pared to static controls, we also demonstrated that preconditioning cells affected the distribution of the adhesion molecule ICAM-1, as determined by immunocytochemical staining. In an adhesion assay, preconditioning further affected the adherence of THP-1 monocytes to HUVECs.
In summary, this system allows the assessment of responses of the vascular endothelium to inflammatory factors in a pathophysiologically-relevant setting. Priming of HUVECs with oscillatory flow altered their protein expression and functional profiles and rendered them more sensitive to subsequent treatment with TNFα. This *in vitro* model is amenable to further studies examining the effects of cigarette smoke toxicants on the vascular endothelium.

	non-preconditioned		preconditioned	
Protein	Control	TNFα	Control	TNFα
IL-8	1.3±0.6	29.5±10.0	3.1±1.1	52.2±21.7
MCP-1	1.4±1.2	17.8±4.7	8.6±9.4	42.8±13.3

Table 1. Concentrations of inflammatory and chemoattractant proteins in media following exposure of HUVECs for 18-22 hours to 5 ng/ml TNFα. Data were obtained in cells cultured in static conditions (non-preconditioned) or preconditioned with oscillatory flow. Protein levels were determined by multiplex electrochemiluminescence detection using the MesoScale Discovery (MSD) platform. All concentrations are μg/ml. Data are means ± S.D. (n = 5-7 in each case).

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C12

Extracellular calcium-sensing receptor-mediated signalling and its role in human vascular smooth muscle cell proliferation and apoptosis

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Vascular smooth muscle cells (SMC) play a pivotal role in the pathogenesis of medial calcification (major cause of cardiovascular mortality in chronic kidney disease). We have recently demonstrated expression of the calcium-sensing receptor (CaSR) in human aortic SMC (HAoSMC) and human arteries and demonstrated a correlation between CaSR expression and medial calcification. The CaSR is involved in a number of diverse processes as hormone secretion, modulation of inflammation, proliferation, differentiation and apoptosis, however, functional significance of this receptor in vascular SMC is not fully understood. Here we examined CaSR-mediated intracellular signalling pathways and investigated the potential role of CaSR in regulating SMC proliferation and apoptosis.
HAoSMC were incubated with CaSR agonists (neomycin and gentamycin) and signalling inhibitors. ERK1,2 activation was assessed by Western blot. Inositol triphosphate (IP3) production was measured using Biotrak assay (Amersham). Cell proliferation was determined by BrdU incorporation and apoptosis assessed by flow cytometry of propidium iodide stained cells. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test.
Incubation of HAoSMC with 300μM neomycin resulted in 7.5-fold ($p<0.05$) increase in ERK1,2 phosphorylation. This induction was reduced ($p<0.01$) in the presence of 10μM PD98059 (MEK1 inhibitor), indicating that CaSR agonist-induced effects were mediated via classic MEK1/ERK1,2 pathway. ERK1,2 phosphorylation was almost completely abolished by 5μM U73122 (PLC inhibitor), indicating that PLC signalling was crucial for MEK1/ERK1,2 activation. No changes were observed with PI3K and PKC inhibitors. Confirming PLC activation, IP3 production was increased by neomycin/gentamycin ($p<0.05$) and reduced in the presence of U73122 ($p<0.05$). To confirm that ERK1,2 and PLC signalling were mediated via the CaSR, HAoSMC were transfected with CaSR siRNA. CaSR-knockdown resulted in attenuated ERK1,2 phosphorylation in response to neomycin (>50% of neomycin induction in control cells, $p<0.01$) while IP3 production was almost completely abolished. Treatment with neomycin increased HAoSMC proliferation >3-fold ($p<0.01$). This was reduced in CaSR-knockdown cells ($p<0.01$) and further inhibited by PD98059 and U73122 ($p<0.05$). Apoptosis was not affected by neomycin treatment or CaSR expression. However, inhibition of PLC signalling (incubation with U73122) produced a 3.5-fold increase in HAoSMC apoptosis ($p<0.05$), which was further increased by CaSR-knockdown (4.8-fold versus control siRNA, $p<0.05$).
In conclusion, these data suggest that CaSR stimulation leads to activation of the MEK1/ERK1,2 and PLC pathways and increases cell proliferation. CaSR-mediated PLC activation is crucial for SMC survival and protection against apoptosis.

