C3

Caveolin-1 and age-related changes in vascular contractility

N. Hausman[1], M. Taggart[2] and C. Austin[1]

[1]Cardiovascular Research Group, The University of Manchester, Manchester, UK and [2]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK

Caveolin-1 knock-out (KO) mice have a shorter life-span than wild-type (WT) mice (Park et al., 2003). Caveolae, and caveolin-1, can modulate vascular function while normal ageing has been associated with changes in structure and function of resistance arteries. We examined the effect of caveolin-1 ablation on vascular ageing.

Wild type (WT) and caveolin-1 knockout (KO) mice were killed by cervical dislocation at 3 and 12 months of age (3m, 12m). Third order mesenteric arteries were dissected, mounted on a pressure myograph (60mmHg, superfused with physiological salt solution (PSS), 95% air/5% CO_2, 37°C) and contractile responses to noradrenaline (NA) (10^{-9}-10^{-5}M) and KPSS (100mM) examined. Passive pressure-diameter/wall thickness relationships (5-140 mmHg) were determined in the absence of calcium and further calculations performed (Schofield et al., 2002) (n=30 3m WT, n=36 3m KO, n=23 12m WT, n=17 12m KO). Data is expressed as maximal responses (mean ± SEM) with statistical differences ($p<0.05$) examined by ANOVA and Bonferroni.

Caveolin-1 ablation significantly reduced NA constriction of vessels from 3 month mice (69 ± 2% KO (n=24); 81 ± 1% WT (n=30)). Ageing significantly reduced constriction in WT arteries (66 ± 2% 12m n=24), but had no effect on KO vessels (68 ± 2% 12m n=19). Constriction of arteries from 12 month WT and 3 and 12 month KO mice was comparable. Similar changes were observed with KPSS; while ageing in the WT mouse reduced constriction (72 ± 3% (3m, n=20) to 53 ± 4% (12m, n=19). Responses in KO mice at 3m were reduced *c.f.* WT (51 ± 3% n=29) but there were no further significant age-related changes (39 ± 4% n=19).

Ageing was associated with increased diameter and decreased wall: lumen ratio in arteries from WT and KO mice. However, the wall thickness and cross sectional area of KO mice were increased *c.f.* WT vessels indicating growth (growth index = $((CSA_1\text{-}CSA_2)/CSA_2)^*100$; 43% 3m; 21% 12m). Vessel distensibility was reduced by ageing in WT mice as shown by a leftward shift in stress-strain curves. Distensibility was also reduced in vessels from 3 month KO mice, however, these vessels were resistant to any further age-related changes.

Thus caveolin-1 ablation causes structural and functional changes in resistance arteries which are not altered by ageing. This suggests that caveolae play an important role in the normal vascular ageing process.

Park DS, Cohen AW, Frank PG, Razani B, Lee H, Williams TM, Chandra M, Shirani J, De Souza AP, Tang B, Jelicks LA, Factor SM, Weiss LM, Tanowitz HB, Lisanti MP (2003) Caveolin-1 null (-/-) mice show dramatic reductions in life span. Biochem., 42, 15124-31

Schofield, I., Malik, R., Izzard, A., Austin, C., Heagerty, A. (2002) Vascular structural and functional changes in type 2 diabetes mellitus. Circ., 106, 3037-43

Supported by the BHF

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

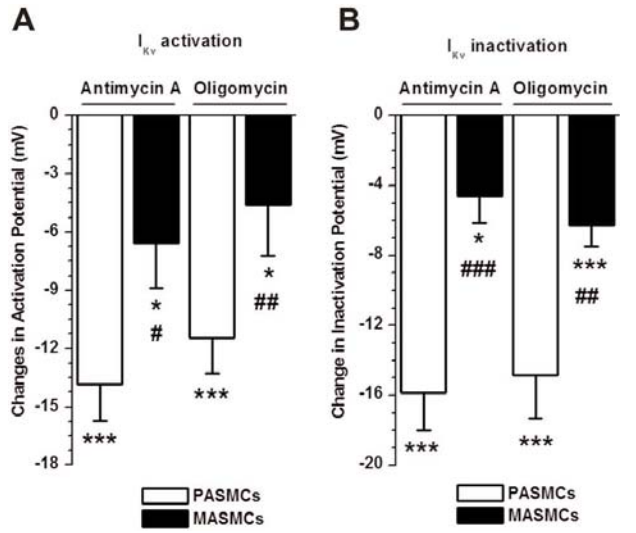
C4

Cellular localization of mitochondria contributes to the regulation of voltage-dependent potassium currents in the rat pulmonary, but not mesenteric arterial myocytes

A.L. Firth[2], D.V. Gordienko[3,4], K.H. Yuill[1] and S.V. Smirnov[1]

[1]Pharmacy & Pharmacology, University of Bath, Bath, UK, [2]Department of Medicine, University of California, San Diego, CA, USA, [3]Division of Basic Medical Sciences, Ion Channels and Cell Signalling Centre, St George's University of London, London, UK and [4]Laboratory of Molecular Pharmacology and Biophysics of Cell signalling, Bogomoletz Institute of Physiology, Kiev, Ukraine

