltration barrier within the glomerulus (podocytes and glomerular endothelial cells (GEnCs)). This channel activity is dependent on nephrin, a cell adhesion molecule (and podocyte marker) located at the podocyte slit diaphragm. FFA, which has been suggested to activate TRPC6 whilst non-specifically blocking other cation channels (3) was used to stimulate channel activity. Human conditionally immortalised podocytes (hCIPs), nephrin-deficient (ND) hCIPs and hGEnCs were loaded with 10μM Fura2-AM. They were incubated in buffer containing 200nM (low) or 5mM (high) Ca^{2+} and stimulated with 200μM FFA in the presence or absence of 200nM thapsigargin to deplete intracellular calcium ($[Ca^{2+}]_i$) stores, in varying amounts of FCS. Changes in $[Ca^{2+}]_i$ were measured using the normalised fluorescence intensity ratio at excitation wavelengths of 340nm to 380nm (R_{norm}). FFA increased the R_{norm} in hCIPs in the presence and absence of a Ca^{2+} concentration gradient (5mM $[Ca^{2+}]_o$ 1.44±0.11 fold increase, $p<0.01$, n=7; 200nM $[Ca^{2+}]_o$; 1.58±0.01 fold increase, $p<0.01$, n=6 unpaired t-tests), which was significantly reduced when $[Ca^{2+}]_i$ stores were depleted (post-thapsigargin 1.08±0.06 fold increase, $p<0.05$, n=6). However, the presence of FCS post-thapsigargin restored the FFA induced increase in R_{norm} (1.78±0.15 fold increase, $p<0.01$, n=6 unpaired t-test). In contrast the response to FFA in hGEnCs post-thapsigargin was not blocked (2.01±0.12 fold increase, $p<0.01$ paired t-test, n=4). Similarly, in the absence of nephrin (NDhCIPs) the response to FFA was not blocked post-thapsigargin (1.6±0.06 fold increase, $p<0.01$, n=4, paired t-test). The post-thapsigargin response to FFA was FCS dose dependent. In conclusion in hCIPs the FFA induced increase in $[Ca^{2+}]_i$ was dependent on thapsigargin-sensitive $[Ca^{2+}]_i$ stores, except in the presence of FCS whereby it became store independent in an FCS dose-dependent manner. $[Ca^{2+}]_i$ store-dependent activation by FFA was also nephrin dependent and was not seen in hGEnCs. This evidence suggests novel activity of a not-yet specified calcium channel in hCIPs, which is nephrin and FCS dependent and potentially involved in progression of renal disease.

Winn MP et al. (2005). Science 308, 1801-4.

Reiser J et al. (2005). Nat Genet 37, 739-44.

Inoue R et al. (2001). Circ Res 88, 325-32.

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

PC2

Characterization of the TRPM8 calcium channel in rat aorta and tail artery

D. Melanaphy[1], C.D. Johnson[1], D. Paula[1], P. Anderson[2] and Z. Alexander[1]

[1]Cell and Metabolic Signalling Research Group, Queen's University, Belfast, UK and [2]Respiratory Research Group, Queen's University, Belfast, UK

Ca^{2+}-permeable cation channels not only control excitability of vascular myocytes but also form an important Ca^{2+} channeling route. Several mammalian homologues of Drosophila transient receptor potential (TRP) proteins are strong candidates for such channels (Beech, 2005). Recent studies of novel melastatin TRPM8 channels have focused on their role in cold transduction in sensory neurones. However, there are now reports that these receptors are present in blood vessels and may be active in vascular tone (Yang et al., 2006). We have examined this possibility in detail using functional and molecular techniques.

Vascular tissue was taken from humanely-dispatched Wistar rats (12 weeks). Sections of precontracted proximal tail artery and thoracic aorta with endothelia removed were mounted in Krebs for isometric contraction study. Vasoconstrictions induced by KCl (60 mM; tail: 0.85±0.13 g, n=7; aorta: 0.88±0.09 g, n=6) were inhibited (paired Student's t-test, performed on absolute data) by addition of selective TRPM8 channel agonists, menthol (300 μM; tail: 43±9 % control, $P< 0.01$; aorta: 61±10 %, $P< 0.01$) or icilin (50 μM; tail: 39±16 % control, n=3, $P< 0.05$; aorta: 42±7 %, n=3, $P< 0.05$). Similar results were obtained with contractions evoked by noradrenaline (2 μM). Sympathetically-evoked constrictions (0.6±0.32 g, n=3) in tail artery were significantly reduced by menthol (56±9 % control, $P< 0.05$).