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

C13

Proteomic analysis reveals distinct role of smooth muscle progenitors in extracellular matrix production

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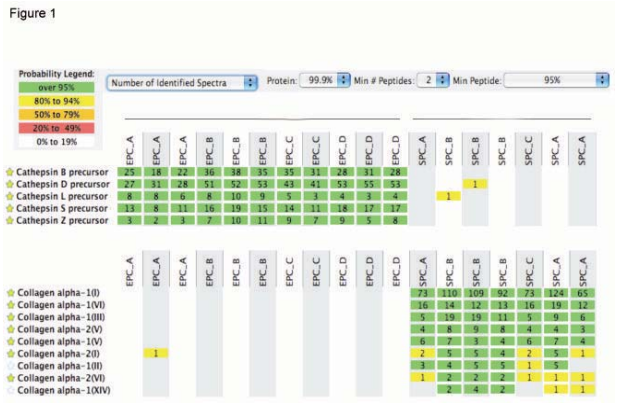
Background. Recent studies on cardiovascular progenitors have led to a new appreciation of their secretome, as paracrine factors may support the regeneration of damaged tissues.
Methods and Results. Here we utilized a shot-gun proteomics strategy to define endothelial and smooth muscle progenitors (EPCs and SPCs) based on their distinct proteomic profiles in the secretome. Our data confirms previous results from

microarray experiments (Urbich C et al. 2005) that EPCs express high levels of cathepsins (Fig 1). In contrast, no cathepsins were detected in SPCs conditioned medium. SPCs also showed attenuated production of proteolytic enzymes and inflammatory cytokines, but secreted extracellular matrix molecules such as a variety of collagen chains and fibronectin. Compared to their mature smooth muscle counterparts, SPCs produced different isoforms of matrix proteins as evidenced by the truncation of angiogenic domains in collagen alpha-1 (I). Moreover, SPCs retained specific proteins from the bovine serum supplement, including insulin-like growth factor-binding protein 2, a known target gene of the hypoxia-inducible factor, pigment epithelium-derived factor, a potent inhibitor of angiogenesis, and proteoglycans regulating collagen assembly. As a functional consequence, SPCs showed reduced invasive capacity and their conditioned medium inhibited endothelial tube formation. Conclusion. The present study represents an important conceptual development in vascular biology suggesting that SPCs can regulate tissue remodeling and demonstrates the utility of contemporary proteomics to better characterize vascular progenitors intended for therapy in clinics.

Figure 1



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Sources of Funding: This work was funded by the European Vascular Genomics Network (LSHM-CT-2003-503254; Brussels, B) as part of the 6th European Framework Programme and grants from the British Heart Foundation and Oak Fundation. D.S. was supported by a grant from Juvenile Diabetes Research Foundation and by resources and facilities at the Carl T. Hayden VA Medical Center, Phoenix, AZ, USA.

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C14

Resistance artery structure and mechanics in hypertension and aging. Influence of integrin-extracellular matrix interactions

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Vascular structural and mechanical alterations in hypertension and aging contribute towards cardiovascular morbidity/mortality[1-3]. The aim of this study was to determine whether integrin-extracellular matrix (ECM) interactions contribute towards altered vascular structure and mechanics in hypertension and aging. The effect of disintegrin-treatment (kistrin, or echistatin) *ex vivo* on mesenteric artery structure and mechanics was assessed using wire-myography in spontaneously hypertensive rats (SHRs) and wistar-kyoto rats (WKYs). Integrin αV and α5 expression was also quantified using immunohistochemistry. Differences between data were tested using 2-tailed unpaired student's t-test, or one-way ANOVA followed by Bonferroni's correction for multiple comparisons. In arteries from 8 and 15 week-old SHRs versus WKYs, media/lumen ratios were greater (by 108 and 70% respectively; $P<0.01$) and media cross-sectional area (MCSA) and stiffness unaltered. Disintegrin-treatment of arteries did not alter structure or mechanics in SHRs versus control-treatment. With aging in WKYs, media thickness and MCSA were significantly increased (by 25 and 44% respectively in 15 week-old versus 8 week-old WKYs, $P<0.05$; by 43 and 46% respectively in >1 year-old versus 8 week-old WKYs, $P<0.01$) and stiffness unaltered. This was associated with unaltered integrin αV and α5 expression. In WKYs at all ages, disintegrin-treatment of arteries did not alter structure; however, stiffness was significantly reduced with echistatin-treatment versus control-treatment ($P<0.05$). These results suggest that integrin-ECM interactions are important determinants of passive stiffness in aging WKYs. Further insight into the role of integrin-ECM interactions on vascular structure and mechanics may have important implications for the reduction of cardiovascular morbidity/mortality.