Mitochondria are one of the major oxygen sensors triggering hypoxic pulmonary vasoconstriction (HPV), a unique response of the pulmonary circulation to low oxygen unique. Various mitochondrial factors including reactive oxygen species, cytochrome c, ATP and magnesium, are also potent modulator of voltage-gated K^+ (Kv) channels in plasmalemmal membrane of pulmonary arterial smooth muscle cells (PASMCs). The main aim of this investigation was to determine whether differences between mitochondria localization in art PASMCs and systemic mesenteric arterial SMCs (MASMCs) could be a contributing factor to the divergent oxygen sensitivity in two different circulations. Myocytes were enzymatically isolated from arteries dissected from male Wistar rats (250-300g)(Smirnov *et al*., 2002; Firth *et al*., 2008); experiments were performed at room temperature. Cellular localization of mitochondria was compared using confocal imaging and immunofluorescent labelling (Gordienko *et al*., 2001; Gordienko & Zholos, 2004). It was found that mitochondria (labelled with MitoTracker Green) were located significantly closer to the plasmalemmal membrane (labelled with Di-8-ANEPPS) in PASMCs compared to MASMCs. On the other hand, sarcoplasmic reticulum (labelled with Brefeldin A BODIPY) was more closer to the plasmalemmal membrane in MASMCs than in PASMCs. Differences in the functional coupling between mitochondria and Kv channels were then evaluated with the patch-clamp technique and two specific mitochondrial inhibitors, antimycin A (a Complex III inhibitor) and oligomycin (an ATP synthase inhibitor)(Smirnov *et al*., 2002; Firth *et al*., 2008). These inhibitors were chosen as they have most pronounced effect on I_{Kv} amplitude and steady-state activation in rat PASMCs (Firth *et al*., 2008). Electrophysiological results showed that relative changes in the steady-state activation, inactivation of I_{Kv} (Fig. 1) and I_{Kv} block (not shown) induced by antimycin A and oligomycin were significantly greater in PASMCs than in MASCMs. These findings suggest a greater structural and functional coupling between mitochondria and Kv channels specifically exists in PASMCs, which could play an important role in HPV.



Mitochondrial inhibition has significantly greater effects on I_{Kv} in PASMCs than in MASMCs. **A** and **B**, Comparison of antimycin A and oligomycin induced changes in the half activation and half inactivation potentials, respectively. Open and filled bars represent the relative changes in the half activation and half inactivation potential in PASMCs (n=8-12) and MASMCs (n=9-11) for antimycin A and oligomycin, respectively. * P<0.05 and *** P <0.001. * compares the difference in the parameters between the control condition and in the presence of an inhibitor measured in the same cell using paired statistical test. # P<0.05, ## P<0.01, ### P<0.001. # compares the difference between the two cell types using non-paired statistical test.

Firth AL *et al*. (2008). *Am J Physiol Lung Cell Mol Physiol* **295**, L61-L70.

Gordienko DV *et al*. (2001). *Cell Calcium* **29**, 13-28.

Gordienko DV & Zholos AV (2004). *Cell Calcium* **36**, 367-386

Smirnov SV *et al*. (2002). *J Physiol* **538**, 867-878

This work was supproted by the British Heart Foundation (grants PG03/059 & PG04/069 supported KHY and PG08/062/25382 awarded to DVG) and by the Wellcome Trust (grant 075112, awarded to DVG)

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

C5

Expression of two pore domain potassium channels (K2P) in the placenta

T. Ali[1], F. Broughton Pipkin[2] and R.N. Khan[1]

[1]Academic Division of Obstetrics & Gynaecology, University of Nottingham, Derby, Derbyshire, UK and [2]Obstetrics & Gynaecology, University of Nottingham, Nottingham, Nottinghamshire, UK

Vascular function of the placenta is closely linked with the well being of the fetus. Key to this is the effective transport of blood and gases which is possible due to the low resistance of the placenta. There is a poor understanding about the specific ion channels that are involved in regulating the vessel tone across the placenta. Two members of the K2P family TWIK-2 and TREK-1 are important for maintaining the resting membrane potential. This study aims to investigate the expression of these ion channels in arteries taken from the human chorion and villi. Hypothesis: TWIK-2 and TREK-1 expression is differentially modulated across the fetal and maternal interface of the placenta. Placentae were collected with written informed consent from patients undergoing elective Caesarean section at term (~37 weeks). Explants taken from the stem villous artery (SVA) and the third branch of the chorionic plate artery (CPA) were used to culture smooth muscle cells (SMC). After 20 days the cells were harvested and immunofluoresence was used to detect the expression of TWIK-2 and TREK-1. Briefly SMC were fixed with 3% PFA and pre incubated with primary antibody to detect expression of the K2P channels (Alomone, Jerusalem).Western blotting was performed on crude tissue lysate and separated by 12% SDS-PAGE to quantify expression of TWIK-2 and TREK-1 in CPA and SVA. The blots were stripped and re-probed with beta actin (AbCam, UK) to measure protein loading. Immunofluorescence for TWIK-2 and TREK-1 was detected in both CPA and SVA and staining for alpha actin confirmed the smooth muscle cell phenotype (n = 4 placentae). Staining was concentrated in the nuclei of SMC cultured from CPA, while in SVA, the staining was cytosolic and showed actin like stress fibres. Western blot analysis on isolated placental arteries showed that the TREK-1 dimer (100 kDa) band density is significantly higher in SVA (n =6 placentae; P= 0.04). No significant difference was seen with TWIK-2 (37 kDa) in the two different vessel types (n =6 placentae; P=0.47). We have successfully shown that TWIK-2 and TREK-1 are expressed in both the SVA and CPA. Our early findings suggest TREK-1 is up regulated in the SVA and the immunofluorescence staining shows the channel may be differentially regulated within the two vascular beds. These findings suggest that TWIK-2 and TREK-1 may have an important role in regulating vasodilatation of placental arteries and requires further investigation. This also raises the possibility that K2P channels are a potential target for treating placental disorders such as pre eclampsia where the vascular arteries are poorly perfused and do not respond well to vasodilators.