Single vascular smooth muscle cells (VSMCs) were isolated from proximal tail artery for patch-clamp recordings. With high K^+ (125 mM) and low intracellular Ca^{2+} buffering (0.3 mM EGTA in the pipette solution), STOCs discharge (reflecting BKCa channel activation due to spontaneous localised Ca^{2+} release events) was observed at 1.4-4.4 Hz at holding potentials from -40 to -10 mV. These STOCs were significantly accelerated by 100 μM menthol application (75±19% frequency increase, $P<0.01$, n=4), while the amplitude of these currents was markedly reduced. Eventually (1-2 min after menthol application) STOCs discharge was terminated.

PCR studies in VSMCs from thoracic aorta, tail, femoral, renal and mesenteric arteries with endothelia removed all showed the presence of mRNA for TRPM8 receptor proteins.

We conclude that TRPM8 channels are present and functional in a range of different blood vessels. Since they can be localised not only in the plasma membrane, but also in the SR membrane (Abeele et al., 2006) Ca^{2+} store release and depletion evidenced by STOCs initial acceleration and eventual suppression seems the most likely mechanism of vasoconstrictor inhibition by TRPM8 agonists.

Abeele FV et al. (2006). J Biol Chem 281, 40174-40182.

Beech D (2005). Clin Exp Pharmacol Phys 32, 597-603.

Yang XR et al. (2006). Am J Physiol Cell Mol Physiol 290, L1267-76.

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

PC3

Ionic currents, STICs, STOCs and membrane potential in rat coronary artery smooth muscle cells

C. Prendergast[1], J. Quayle[2], T. Burdyga[1] and S. Wray[1]

[1]The Physiological Laboratory, University of Liverpool, Liverpool, UK and [2]Human Anatomy and Cell Biology, University of Liverpool, Liverpool, UK

Using confocal microscopy on preparations of intact rat coronary artery (CA), we have observed that CA smooth muscle cells *in situ* generate frequent Ca^{2+} sparks from multiple discharging sites. In order to examine the SR Ca^{2+} releases and associated currents in more detail, an enzymatic isolation procedure was employed to produce isolated cells. Ca^{2+} sparks could still be observed in these cells following isolation. Cells were patch clamped and whole cell outward current measured. In all cells, a large sustained outward current was observed that exhibited oscillatory behaviour at very positive potentials. We have shown that approximately 70% of this whole cell current is mediated by voltage-dependent delayed rectifier K^+ channels (K_v) and large conductance Ca^{2+}-dependent K^+ (BK_{Ca}) channels. Spontaneous transient outward currents (STOCs) were observed in these cells, the amplitudes of which were voltage-dependent. At 0 mV, STOCs attained amplitudes of up to 250 pA, whereas at -40 mV, amplitudes of 5-40 pA were observed. At very negative potentials (-80 mV), STOCs were no longer generated, but small spontaneous transient inward currents (STICs: 5-30 pA) were observed instead, which exhibited sensitivity to the Ca^{2+}-activated Cl^- channel (Cl_{Ca}) blocker, niflumic acid (10 μM). Using current clamp conditions, the resting membrane potential was determined to be -41.6 ± 2.5 mV (n = 25). The effect of ion channel inhibition on membrane potential was examined and the influence of resting membrane potential on the generation of spontaneous currents, in response to SR Ca^{2+} releases, was studied. Membrane potential was shown to be dependent on K_v, BK_{Ca} and Cl_{Ca} channels. The response to the generation of Ca^{2+} sparks by 1 mM caffeine exhibited membrane potential dependence, with STOCs occurring when membrane potential was relatively depolarised and STICs occurring when membrane potential was relatively hyperpolarised. At a holding potential of -40 mV, similar to the physiological resting membrane potential, spontaneously produced Ca^{2+} sparks generated STOCs in almost all cells (17/18 cells); however, STICs were also observed on some occasions (4/18 cells). To our knowledge, this is the first time that both STOCs and STICs have been observed in coronary artery smooth muscle cells and both events were shown to be highly membrane potential sensitive.