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by NO but also by some other factors (e.g. hyperpolarizing factor).

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PC25

Cardiac protein thiol modification by 15-deoxy-delta 12, 14 prostaglandin J2

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Cellular redox signalling is in part mediated by post translational modification of proteins by reactive oxygen species, nitrogen species or by products of their reactions. Enhanced lipid peroxidation has traditionally been causatively associated with many diseases; although important roles in redox signalling are now being recognised. Many lipid peroxidation products are reactive and are capable of reacting with and modifying protein thiols. Cyclopentenone prostaglandins (CyPGs) are model examples of reactive oxidised lipids, containing electrophilic carbon centres that allow covalent adduction with target proteins. To determine if lipid-protein adducts form in a cellular setting, a biotinylated derivative of 15-deoxy-delta 12, 14 prostaglandin J2 (15d-PGJ2) (an electrophilic lipid) was used to treat adult rat ventricular myocytes. There was a dose- and time-dependent increase in biotin-15d-PGJ2 labelling which was maximal at 50μM 15d-PGJ2 and after 120 minutes. 15d-PGJ2 treatment of isolated rat hearts decreased coronary perfusion pressure (vasodilation). Mesenteric vessels were treated with biotin-15d-PGJ2 in order to identify proteins that formed an adduct with 15d-PGJ2. These proteins were purified with avidin-agarose and identified by separation of tryptic digests by liquid chromatography with online analysis by mass spectrometry. Several proteins were identified that formed an adduct. However one particular protein that was modified, soluble epoxide hydrolase (sEH), we hypothesized might account for the vasodilation observed in isolated hearts. sEH catalyses the hydroysis of epoxides, such as epoxyeicosatrienoic acids (EETs), to diols (dihydroxyeicosatrienoic acids). We assayed sEH activity, and found 15d-PGJ2 inhibited its activity, both in vitro with recombinant sEH, as well in cardiac homogenates after hearts were exposed to this lipid. Furthermore, when an inhibitor of sEH, (AUDA), was perfused through isolated rat hearts, it mimicked the vasodilatory response of 15d-PJ2. Similarly, 14, 15-epoxyeicosatrienoic acid (14, 15-EET) also decreased coronary perfusion pressure. These results suggest that 15d-PGJ2 is able to form an adduct with sEH, which inactivates it. This results in cellular accumulation of 14, 15-EET, allowing them to exert their vasodilatory response.

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PC26

Expression and role of RhoA/ROK pathway in control of agonist-induced Ca^{2+} signaling in endothelial cells of intact rat tail artery

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The endothelium is a confluent monolayer, lining the inner surface of blood vessels which acts as the main local regulator of vascular wall homeostasis. Binding of inflammatory mediators to G-protein coupled receptors increases endothelial cells permeability by increasing intracellular Ca^{2+} concentration. Several studies have shown that RhoA/Rho-kinase plays a key role in modulation of barrier properties of cultured endothelial cells and intact microvessels [W-S. Beata *et al*, 1998: C.M. Joes *et al*, 1999: R.H. Adamson *et al*, 2002]. Therefore we investigated role of Rho-A and its main effector, Rhokinase in control of calcium signaling in intact endothelial cells of rat tail artery using immunohistochemistry and confocal imaging. Rats were humanely killed under CO_2 anaesthesia; their tail removed from the ventral grove, cleaned of fat and loaded with Fluo-4 AM (Molecular Probes, 15um) with pluronic. Confocal imaging was done using Nipkow disc based confocal imaging system (Ultraview Perkin Elmer, UK). Minimum of 3 animals were used in each set of experiments. We have found that in endothelium of rat tail artery ROK-α but no ROK-β was expressed. Carbachol a muscurinic receptor agonist was used in our study to stimulate the intact endothelial cells. Application of carbachol (0.1uM, 1uM, 10uM) to intact endothelial cells produced a calcium transient which consisted of two components: initial fast - dependent on Ca^{2+} release and subsequent, sustained dependent on Ca^{2+} entry. Sustained component of CCh induced Ca^{2+} transient was 41 $\pm$ 0.7 of the peak taken for 100% (n= 372cells, 7vessels). The frequency of oscillation ranges from 0.05 to 0.3 Hz (n=372cells, 7vessels). Inhibition of Rho-A by C3-transferase (1μg/ml) or Rho-kinase by H-1152 (200 nM) reduced the initial fast component of CCh induced Ca^{2+} transient to 55$\pm$1.5 (n=134 cells, 3 vessels) and 52$\pm$2.1 (n=136 cells, 3 vessels) of the peak, respectively and either fully abolished or significantly decreased the amplitude and the duration of the Ca^{2+} oscillations while the amplitude of the sustained component expressed as a percentage of peak was 65$\pm$1.8% (n=134cells, 3vessels) and 29$\pm$1.6% (n=136cells, 3vessels), respectively. Taken together, the data obtained suggest that Rho-A/ROK pathway is involved in control of calcium signaling induced by CCh in intact endothelium of large conduit arteries.