The authors would like to thank all the women who agreed to participate in this study along with all the labour ward staff at the Derby City General Hospital. Funded by the British Heart Foundation

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

C6

Spontaneous ATP release from nerves is the predominant determinant of spontaneous action potentials in the mouse urinary bladder

J.S. Young[1], E. Meng[1,2], T.C. Cunnane[1] and K.L. Brain[1]

[1]Department of Pharmacology, University of Oxford, Oxford, UK and [2]Department of Urology, Tri-Service General Hospital, Taipei, Taiwan

Spontaneous purinergic neurotransmission was characterised in the mouse urinary bladder. Intracellular electrophysiological recording from smooth muscle cells of the detrusor muscle revealed spontaneous depolarizations, distinguishable from

PC50

Pharmacological properties of pulmonary arteries from TASK-1 knockout mice

B. Manoury, C. Lamalle and A.M. Gurney

Faculty of Life Sciences, University of Manchester, Manchester, UK

Growing evidence suggests that the TWIK-related Acid Sensitive K^+ (TASK) channels have a functional role in the cardiovascular system. In pulmonary artery (PA), compelling evidence has suggested that TASK-1 is a major contributor to the background K^+ current that support the membrane potential (E_m) of the smooth muscle cells. However, due to poor selectivity of its modulators, the role of TASK-1 channels in the functional regulation of tone in pulmonary arteries remains elusive. The purpose of this study was to investigate the properties of PA isolated from mice in which the TASK-1 gene has been deleted, compared with their wild type controls (C57BL/6 mice).

Intra-PAs (200μm diameter) were isolated from C57BL/6 mice and mice in which the TASK-1 and TASK-3 genes had been deleted (TASK1/3 KO, Aller et al., 2005). TASK-3 is not expressed in PA (Gardener, Johnson et al., 2004). Vessels were mounted on a small vessel myograph for isometric tension measurement. The responses to various drugs were recorded and expressed relative to a reference contraction elicited by application of 50mM KCl at the beginning of each experiment. Statistical analysis was performed using Student's t-test, with 0.05 as a critical value.

Responses of PA to 50mM KCl were not significantly different between C57BL/6 mice (292 ± 54 mN, N=13 vessels) and TASK1/3 KO mice (237 ± 42 mN, N=11). The addition of 10mM KCl, the K^+ channel modulators 4-AP (1mM) and levcromakalim (10μM), or the L-type calcium channel blocker nifedipine (1μM), did not elicit any significant response in either the TASK1/3 KO (N=6) or C57BL/6 mice (N=6). The contractile response of PA to phenylephrine (PE) had a pEC_{50} of 6.6 ± 1.3 in C57BL/6 (N=4) and 6.8 ± 1.8 in TASK1/3 KO mice (N=4), with maximum responses (10μM) of 173 ± 37 % and 178 ± 15 %, respectively, of the response to 50mM KCl. For serotonin the pEC_{50} and maximum responses were, respectively, 6.9 ± 0.3 and 379 ± 94 % in C57BL/6 mice (N=4) and 7.1 ± 0.1 and 267 ± 31 %, respectively, for TASK1/3 KO (N=4). Statistical analysis of both pEC_{50} and the efficacy of PE and serotonin showed no difference between the TASK1/3 KO and their wild type control.

The PA isolated from TASK1/3 KO mice does not display any response to 10mM KCl, K^+ channel modulators or nifedipine, suggesting that the E_m is not depolarised and that artery is fully dilated as in the C57BL/6 mice. Responses to the agonists PE and serotonin were also unchanged. Further experiments are in progress to investigate other possible effect of TASK-1 deletion, in order to discriminate the contribution of the TASK channels to the overall background K^+ channels in PA smooth muscle cells. The results thus far do not, however, support a major physiological role for TASK-1 in mouse PA.

Aller MI et al.(2005). *J Neurosci* **25**, 11455-11467.

Gardener MJ, Johnson IT et al. (2004.) *Br J Pharmacol* **142**, 192-202.

The authors aknowledge Prof. William Wisden (University of Aberdeen, UK) for kindly providing the TASK-1/3 knockout mice.

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

PC51

Modulation of Kv2.1 assembly contributes to the changes in vascular smooth muscle cell excitability in a hypertensive mouse strain

A. Moreno-Dominguez[1,2], P. Cidad[1,2], E. Alonso[1,2], J. Lopez Lopez[1,2] and T. Perez-Garcia[1,2]

[1]Biochemistry, Molecular Biology and Physiology, Universidad de Valladolid, Valladolid, Spain and [2]Institute of Biochemistry and Molecular Genetics (IBGM), Universidad de Valladolid and CSIC, Valladolid, Spain

Essential hypertension involves a gradual and sustained increase in total peripheral resistance reflecting an increased vascular tone, which 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 Cav and Kv channels) that promotes arterial contraction. 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. Here we have undertaken the study of the molecular and functional characterization of Kv and KCa channels in VSMCs in essential hypertension by using VSMCs from mesenteric arteries obtained from a hypertensive inbred mouse strain, BPH, and the corresponding normotensive strain, BPN. Mice were killed by decapitation after isofluorane anesthesia. Real-time PCR using low-density taqman® arrays revealed a differential distribution of K^+ channel subunits mRNA between both strains, the most conspicuous change being the "de novo" expression of Kcng3 in hypertensive phenotype. To study the functional relevance of this change, we characterized the electrophysiological properties of freshly dissociated VSMCs of BPN and BPH mice with the patch-clamp technique. Both KCa current density, (500 nM paxilline-sensitive current) and Kv current density (paxilline-resistant current) were significantly smaller in BPH cells at all voltages. Application of selective Kv2 blockers as 50 nM stromatoxin or 20 nM guangxitoxin induced a reduction of the current amplitude that was significantly smaller in BPH cells, suggesting that the reduction in the functional expression of Kv2 currents in BPH cells contribute to the decreased Kv current in these cells. These findings could be explained by the "de novo" expression of Kv6.3 mRNA in BPH VSMCs, as Kv6.3 subunits associate with Kv2 channels to form heteromultimers with decreased current amplitude. In agreement with this, Kv6.3 protein could be detected by western-blot in BPH but not in BPN arteries. Moreover, intracellular application of anti-Kv6.3 antibody decreased Kv current amplitude in BPH VSMCs but had no effect in BPN ones, demonstrating a functional role of this subunit in hypertensive VSMCs. These data suggest that the expression of Kv6.3 channels in resistance vessels could contribute to the natural development of hypertension, and can help to understand the molecular basis of this complex and multifactorial disease.