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PC4

Evidence for a high sensitivity of large conductance Ca^{2+}-activated K^+ channels in rat aortic smooth muscle cells to ionized irradiation

A.I. Soloviev, S.M. Tishkin and I.V. Ivanova

Experimental Therapeutics, Institute of Pharmacology and Toxicology, Academy of Medical Science, Kiev, Ukraine

It is known that γ-irradiation significantly suppresses large conductance Ca^{2+}-activated K^+ channels (BKCa) in rat coronary artery endothelial cells (Soloviev et al. 2005), and may contribute to radiation-induced endothelium-dependent vascular disorders (Soloviev et al. 2003). The goal of this study was to evaluate the influence of whole body γ-irradiation (6 Gy) on BKCa function in rat thoracic aorta smooth muscle cells using patch-clamp technique in whole-cell modification. During irradiation, Wistar rats (250 g b.w.) were restrained in a plastic box, and the radiation beam was focused on the animal's chest. There was no change in housing, standard food or drinking water following irradiation. The animals were closely observed for unwanted effects and there were no visible signs of discomfort or illness. On the 9th and 30th days post-irradiation the thoracic aorta was taken from animals anaesthetized with ketamine/xylasine (1 ml/kg b.w.) to obtain isolated aortic smooth muscle cells.

The stimulation of freshly isolated smooth muscle control cells by increasingly depolarized voltage steps showed the current-voltage relationship which demonstrated clearly expressed outward rectification with the reversal potential of -40 ± 5 mV. The current density amplitude was 52±6 pA/pF (n=10) at +70 mV. Paxillin (500 nM), selective inhibitor of BKCa channels, being added to the external solution, decreased outward potassium current density to 21 ± 6 pA/pF. Outward currents in smooth muscle cells obtained from irradiated animals on the 9th and 30th days post-irradiation demonstrated a significant decrease of K^+ current density amplitudes to 35±8 pA/pF (n=7) and 20±3 pA/pF (n=8), respectively. There was no significant shift in reversal potentials under irradiation for these whole-cell currents. Paxillin decreased K^+ current from 35±8 to 20±9 pA/pF in cells obtained on 9th day post-irradiation, and was without effect on irradiated cells on 30th day post-irradiation indicating the absence of conductance through BKCa channels.

In conclusion, the data obtained clearly demonstrate that non-fatal whole-body γ-irradiation suppresses a large conductance Ca^{2+}-activated K^+ channels in vascular smooth muscle cells. Radiation-induced inhibition of BKCa channels could contribute to vascular hypercontractility and an increase in arterial blood pressure.

Soloviev A, Tishkin S, Rekalov V, Ivanova I & Moreland R (2005). J Physiol 568P, 14P-15P.

Soloviev A, Tishkin S, Parshikov A, Ivanova I, Goncharov E & Gurney A (2003). Br J Pharmacol 138, 837-844.

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PC5

Molecular identification of transient receptor potential channels in rat retinal arterioles

T. Curtis[1], J. McKee[1,2], D.P. Dash[1], A. Arora[1], C.N. Scholfield[2], J.G. McGeown[2] and D.A. Simpson[1]

[1]Centre for Vision Sciences, Queen's University of Belfast, Belfast, UK and [2]Cell and Metabolic Signalling Group, Queen's University of Belfast, Belfast, UK

Our previous studies using electrophysiological and microfluorimetry techniques have suggested that several Ca^{2+} influx pathways exist in rat retinal arterioles (Scholfield et al. 2007). These include voltage-dependent L-type Ca^{2+} channels activated by cell membrane depolarization and non-voltage-gated receptor- and store-operated Ca^{2+} channels. Recent work has identified members of the transient receptor potential (TRP) channel superfamily as important molecular entities underlying non-voltage-gated Ca^{2+} influx pathways in different tissues, including those of the vasculature (Inoue et al. 2006). In the present study we sought to identify systematically the TRP channel subtypes expressed in rat retinal arterioles. Male Sprague-Dawley rats (200-300g) were anaesthetized with CO_2 and killed by cervical dislocation. Retinas were removed, arterioles mechanically isolated and TRP channel mRNA expression evaluated by RT-PCR. Detection of TRP channel family members was verified in cDNA reverse-transcribed from three different RNA isolations (10 vessels per isolation). Rat brain and kidney samples were used as positive controls. Immunofluorescence staining with commercially available polyclonal antibodies was also used to test for cell-specific expression of TRP channels in retinal arterioles embedded within retinal flatmount preparations. To ensure that the images collected originated from the arterioles the retinas were counter-stained with propidium iodide nuclear stain. Using RT-PCR, 13 TRP channel subtypes were detected in retinal arterioles: TRPC1, TRPC3, TRPC4, TRPC7, TRPV1, TRPV2, TRPV4, TRPM1, TRPM2, TRPM3, TRPM7, TRPML1 and TRPML3. Lack of expression of other TRP channels could not be attributed to primer design since all of the other TRP channel family members could be detected in brain or kidney. Immunofluorescence labelling revealed a punctuate distribution of TRPV2 throughout the vascular smooth muscle cell layer of retinal arterioles, while TRPV4 was specifically localized to the plasma membrane of endothelial cells. Consistent with the RT-PCR results, TRPC5 could not be detected in retinal arterioles. This was not due to a lack of antibody reactivity because intense staining was observed in the ganglion cell layer of the retina. This study defines for the first time the main TRP channel subtypes expressed in retinal arterioles. These channels represent promising candidates for non-voltage-gated Ca^{2+} entry pathways within the retinal microcirculation.