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0.33±0.02 when iberiotoxin was also added (N=51 cells in 4 vessels from 4 animals; P<0.001). Iberiotoxin, however, had no additional effect on oscillation frequency. It appears, therefore, that Ca^{2+}-activated Cl^- currents play an important role in determining the frequency of the Ca^{2+}-oscillations elicited by endothelin, while Ca^{2+}-activated K^+-currents limit the amplitude of these oscillations.

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The Wellcome Trust, JDRF

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PC13

The vasorelaxant effect of *Viscum album* leaf extract is mediated by calcium-dependent mechanisms

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Viscum album leaf extract (VA) has a folk reputation as an anti-hypertensive agent in Nigeria. Evidence suggests that it has a vasorelaxant effect that is endothelium dependent involving the release of nitric oxide (Ekpenyong et al, 1999). However, VA showed a relaxant effect in endothelium-denuded preparations (Ekpenyong et al, 1999) suggesting that it might also act on the vascular smooth muscle via calcium-dependent mechanisms. The present study was designed to investigate this possibility.

Fresh leaves of *Viscum album* were harvested and ground into powder. An aqueous extract was prepared and phytochemical analyses done. Sprague-Dawley rats (n=6) were anaesthetized using a 25% urethane and 1% chloralose mixture given intraperitoneally at a dose of 5ml/kg. Thoracic aortae were obtained and cut into 2 mm ring segments in Physiological salt solution (PSS; composition ($mmol.l^{-1}$): NaCl, 119; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 14.9; $CaCl_2$, 1.6; glucose, 11.5). Each aortic ring was mounted in a tissue bath containing PSS and continuously bubbled with a 95% O_2-5% CO_2 gas mixture at a temperature and pH of 37°C and 7.4 respectively (Adegunloye and Sofola, 1997). Relaxation response studies to VA (2-16 $mg.ml^{-1}$) were done following pre contraction with 10^{-7} $mol.l^{-1}$ noradrenalin or 60$mmol.l^{-1}$ KCl. The IC_{50} of VA in noradrenalin pre-contracted rings was 5.2 ± 0.79$mg.ml^{-1}$ while the IC_{25} in KCl pre-contracted rings was 7.1±0.56 $mg.ml^{-1}$. These concentrations of V. album were used in subsequent experiments. Concentration response curves (CRCs) to noradrenalin (10^{-9} to $10^{-5}mol.l^{-1}$), KCl (10-80$mmol.l^{-1}$) and $CaCl_2$ ((2.5×10^{-4} to 1.6×10^{-1} $mol.l^{-1}$) were constructed with and without VA. Phasic contraction to 10^{-5} $mol.l^{-1}$ noradrenalin was carried out in Ca-free EGTA (N′N′-ethylene glycol tetra acetic acid) PSS (Perry & Webb, 1991). The results are presented as mean ± SEM. The student's t test for paired data was used for statistical analysis. P<0.05 was taken as statistically significant. Phytochemical analyses showed the presence of flavonoids and tannins. The CRCs to noradrenalin or KCl were significantly (P<0.05) attenuated and shifted to the right in the presence of the VA. Also the CRC to $CaCl_2$ in the presence of noradrenalin or KCl was attenuated and shifted to the right by VA, while the phasic response to noradrenalin was diminished (P<0.05).