Supported by grants R006/009 and PI041044 (ISCIII) and grants BFU2004-05551 and BFU2007-61524 (MCYT).

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

PC52

Inhibitory actions of mibefradil and flunarizine in human vas deferens

N. Amobi[1], J. Guillebaud[2] and I. Smith[3]

[1]Physiology - Applied Biomed. Sci., King's College London, London, UK, [2]Elliot-Smith Clinic, Churchill Hospital, Headington, Oxford, UK and [3]Physiology - Applied Biomedical Science Research, King's College, London, UK

It has been reported that Ca^{2+} currents in isolated human vas deferens smooth muscle cells exhibit components that are differentially sensitive to Ni^{2+} (T-type current) and verapamil (L-type current). Thus, Park et al., (2004) suggested that T-and L-type VOCs contribute to the tissue contractility. This study investigated the effects of T-type Ca^{2+}-antagonists, mibefradil and flunarizine compared to nifedipine, a prototype L-type Ca^{2+} antagonist. Given that mibefradil and flunarizine also block L-type VOCs, their potencies in this tissue as L-type Ca^{2+} antagonists were determined.

Human vas deferens specimens obtained after elective vasectomies (with consent of patients and College Ethical approval) were cut into strips of longitudinal muscle or rings of circular muscle. These were set up for tension recording and superfused with Krebs medium (36°C). Contractions were evoked by noradrenaline (NA) in the presence of Ca^{2+} antagonists and expressed as a percentage of drug-free controls (mean ± S.E.M). Statistical analysis was by one-way ANOVA followed by *a priori* t-test using within-groups variance from ANOVA. Differences between time-matched controls and drug groups were considered significant at $P < 0.05$.

NA (0.1-100 μM) evoked rhythmic and tonic contractions, which were inhibited by nifedipine (0.01-0.1 μM) or by mibefradil (1-10 μM) such that contractions to NA (100 μM) in longitudinal and circular muscles were reduced respectively by 88.4 ± 1.3%, (n=9) and 87.03 ± 2.7%, (n=8) in nifedipine (0.1 μM); by 89.1 ± 3.4%, (n=5) and 68.5 ± 3.8%, (n=7; $P< 0.05$) in mibefradil (10 μM) and modestly by 18.3 ± 6.3% (n=6) and 27.0 ± 10.8% (n=5) in flunarizine (10 μM). The drugs' potencies as antagonists of L-type VOCs were determined against contractions to high K^+ (120 mM, in the presence of FPL 64176, 1 μM). The contractions in longitudinal and circular muscle had different times to peak and decline but were inhibited comparably by nifedipine (IC_{50} longitudinal and circular muscle, ~2 nM) or by mibefradil (IC_{50} longitudinal muscle, 1.1 μM; circular muscle, 2.4 μM) and were insensitive to flunarizine (up to 30 μM). These results suggest that the effects of mibefradil and flunarizine, at concentrations found to be effective against noradrenaline-induced contractions of human vas deferens involve the blockade of L-type VOCs. The difference in the effect of mibefradil in longitudinal and circular muscle may involve factors that modulate the activation and/or sensitivity of L-type VOCs to the drug. Overall, the results indicate that noradrenaline-induced contractions of human vas deferens depend primarily on nifedipine-sensitive L-type VOCs than on mibefradil or flunarizine-sensitive T-type VOCs.

Park SY et al. (2004) J Urol 172, 628-633.

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

PC53

Human saphenous vein vascular smooth muscle cells mechanically damaged during vein harvesting as coronary artery bypass graft (CABG) co-express iNOS and ET-1

A. Loesch[1], A. Dooley[1], X. Shi-Wen[1], D.J. Abraham[1], M.R. Dashwood[1], M. Dreifald[2] and D.S. Souza[2]

[1]University College London, London, UK and [2]Orebro University Hospital, Orebro, Sweden

The human saphenous vein (SV) suffers trauma during harvesting for CABG which reflects on the graft patency (Souza 1996, Souza et al. 2006, Dashwood and Loesch 2007). Commonly used harvesting procedures involve stripping the pedicle of connective tissue from the vein followed by the distension to overcome venospasm (Souza 1996). Such procedures cause structural damage of the vein vascular smooth muscle cells (VSMC) and rapid expression of immunoreactive iNOS (Loesch et al. 2006). Here, we used standard protocols of immunolabelling for confocal and electron microscopy (EM) and Western blot analysis to study the co-expression of iNOS and ET-1 in medial VSMC of SV harvested in patients undergoing CABG.

Following local ethics committee approval and patients' informed consent, segments of SV harvested for CABG (n=10) were collected during surgery. For EM, SV samples were fixed in 4% paraformaldehyde and 0.1% glutaraldehyde prior to single and double immunolabelling with ExtrAvidin and immunogold methods using antibodies to iNOS (polyclonal SC-651, Santa Cruz Biotech) and ET-1 (monoclonal MCE-6901-01, Peninsula Labs). For the confocal microscopy frozen SV sections were labelled with iNOS and ET-1 antibodies in conjunction with Alexa Fluor® 568 and 488 (Molecular Probes), respectively.

Both confocal and EM revealed the co-expression of iNOS and ET-1 in VSMC of SV harvested for CABG (Fig. 1A-C and 2A-C). The immunoreactivity was predominantly localised in structurally damaged VSMC (Fig. 2). Western blot analysis showed increased iNOS and ET-1 levels in extracts from stripped and distended SV as compared to SV harvested without stripping and distension (Fig. 2D). Conventional harvesting of SV for CABG induces both iNOS and ET-1 in structurally distorted VSMC. The functional consequences of co-expressed iNOS and ET-1 in venous VSMC require further investigation.