Inoue R, Jensen LJ, Shi J, Morita H, Nishida M, Honda A & Ito Y (2006). Circ Res 99, 119-31.

Scholfield CN, McGeown JG & Curtis TM (2007). Microcirculation (in press).

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PC6

TASK-2 K^+ channel expression in rat mesenteric and femoral arteries

L. Goonetilleke[1,2], T.P. Green[2] and J. Quayle[1]

[1]Human Anatomy and Cell Biology, University of Liverpool, Liverpool, UK and [2]Pharmacology, Liverpool University, Liverpool, UK

Two-pore domain (K_{2P}) K^+ channels may have a role in regulating smooth muscle contraction. For example, TASK-2 channels contribute to setting the membrane potential in smooth muscle cells from murine gastrointestinal tract (Cho *et al.* 2005) and rat pulmonary artery (Gönczi *et al.* 2006). We have investigated the expression of TASK-2 channels by RT-PCR and immunocytochemistry in rat femoral and mesenteric artery. The channel was cloned from rat kidney cDNA and expressed in *Xenopus* oocytes, to further define the properties of this conductance.

RT-PCR analysis using TASK-2 specific mouse primers showed the presence of TASK-2 transcripts in both rat femoral and mesenteric artery whole tissue mRNA. To investigate expression at the protein level we used immunocytochemistry. Single arterial smooth muscle cells were isolated and incubated overnight with primary antibody (rabbit anti-human, 1:100 dilution, Alomone Laboratories). A goat anti-rabbit secondary antibody coupled to Alexa Fluor-488 was used for fluorescent detection in a confocal microscope. Some signal was detected in smooth muscle cells, although this did not appear to be membrane localised. Controls were by omission of primary antibody and by pre-incubation of primary antibody with antigenic peptide. To further investigate expression pattern in the artery, the antibody was tested on 8 μm frozen sections. Once again, a moderate signal was seen in the smooth muscle layer. Some evidence was found for endothelial staining, although definitive evidence awaits co-staining with an endothelial-specific antibody.

In order to further characterise the TASK-2 K^+ conductance, the subunit was cloned from rat kidney cDNA using primers based on the published mouse sequence (submitted to the EMBL Nucleotide Sequence Database, Accession number AM229406). Rat TASK-2 shows 88 and 96% homology to the human and mouse homologues respectively. The differences between the three homologues occur in the C-terminus. TASK-2 formed functional K^+-selective and extracellular pH-sensitive channels when expressed in *Xenopus* oocytes ($pKa = 8.56 \pm 0.03$).

In conclusion, TASK-2 channels were shown to be present in rat femoral and mesenteric arteries by RT-PCR and immunocytochemistry, and may be expressed in both smooth muscle and endothelial cells. The channel was cloned and expressed in *Xenopus* oocytes, and this will facilitate future comparison with native K^+ conductances in vascular cells.

Cho SY, Beckett EA, Baker SA, Han I, Park KJ, Monaghan K, Ward SM, Sanders KM & Koh SD (2005). *J Physiol* 565, 243-259.

Gönczi M, Szentandrássy N, Johnson IT, Heagerty AM & Weston AH (2006). *Br J Pharmacol* 147, 496-505.