These results suggest that the vasorelaxant effect of VA may be mediated by a non-specific non-competitive inhibition of Ca^{2+} influx as well as inhibition of Ca^{2+} mobilization from intracellular stores. These calcium antagonistic effects may be due its flavonoid or tannin content.

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Support of the The Physiological Society is gratefully acknowledged.

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PC14

A novel mechanism for vasorelaxation by S-nitrosothiol-induced activation of PKG1a and type I PKA

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In previous studies we demonstrated a novel redox mechanism for directly activating PKG1a and PKAI by H2O2 mediated interprotein disulphide formation. These are examples of how oxidant signalling can directly integrate into phosphoregulation and alter vasotone. We hypothesised that nitric oxide (NO) species may also induce disulphide formation and hence kinase activation through formation of S-nitrosothiol intermediates. The nitric oxide donors SNAP and CysNO were assessed for their ability to generate protein S-nitrosylation in the isolated perfused rat heart using the biotin-switch method. Isolated rat heart perfusion at constant flow with the nitric oxide donor SNAP (100μM-1mM) did not alter protein S-nitrosylation, whereas CysNO (5-20μM) increased this protein modification in a dose-dependent manner. In addition to S-nitrosylation, CysNO also caused a dose-dependent increase in PKG1a and PKA-RI disulphide dimerisation, which was absent in SNAP perfused hearts. However, treatment of heart homogenate in vitro with SNAP induced an increase in both protein S-nitrosylation and PKG1a disulphide dimerisation, indicating that S-transnitrosylation is crucial for increased disulphide formation. When purified recombinant N-terminal PKG1a was exposed to CysNO, it induced intermolecular disul-

phide formation. This indicates that PKG1a disulphide occurs via formation of an S-nitrosothiol intermediate. It was demonstrated using studies measuring tension in isolated aorta that both NO donors are able to induce dose-dependent vessel relaxation. However, only SNAP mediated relaxation was blocked by the guanylate cyclase inhibitor ODQ, indicating that CysNO induces cGMP independent relaxation. Using PKG and PKA inhibitors in combination with ODQ is was shown that the cGMP independent relaxation generated by CysNO was dependent on PKG and PKA activation. This is consistent with CysNO directly activating PKG and PKA through the formation of a interprotein disulphide bond. These studies highlight the potential for beta-adrenergic like signalling, independently of cAMP elevation, by increased S-nitrosylation of PKA-RI.

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PC15

Endogenous superoxide dismutase (SOD) plays a greater role in hypoxia-induced muscle vasodilatation in rats exposed to 12% O_2 for 1-7 days than in normoxic rats

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The hindlimb vasodilatation evoked by acute systemic hypoxia is ~50% mediated by adenosine and 90% nitric oxide-dependent[1]. In normoxic (N) rats we showed that during acute hypoxia, xanthine oxidase (XO) metabolises adenosine to generate O_2^-, which is converted to H_2O_2 by endogenous SOD and contributes to the hindlimb vasodilatation[2]. Chronic hypoxia is likely to increase O_2^- release via XO and the mitochondria. Thus, we have now investigated in chronically hypoxic rats, housed in 12% O_2 for 1, 3 and 7 days (1, 3 & 7CH), the contribution of H_2O_2 generated by endogenous SOD in hindlimb.

Male Wistar 1, 3 and 7CH (n=9, 10 & 10) rats were anaesthetised with Alfaxan (12 mg.kg^{-1}.hr^{-1} i.v.) and routinely breathed 12% O_2. Arterial blood pressure (ABP) and femoral blood flow (FBF) were recorded and FVC was computed online (FBF/ABP) before and during a 5 min period of breathing 8% O_2 (acute hypoxia) before and during infusion of the cell permeant SOD inhibitor sodium diethyldithiocarbamate trihydrate (DETC; 5 mg.kg^{-1}.min^{-1} i.a.). Responses before & after DETC were compared with Student's paired t-test, & between groups by factorial ANOVA with Scheffe's post hoc test; *: P<0.05.