PC47

Contribution of inward rectifier K^+ currents to the excitability of vascular smooth muscle cells from BPN and BPH mice

S. Tajada[1,2], C. Ruiz-Mc Davitt[1,2], A. Moreno-Domínguez[1,2], T. Perez-Garcia[1,2] and J. Lopez-Lopez[1,2]

[1]Biochemistry, Molecular Biology and Physiology, Universidad de Valladolid, Valladolid, Spain and [2]Institute of Biology and Molecular Genetics (IBGM), Universidad de Valladolid and CSIC, Valladolid, Spain

Inward rectifier (K_{IR}) and ATP-sensitive (K_{ATP}) K^+ channels play essential roles in vascular smooth muscle cells (VSMCs) as they contribute to maintain the resting membrane potential (E_M) and in this way regulate the contractile tone of resistance arteries. K_{ATP} channels underscore the functional bond between cellular metabolism and membrane excitability, and their blockade results in vasoconstriction and depolarization in various types of VSM. K_{IR} channels are known to be abundant in the VSMCs of resistance vessels, where their roles are far from being completely understood, although it has been established that they contribute to the resting E_M and resting tone of these cells and that their activation in response to moderate increases in extracellular K^+ induces vasodilation.

We designed this study to examine the expression and function of these channels in VSM and their regulation in essential hypertension, using VSMCs from mesenteric arteries obtained from a hypertensive inbred mice strain, BPH, and the corresponding normotensive strain, BPN. All animal protocols were approved by the Institutional Care and Use Committee of our Institution. Mice were killed by decapitation after isofluorane anesthesia. Real-time PCR using low-density TaqMan® arrays reveals mRNA expression of several K_{IR} and K_{ATP} genes in mesenteric VSMCs and also shows a reduced expression of some of these genes under hypertensive conditions. Functional characterization of K_{IR} and K_{ATP} channels was performed in freshly dispersed VSMCs with the whole-cell configuration of the patch-clamp technique, using $BaCl_2$ (100 μM) to block K_{IR} channels and pinacidil (10 μM) to activate K_{ATP} channels. Pinacidil application induced an increase of the current amplitude that was larger in BPN than in BPH VSMCs. We also found a decrease in the amplitude of the $BaCl_2$-sensitive current in VSMCs from BPH mice. The contribution of K_{IR} channels to the resting E_M was evaluated in perforated-patch experiments, where we observed a significant reduction of the $BaCl_2$-induced depolarization in hypertensive VSMCs.

Finally, the contribution of K_{IR} and K_{ATP} channels to determine vascular tone in mesenteric arteries of BPN and BPH mice was explored in pressurized arteries. Altogether, our findings indicate that the observed decrease in the functional expression of K_{IR} and K_{ATP} channels in BPH VSMCs from resistance arteries could participate in the genesis of the altered vascular tone during hypertension.

Supported by grants R006/009 and PI041044 (ISCIII) and BFU2007-61524 (MCYT).

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

PC49

Effects of elevated glucose and AGE-modified recombinant human serum albumin on antioxidant gene expression in human endothelial cells

X. Cheng[1], A. da Silva[2], R. Hider[2], R.C. Siow[1] and G.E. Mann[1]

[1]Cardiovascular Division, King's College London, London, UK and [2]Pharmaceutical Sciences Division, King's College London, London, UK

Hyperglycaemia and advanced glycation end-products (AGE) are associated with oxidative stress and endothelial dysfunction in diabetes mellitus (Mann et al., 2003). We previously hypothesised that the generation of reactive oxygen species (ROS) by NAD(P)H oxidase, mitochondria or uncoupled endothelial nitric oxide synthase may upregulate antioxidant gene expression in endothelial cells via activation of the redox sensitive transcription factor Nrf2, which binds to the antioxidant response element (ARE) in the promoter region of target genes (Mann et al., 2007). In the present study, we examined the effects of elevated glucose and AGE-modified recombinant human serum albumin (AGE-HSA) on antioxidant gene expression in human umbilical vein endothelial cells (HUVEC).

Endothelial cells were isolated by collagenase digestion and cultured in M199 containing 20% FCS. Confluent monolayers serum-deprived (1% FCS) for 4 h and then treated for 3-12 h with (i) elevated D-glucose (25 mM) and D-mannitol (20 mM + 5 mM glucose) as osmotic control) or (ii) AGE-HSA (100 μg/ml) and HSA (100 μg/ml) as a control. Elevated glucose increased heme oxygenase-1 (HO-1) expression after 6-12 h (Fig. 1), but had negligible effects on induction of either glutathione peroxidase-1 (GPx-1) or the phase II defence enzyme NAD(P)H:quinone oxidoreductase-1 (NQO1) (data not shown). Treatment of cells with AGE-HSA increased HO-1 expression after 3-6 h, which returned to basal levels after 12 h. AGE-HSA also increased expression of NQO1 but had negligible effects on GPx-1 expression. Our findings that hyperglycaemia and AGE-HSA induce adaptive antioxidant responses in fetal endothelial cells via the Nrf2/ARE pathway provide a basis for examining whether the phenotype of fetal endothelial cells is altered by in utero programming in gestational diabetes.