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PC7

Ca^{2+}-sensitive Cl^- currents play a key role in endothelin-induced Ca^{2+} responses in myocytes within rat retinal arterioles

M. Stewart[1], T. Curtis[2], S. Norman[1] and J.G. McGeown[1]

[1]Cell and Metabolic Signalling Group, Queen's University Belfast, Belfast, UK and [2]Centre for Vision Science, Queen's University Belfast, Belfast, UK

Endothelin (Et-1) is an important paracrine vasoconstrictor, which is synthesized by the endothelium and stimulates increases in $[Ca^{2+}]$ and contraction in adjacent vascular myocytes. Electrophysiological studies have revealed that Et-1 promotes spontaneous transient depolarisations in retinal arterioles. These responses are mediated by Ca^{2+}-activated Cl^- currents which can be blocked by niflumic acid and anthracene 9-carboxylate (Scholfield et al. 2006). In this study we tested the hypothesis that positive feedback via activation of Ca^{2+}-sensitive Cl^- currents contributes to Et-1-induced Ca^{2+} signals. Male Sprague-Dawley rats (200-300g) were anaesthetized with CO_2 and killed by cervical dislocation. Retinae were rapidly removed and arterioles isolated mechanically. Vessel segments were incubated with fluo-4 AM, placed in an organ bath on an inverted microscope and superfused with physiological solutions at 37°C. High speed confocal imaging (20 fps) was used to record cellular Ca^{2+} signals and normalized fluorescence (F/F_0) was used as a measure of $[Ca^{2+}]$. For each protocol, changes in mean fluorescence were recorded for 3 representative cells in each of 4 repeat experiments. Summary data are presented as mean±SEM. Et-1 alone (10nM) stimulated Ca^{2+} oscillations. Oscillation frequency was increased from 0.167±0.028 s^{-1} under control conditions, to 0.342±0.036 s^{-1} during the last 10 s in the presence of endothelin ($P<0.01$, non-parametric ANOVA). Oscillation amplitude ($\Delta F/F_0$) was also increased from an average of 0.12±0.01 to 0.29±0.04 for control and Et-1 treatment periods, respectively ($P<0.001$). Baseline $[Ca^{2+}]$ was elevated, rising from a mean of 1.01±0.03 during the control period to 1.38±0.12 during Et-1 superfusion. Addition of the Cl^- channel blocker anthracene 9-carboxylate (10 mM) abolished the effects of Et-1 on oscillations. Oscillation frequency was decreased from 0.342±0.036 s^{-1} at the end of the Et-1 treatment period, to 0.142±0.039 s^{-1} when Et-1 and anthracene 9-carboxylate were both present ($P<0.001$). Similarly, mean oscillation amplitude was reduced from 0.29±0.04 in the presence of Et-1 to 0.13±0.02 when anthracene 9-carboxylate was also added ($P<0.001$). There were no significant differences in oscillation frequency or amplitude between the control period and the period of treatment with both Et-1 and anthracene 9-carboxylate ($P>0.05$). In contrast, anthracene 9-carboxylate had no effect on the elevated baseline $[Ca^{2+}]$. It appears, therefore, that activation of Ca^{2+}-activated Cl^- currents plays an important role in the stimulation of Ca^{2+} oscillations by Et-1.

Scholfield CN, McGeown JG & Curtis TM (2006). *Microcirculation* (in press).

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PC8

High glucose downregulates the BKβ1 potassium channel subunit and activates NFATc3 in vascular smooth muscle cells

X. Zhang[1], A. Arora[1], M.K. McGahon[1], C.N. Scholfield[2], J.G. McGeown[2] and T. Curtis[1]

[1]Centre for Vision Sciences, Queen's University of Belfast, Belfast, UK and [2]Cell and Metabolic Signalling Group, Queen's University of Belfast, Belfast, UK