In 1, 3 and 7CH rats baseline P_aO_2 was lower than in N rats (47±1*, 45±2*, 46±2* vs 88±2 mmHg; mean±SEM), while baseline FVC was increased (0.011±0.001*, 0.010±0.001*, 0.010±0.001* vs 0.006±0.001 ml.min^{-1}.mmHg^{-1}), indicating a tonic hindlimb vasodilatation in 1-7CH rats. Breathing 8% O_2 caused a further decrease in P_aO_2 (33±1*, 30±1*, 31±1*), and increase in FVC (to 0.017±0.002*, 0.016±0.002*, 0.017±0.002*) in 1, 3 & 7CH rats to the same levels seen in N rats (28±2 mmHg; 0.016±0.001 ml.min^{-1}.mmHg^{-1}). During DETC infusion, baseline FVC was significantly reduced in 3CH, but not in 1 & 7CH rats. Further, acute hypoxia still caused a significant increase in FVC in 1, 3 & 7CH rats, but changes were smaller than before DETC (0.008±0.002 vs 0.003±0.001*, 0.007±0.002 vs 0.003±0.001*, 0.008±0.002 vs 0.003±0.001*). In N rats, the change in FVC tended to be reduced (0.009±0.001 vs 0.007±0.001 ml.min^{-1}.mmHg^{-1}).

Thus the conversion of O_2^- to H_2O_2 by endogenous SOD apparently contributes to tonic muscle vasodilatation in 3CH rats and plays a greater role in the muscle vasodilatation evoked by acute hypoxia in 1, 3 and 7CH than N rats. We propose that during early chronic hypoxia the generation of O_2^- via adenosine and XO or by mitochondria is increased, and/or SOD activity is increased so enhancing the vasodilator role of H_2O_2.

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Funded by BHF.

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PC16

Venular and arteriolar EDHF-mediated dilatation in cremaster muscle of the rat is sensitive to ROS scavenging

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Reactive oxygen species (ROS) are thought to play a role in EDHF, but endothelial sKCa and IKCa channels opening in response to increased endothelial [Ca2+]i, is also known to be a requirement. We have examined the vasodilatation response to carbachol in the isolated perfused cremaster muscle preparation.

The iliac artery of a freshly killed (Schedule 1) Wistar rat was cannulated orthogradely and branches that did not lead to the cremaster muscle were ligated. The cremaster microcirculation was flushed with St. Thomas' cardioplegic solution {in mmol.l-1: NaCl (110); KCl (7.9); MgCl2.6H20 (34); CaCl2 (1); and Hepes (11)} containing heparin (300 IU.ml-1). The skin over the scrotum was removed to reveal the cremasteric sack. The cremaster was cut to isolate it from the enclosed testes, care being taken not to damage any major vessel. The muscle was spread and pinned over a transparent Sylgard (Dow Corning) support and superfused with a Krebs-bicarbonate buffer solution maintained at 37□C at 1-2 ml.min-1. The perfusate was changed to a buffer contain-

ing albumin (10 mg.ml-1) with added FITC-albumin (5 mg.ml-1).
The cremaster muscle arterioles and venules (pre-phenylephrine diameter 47 ± 4.0 μm (MEAN ± SEM; n = 15 and 63.2 ± 6.7; n = 12, respectively) dose-dependently dilated to carbachol (artery 68 ± 0.6%, vein 84 ± 2.9%; p < 0.001 paired 't' test for both) which was significantly reduced by L-NAME and indomethacin (artery 48 ± 1.3, vein 70 ± 1.3%). The effect of these inhibitors in reducing the carbachol-induced dilatation was significantly greater in arterioles than in the paired venules (p < 0.05, paired 't' test), which may be accounted for by the differences in the smooth muscle layer thickness. The dilatation was virtually blocked by apamin and charybdotoxin (artery 6 ± 2.0%, vein 12 ± 1.5%, n = 4) and very much reduced by scavenging ROS with SOD and catalase (artery 13 ± 2.9%, vein 15 ± 3.9%, all n = 4).

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PC17

Human vascular endothelial cells show a time-dependent response to the stress of hypoglycaemia following their isolation and culture