Mann, G.E., Rowlands, D.J., Li, F.Y., de Winter, P. & Siow, R.C. (2007). Cardiovasc. Res. 75, 261-274

Mann, G.E., Yudilevich, D.L. & Sobrevia, L. (2003). Physiol. Rev. 83, 183-252

Supported by China Scholarship Council PhD Award and EU COST ACTION B35

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

PC37

Single channel mechanism of the TRPM8 calcium channel voltage dependence

J.A. Fernandez and A.V. Zholos

School of Medicine, Dentistry and Biomedical Sciences, Queen's University of Belfast, Belfast, UK

TRPM8 channels are present in blood vessels where they are likely to be involved in the regulation of vascular tone (Yang et al., 2006; Inoue et al., 2006). Their activity depends on temperature, voltage and chemical signaling. However, single channel mechanisms of TRPM8 gating remain unknown. We examined the influence of voltage on TRPM8 gating using patch-clamp recording techniques (all values were expressed in means ± S.E.M.). In HEK293 cells stably expressing TRPM8, whole-cell currents during voltage steps (-100 to 150 mV, 10 mV increments) and ramps (-100 to 100 mV) showed a strong outward rectification. Rectification of several TRP channels has been suggested to arise from the voltage-dependence of gating (Nilius et al., 2005). Thus, single channel currents (cell-attached configuration) were measured at different voltages (room temperature, filtered at 2 kHz and sampled at 10 kHz) giving single channel conductance of 60.2±4.3 pS (n=6), and an increase in open probability (Po) from 0.03±0.01 at 40 mV (n=7) to 0.41±0.06 at 160 mV (n=7). To further study the mechanism underlying this voltage-dependent increase in Po, shut and open dwell times at 80 and 140 mV were compared. There was only a modest increase in the mean open time from 0.70±0.08 to 1.10±0.28 ms (n=4 patches; not significant, P=0.229, two-tailed *t*-test) whereas the mean shut time decreased from 15.55±2.15 to 4.04±1.08 ms (n=4; P=0.003) between 80 and 140 mV, respectively. Dwell-time histograms (transitions > 0.16 ms) were fitted with sums of exponentials (2 open and 4 shut in each case). They showed that membrane depolarization caused a reduction in the relative contributions of the long shut components. Thus, the two longest components (τ > 10 ms) accounted for 40% of all closures at 80 mV, but for only 14% at 140 mV. In contrast, the open time values were little affected by voltage. The 2-D dwell-time distributions (Rothberg and Magleby, 1998) and dependency plots (Magleby and Song, 1992) were consistent with voltage-dependent restructuring of channel gating. These results show that the main effect of membrane depolarization on TRPM8 gating is an increase in the frequency of channel opening. Furthermore, voltage-dependence of TRPM8 gating arises from the effects of membrane potential on the closed dwell times, while open times are essentially voltage-independent.

Yang XR et al. (2006). Am J Physiol Cell Mol Physiol 290, 1267-76

Inoue R et al. (2006). Circ Res 99, 119-131

Nilius B et al. (2005). J Physiol 567, 35-44

Rothberg BS and Magleby KL (1998). J Gen Physiol 111, 751-780

Magleby KL and Song L (1992). Proc R Soc Lond B Biol Sci 249, 133-142

We thank European Social Fund for support, Prof. N. Prevarskaya for the gift of HEK293/TRPM8 cells, and Prof. K.L. Magleby for help with data analysis.

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

PC38

Cell cycle-dependent expression of Kv3.4 channels and cell proliferation in human uterine artery smooth muscle cells

E. Miguel-Velado[1,2], O. Colinas[1,2], F.D. Perez-Carretero[1,2], J.R. Lopez-Lopez[1,2] and T. Perez-Garcia[1,2]

[1]Biochemistry, Molecular Biology and Physiology, Universidad de Valladolid, Valladolid, Spain and [2]Institute of Biology and Molecular Genetics (IBGM), Universidad de Valladolid and CSIC, Valladolid, Spain

The vascular smooth muscle cells (VSMCs) of the vessel wall are differentiated cells specialized in the contractile function required for the maintenance of vascular tone. However, VSMCs are not terminally differentiated and in response to local environmental factors can experience a phenotypic switch towards a synthetic phenotype, which leads to cell proliferation, migration and production of extracellular matrix components. Ion channels have been shown to participate in cell proliferation, as they can modulate the progression of the cells through the cell cycle. In this regard, it is known that in different cell types K+ channel expression varies with the cell cycle. Also, several reports indicate that the blockade of some K+ currents in VSMCs is antiproliferative. Recently, our group has demonstrated that the functional expression of Kv3.4 channels is related to proliferation of human uterine VSMCs. In this work, we sough to gain deeper insight on the relationship between the expression and function of Kv3.4 channels and the progression of cultured uterine VSMCs through the cell cycle. We have used selective cell cycle blockers to describe the expression pattern of Kv3.4 mRNA and protein and its functional contribution along the cell cycle in these cells. Besides, we have explored the effects of the blockade of Kv3.4 channels on the distribution of the cells in the different phases of the cell cycle. We found that Kv3.4 mRNA levels increased when cells enter in G1 phase, decline in G2/M and reach the lowest level in quiescent (G0) cells, but Kv3.4 protein levels and Kv3.4 currents remain elevated while cells are proliferating, being maximum during G2/M phase. On the other hand, Kv3.4 channels blockade leads to a decrease in the mRNA levels of D1, A2 and B1 cyclins, an increase in the proportion of cells in the G0/G1 phase, a decrease in the number of ki67-positive cells and a decrease in the proportion of cells incorporating BrdU. Altogether, our data indicates that the blockade of Kv3.4 channels induces a decrease in the number of cells entering the cell cycle, suggesting the existence of a close association between the functional expression of Kv3.4 channels and the proliferation rate of uterine VSMCs.

Supported by grants R006/009 and PI041044 (ISCIII) and grants BFU2004-05551 and BFU2007-61524 (MCYT).

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

PC11

Alpha-adrenoceptor activation and myogenic responsiveness in rat mesenteric arteries: possible role of TRPC channels

A.I. Lurie[1], F. Henriksen[1], D. Beech[2], M. Salomonsson[1] and L.J. Jensen[1]

[1]Department of Biomedical Sciences, University of Copenhagen, Copenhagen N, Denmark and [2]Institute of Membrane and Systems Biology, University of Leeds, Leeds, UK

The myogenic response is an important contributor to the vascular tone in resistance vessels. Though weak, it can be evoked in rat mesenteric arteries in the presence of noradrenalin (NA)[1]. This led to speculation that receptor and/or store-operated cation channels are involved. Canonical Transient Receptor Potential (TRPC) channels are linked to G-protein activation and are candidates for receptor operated Ca2+ permeable cation channels in vascular smooth muscle cells (VSMCs). TRPC1 and TRPC6 channels are expressed and functional in mesenteric artery VSMCs[2,3]. We hypothesized that upon alpha1-adrenoceptor activation, TRPC6 and/or TRPC1 channels are enabled, increasing sensitivity to heightened transmural pressure and leading to myogenic constriction.