Large conductance, calcium-activated potassium (BK) channels play a central role in modulating cell excitability and contractility in vascular smooth muscle (VSM). BK channels are composed of pore-forming α subunits and accessory β subunits. The β subunits influence the calcium sensitivity of BK currents and in VSM the BKβ1 subunit is preferentially expressed. During the course of this meeting we show that in diabetes, the BKβ1 subunit is downregulated in retinal VSM cells. Work from other groups has suggested that high glucose can activate the transcription factor, NFATc3 (Nilsson et al. 2006). Activation of NFATc3 signalling is responsible for downregulating BKβ1 expression during angiotensin II-induced hypertension (Nieves-Cintron et al, 2006). Here we show that high glucose downregulates BKβ1 subunit expression in the A7r5 VSM cell line and that this is associated with nuclear accumulation of NFATc3. A7r5 cells were cultured in DMEM, with 2% calf serum in normal glucose (5 mM D-glucose) or high glucose (25 mM D-glucose) for times ranging between 30 min to 2 weeks. mRNA and protein expression for the β1 subunit was determined by real-time PCR and flow cytometry, respectively. Immunohistochemistry was used to assess NFATc3 activity in cells counter-stained with propidium iodide nuclear stain. For scoring of NFATc3 positive nuclei 30 fields of view were imaged for each time point, and an average of 50 cells per field were counted. All experiments were performed in triplicate. After 6 hours exposure to high glucose, β1 transcript levels decreased by approximately 40%, and this level of downregulation persisted up to 2 week's exposure ($p<0.01$). This was paralleled by a 25% reduction in immunodetected β1 protein which was first evident 16 hours after high glucose treatment and was also sustained throughout the 2 week period ($p<0.01$). No significant differences in β1 subunit mRNA or protein expression were observed in cells exposed for 4 days to 25mM L-glucose ($p>0.05$). Exposure of A7r5 cells to high glucose increased NFATc3 nuclear accumulation in a time-dependent manner. The number of NFAT positive nuclei increased from 21 ± 3% in control cells to 37 ± 2% and 47 ± 3% in cells exposed to high glucose for 30 min and 2 hours, respectively ($p<0.001$). These results suggest that A7r5 cells may represent a useful model system for investigating the molecular mechanisms underlying changes in BKβ1 expression in vascular myocytes during high glucose exposure, and furthermore, highlights the possible contribution of NFATc3.

Nieves-Cintron M, Amberg GC, Nichols CB, Molkentin JD & Santana LF (2006). J Biol Chem 10.1074/jbc.M608822200.

Nilsson J, Nilsson LM, Chen YW, Molkentin JD, Erlinge D & Gomez MF (2006). Arterioscler Thromb Vasc Biol 26, 794-800.

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PC9

Lack of direct coupling between single L-type $Ca_v1.2$ Ca^{2+} channels and RyRs in single calcium release units of arterial vascular smooth muscle cells

K. Essin[1], S. Moosmang[2], A. Welling[2], F. Hofmann[2], F.C. Luft[3] and M. Gollasch[1]

[1]Section Nephrology/Intensive Care, Campus Virchow, Charite Universitätsmedizin, Berlin, Germany, [2]Department of Pharmacology and Toxicology, Technical University München, Munich, Germany and [3]Franz Volhard Clinic HELIOS Klinikum Berlin Campus Buch and Max Delbrück Center for Molecular Medicine, Berlin, Germany

We tested the hypothesis that local and tight coupling between the $Ca_v1.2$ channels and RyRs is not required to initiate Ca^{2+} sparks in arterial vascular smooth muscle cells (VSMC). Cross-signalling between $Ca_v1.2$ channels and RyRs was studied using an approach based on Poisson statistical analysis of elementary calcium release events (ECRE). ECRE were recorded as Ca^{2+} sparks or STOCs in tibial VSMCs of smooth muscle-specific L-type $Ca_v1.2$ channel knockout (SMAKO) mice. $Ca_v1.2$ channel gene inactivation reduced Ca^{2+} spark frequency and amplitude by ~50% and ~80%, respectively. The first-latency histograms of sparks only slightly depended on the depolarization level and peaked at a relatively positive potential in wild-type VSMCs, i.e. ~+20 mV. Furthermore, average latencies between -30 mV and +50 mV occurred at ≥ 100 ms. Average latency of the first identified STOCs was similar and ≥ 100 ms in wild-type, nimodipine-treated and SMAKO VSMC and only slightly depended on the depolarization level. All-latency histograms in all three VSMC cell groups did not show a tendency to decline upon depolarization time. The observation that the first-latency and all-latency histograms have different waveforms implies that the release waveform is not determined by the time course of first event activation, with relatively fewer re-openings, as is the case in skeletal and cardiac muscle. Thus, $Ca_v1.2$ channels are important for frequency and amplitude modulation of VSMC Ca^{2+} sparks. However, local and tight coupling between the $Ca_v1.2$ channels and RyRs is not required to initiate Ca^{2+} sparks from single calcium release units in arterial VSMC.

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