Male Wistar rats were ethically euthanized. 2nd or 3rd order branches of the superior mesenteric artery were isolated and mounted in a pressure myograph at a physiological pH (7.35-7.45; 5% CO_2 / 25 mM HCO_3^-) and temperature. Vessel diameters were measured using Myoview software (Danish Myotechnology). At 60 mm Hg and in the presence of 2 nM neuropeptide Y, increasing concentrations of NA were added to the bath to achieve a stable vascular tone (8 - 12% under relaxed levels). A myogenic response was then evoked by increasing transmural pressure to 100 mm Hg. This was repeated in the presence of a TRPC channel inhibitor. Passive diameters were measured in a Ca^{2+} free solution for calculation of vascular tone (%).

The overall effect of pressure increase in the presence of NA was an increase in the amount of vascular tone from 12.3 ± 0.7 (60 mmHg) to 17.6 ± 0.8 % (100 mmHg, P<0.001; N=46). Gd3+ (2 μM), an inhibitor of non-selective cation channels, did not substantially affect 75 mM-KCl induced vasoconstriction (7.6 ± 9.0 % inhibition; N=9), but abolished the pressure-induced increase in vascular tone: 12.7 ± 2.4 to 18.5 ± 2.7 % (control) vs. 8.9 ± 3.1 to 7.8 ± 3.3% (Gd3+) (P<0.05 vs. ctrl. at 100mm Hg; N=6). The non-specific TRPC channel blocker SKF96365 (5 μM) did not substantially affect KCl induced vasoconstriction (1.9 ± 1.4 % inhibition; N=5), but inhibited the myogenic response: 13.5 ± 1.6 to 21.6 ± 2.2 % tone (control) vs. 12.9 ± 1.5 to 14.9 ± 3.0 % tone (SKF96365) (P<0.001 vs. ctrl. at 100mm Hg; N=7). P values were calculated with paired t tests.

Preliminary results using TRPC1 antibody (T1E3[4], 1:250) show no significant blocking effect on the myogenic response in arteries incubated overnight with antibody (9.6 ± 0.8 to 16.2 ± 1.8% tone; N=4) compared to arteries incubated with inactivated (boiled) antibody (8.7 ± 0.7 to 16.4 ± 1.6 % tone; N=4).

These results suggest that TRPC6 channels are involved in evoking myogenic responsiveness in rat mesenteric artery. Future experiments, possibly utilizing siRNA mediated TRPC6 knockdown, could elucidate this role of TRPC6 channels.

Chlopicki S *et al*. (2001). Am J Physiol Heart Circ Physiol **281(5)**, H2176-83.

Hill AJ *et al*. (2006). Cell Calcium **40(1)**, 29-40.

Saleh SN *et al*. (2006). J Physiol **577(Pt 2)**, 479-95.

Xu SZ & Beech DJ (2001). Circ Res. **88(1)**, 84-7.

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

PC12

Ca^{2+}-activated conductances modulate endothelin-induced Ca^{2+} signals in rat retinal arteriolar myocytes

M. Stewart, T. Curtis, N. Scholfield and G. McGeown

School of Medicine, Dentistry and Biomedical Sciences, Queen's University, Belfast, UK

Electrophysiological recordings in retinal arterioles have demonstrated that application of endothelin promotes Ca^{2+}-sensitive, spontaneous transient inward and outward currents (Scholfield *et al*, 2007). The present study provides evidence that these currents act as important feedback mechanisms, modulating endothelin induced Ca^{2+}-signals. Retinal arterioles mechanically isolated from male Sprague-Dawley rats were loaded with fluorescent Ca^{2+}-indicator by incubation with fluo-4 AM (5 μM). Loaded vessels were placed in an organ bath on an inverted microscope and superfused with physiological solutions at 37ºC. High-speed confocal imaging (20 fps) was used to record cellular Ca^{2+}-signals from arteriolar myocytes. Normalized fluorescence (F/F_0) was calculated as a measure of [Ca^{2+}]. Ca^{2+}-oscillations were stimulated when endothelin (Et1, 10nM) was added. Oscillation frequency was increased from 0.123±0.018 s^{-1} (mean±SEM) under control conditions, to 0.340±0.021 s^{-1} in the presence of Et1 (N=89 cells in 4 vessels from 4 animals; P<0.001, non-parametric ANOVA, Dunn's multiple comparison post-hoc test). Oscillation amplitude ($\Delta F/F_0$) was also increased from an average of 0.28±0.02 to 0.57±0.03 for control and Et1 treatment periods, respectively (P<0.001). We have previously shown that 4,4'- diisothiocyanatostilbene-2,2'-disulphonic acid (DIDS) inhibits a Ca^{2+}-activated Cl^--conductance in these cells (McGahon *et al*, 2008). Addition of DIDS (1mM) in the continued presence of Et1 reduced the mean frequency of Ca^{2+}-oscillations back to control values (0.062±0.001 s^{-1}; P<0.001 vs Et1 alone; NS vs Control). DIDS also reduced the mean amplitude of the Ca^{2+}-oscillations to 0.40±0.04, but this was not statistically significantly different from the amplitude in the presence of ET1 alone. Iberiotoxin (100 nM), an inhibitor of large-conductance Ca^{2+}-activated K^+-channels, exaggerated the effects of Et1. Oscillation amplitude was increased from 0.24±0.01 in the presence of Et1 alone, to

PC2

UTP activates P2X receptor and regulate vascular tone in rat arterial smooth muscle

M. Sugihara[1,2], M. Matsuda[2], S. Kajioka[3], K. Abe[1], M. Hirata[2], Y. Ito[4] and H. Morita[1]

[1]Special Patient Oral Care Unit, Kyushu University Hospital, Fukuoka, Japan, [2]Laboratory of Molecular and Cellular Biochemistry, Faculty of Dental Science, Kyushu University, Fukuoka, Japan, [3]Department of Urology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan and [4]Department of Health Science, Kumamoto Health Science University, Kumamoto, Japan

It is well documented that uridine 5'-triphosphate (UTP) is released from several cells surrounding vascular smooth muscle cells including endothelial cells, platelets and sympathetic nerve terminals, and generally considered that UTP regulates vascular tone through selective activation of G-protein coupled P2Y receptors (Burnstock G, 2006). However, here we present evidence supporting that UTP mediates contraction of vascular smooth muscle through P2X receptor activation. Namely, we found that extracellularly applied UTP (~10μM) induced transient inward current in arterial (cerebral, mesenteric or aortic) myocytes, by use of whole-cell patch clamp configuration at a holding potential of -60mV. The current amplitude was enhanced dose-dependently, and current-voltage relationship of the current showed inward-rectification, which was similar to those evoked by activation of P2X channel. The current was also activated by high concentration of uridine 5'-diphosphate (UDP; 1mM), however, uridine 5'-monophosphate (UMP; 1mM) and uridine(1mM) were ineffective. Furthermore, the current induced by UTP (1mM) was inhibited by Gd^{3+} or La^{3+} (IC50=51.3μM or 21.8μM, respectively; n=3~5). Suramin and PPADS, both P2 receptor antagonists, also inhibited the current, and their IC50 were 0.43μM and 0.36μM, respectively (n=3~5). A previous study suggested that UTP activates TRPC3 channel through P2Y receptors in vascular smooth muscle cells (Reading SA et al., 2005). However, SKF96365 (30μM) and 2-APB (100μM), which are potent TRPC3 antagonists, failed to inhibit the UTP-induced current (n=3~5). Application of α,β-methylene ATP (10μM), a potent P2X receptor agonist, slightly potentiated the UTP (1mM) -evoked current (13.1±5.4% (n=8) from the basal current), but inversely, application of UTP (1mM) did not facilitate the α,β-methylene ATP (10μM) -evoked current. Similar effect was observed in the tension recordings in endothelium-denuded rat aorta ring preparations. These results indicate that UTP activates the same receptor which is sensitive to α,β-methylene ATP. Moreover, intracellular application of GDPβS or GTPγS (each 1mM), which is G-protein inhibitor or activator, respectively, did not modify the current at all. This result also indicates that the current was independent of G-protein coupled receptors.

Single channel analysis revealed that both UTP (1mM) and α,β-methylene ATP (10μM) activate channels with similar conductance (10.5pS) in the outside-out mode.

RT-PCR and Western blot analysis showed high expression of P2X1 subtype in cerebral and mesenteric arteries and aorta. However, TRPC3 transcript was not expressed in mesenteric artery and aorta. In cerebral artery, a faint band was observed for TRPC3.

Taken together, our results suggest that UTP regulates arterial tone through P2X receptor activation, but not through activation of P2Y receptor.

Burnstock G (2006). Pharmacol Rev 58, 58-86.

Reading SA et al. (2005). Am J Physiol Heart and Circ Physiol 288, 2055-2061.

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

PC4

Visualisation of vascular cannabinoid receptors and their potential interaction with α_1-adrenergic receptors

C.J. Daly[1], G. Wallace[1], K. White[1], H. Chris[2], A. Irving[2] and J.C. McGrath[1]

[1]Integrative & Systems Biology, Faculty of Biomedical and Life Sciences, Glasgow University, Glasgow, UK and [2]Centre for Neuroscience, Division of Molecular and Translational Medicine, University of Dundee, Ninewells Hospital & Medical School, Dundee, UK

The role of cannabinoid receptors (CB1 & CB2) within the cardiovascular system is unclear. The endogenous cannabinoid anandamide (AEA) mediates vasodilation *in vitro*[1] whilst *in vivo* a triphasic blood pressure response comprising pressor and depressor components has been reported[2]. A possible role for CB1 and CB2 receptors exists within vascular tissue. However, non-CB mediated responses in the vasculature have been observed and a role for the orphan receptor GPR55 has been postulated[3]. The aims of this study were to a) investigate the role of endogenous cannabinoids in mouse tail artery, a thermoregulatory vessel rich in α_1-adrenoceptors and b) examine, for the first time, the binding of a novel fluorescent ligand for CB receptors (Tocrifluor 1117).

Tail arteries were removed from 4 month old male C57 black mice. Vessel segments were either mounted in a wire myograph for functional studies or incubated in Tocrifluor 1117 (0.5μM) & QAPB (1μM, fluorescent α_1-adrenoceptor antagonist) for confocal analysis. Concentration response curves (CRC) to noradrenaline (NA) were performed in the presence and absence of the endocannabinoids AEA (1μM) and 2-arachidonylglycerol (2-AG, 1μM). Tocrifluor 1117 and QAPB were imaged under 488nm and 529nm excitation respectively. Tocrifluor 1117 binding was also examined in HEK293 cells stably expressing GPR55.

AEA 1μM caused a transient contraction in isolated tail artery segments (0.08g). The NA CRC was shifted to left in the presence of 1μM AEA (Log EC50 control –6.80 vs –7.82, $p<0.05$). The maximum contractile response was unchanged. In the presence of 2-AG (1μM) a small leftward (non -significant) shift of the NA CRC was observed. However, comparison of the effect of 2-AG alone (Log EC50 –7.36) and 2-AG plus