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Inconsistent data regarding the ability of primary tissue to detect physiological falls in glucose concentration may be due, in part, to the time-dependent ability of a cell in culture to gain energy via oxidative phosphorylation, making it more sensitive to limitations in glucose supply. Thus, hypoglycaemia may induce a non-specific stress response in these cells that could be misinterpreted as low glucose sensing.
Human umbilical vascular endothelial cells (HUVEC) were enzymically and mechanically isolated from umbilical cords within 12 hours of delivery and distributed at high density onto 1% gelatin coated cover slips (Marin et al, 2001). Cells were cultured in Lonza Medium 199 containing foetal calf serum supplemented with penicillin, streptomycin and fungizone at 37□C in a 5% CO2 incubator, changing medium at every 3 days. Intracellular calcium concentration was measured by ratiometric (340/380nm) labelling with Fura 2 and the response to reducing the superfusate glucose concentration from 10mM to 0mM determined at 1, 2, 4 and 8 days after culture and compared to the response to 10uM ATP at the same time periods. Five cultures, each from 2 umbilical cords, were established and measurements made on 4 cover slips on each day of study. Time dependent differences in calcium response were analyzed by single factor ANOVA. Sheffe post hoc tests were performed as appropriate and significance was taken as P<0.05. Local ethical approval and informed consent were obtained.
A significant time dependent effect was observed in response to both falls in glucose and addition of ATP (P<0.001 and P<0.002 respectively) but the pattern of response differed (P<0.0001, two way ANOVA). The calcium response to zero glucose or ATP at day 1 was low (ratiometric mean±SEM: 0.005±0.003 and 0.004±0.004 respectively). At day 2 the response to zero glucose was significantly increased by a factor of 7.4 (0.037±0.008) but although the mean response to ATP was also increased by around the same amount, to 0.033±0.020, this did not reach significance. At day 4 the response to zero glucose was sustained (0.022±0.008) while the response to ATP (0.238±0.083) was substantially and significantly increased by almost 60-fold compared to day one. On the eighth day of culture, the response to glucose dropped to a level statistically similar to that of day 1 (0.004±0.002) while the ATP response was sustained at a level not significantly different to that on day 4 (0.161±0.035).
Our data shows that cultured HUVEC show a time-dependent response to the stress of hypoglycaemia following isolation and culture that differs in time course and magnitude from the response to ATP. What the mechanism involved is and whether it underlies the variability of the response to hypoglycaemia reported previously in the literature (Kumar, 2007) is not known.

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

PC18

Does nicotinic acid adenine dinucleotide phosphate elicit Ca^{2+} release via two-pore channel subtype 2 in rat arterial smooth muscle cells?

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Nicotinic acid adenine dinucleotide phosphate (NAADP) is a potent Ca^{2+} mobilising messenger in mammalian and non-mammalian cells. Studies on a variety of cell types suggest that NAADP evokes Ca^{2+} release from a lysosome-related store and via activation of a receptor distinct from either ryanodine receptors or inositol 1,4,5-trisphosphate receptors (IP_3R; 1,2). Consistent with this view, studies on pulmonary arterial smooth muscle cells (PASMC) have shown that NAADP elicits Ca^{2+} signals by mobilizing lysosome-related Ca^{2+} stores (3). Recent investigations have suggested that the two-pore channel subtype 2 (TPC2) acts as an NAADP receptor in HEK293 cells that stably over-express human TPC2 (hTPC2; 4). We therefore investigated the possible role of TPC2 in NAADP-mediated Ca^{2+} release in PASMC.

PC2

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

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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.

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PC4

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

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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

indomethacin (10μM, Log EC50 -6.33) revealed a significant difference (p>0.05). Tocrifluor 1117 binding was most evident on perivascular fat, adventital cells, nerves and smooth muscle. In several areas of media, colocalisation of Tocrifluor 1117 and QAPB was observed. In live HEK293 GPR55 cells, Tocrifluor 1117 generated a rise in Ca++ and promoted receptor clustering, visible as punctate fluorescence which developed over time.

Tocrifluor 1117 (fluorescent analogue of the CB1 antagonist AM251) is a potentially very powerful tool for identifying the cellular location of cannabinoid receptors, including GPR55 in living tissues. AM251 has been shown to activate GPR55[4]. The results of the study suggest that endogenous cannabinoids potentiate the actions of noradrenaline in tail artery possibly via co-localised cannabinoid (GPR55?) and α_1-adrenergic receptors in vascular smooth muscle. The importance of the CB/GPR55 receptors in perivascular fat and adventitia requires further study.

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PC5

Quantitative RT-PCR analysis of smooth muscle markers between mature and immature vascular myocytes

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Immature, non-contractile myocytes morphologically similar to the intestitial cells of Cajal, have been found resident in the vasculature[1]. It is thought that these cells are synthetic vascular myocytes and play a major role in vascular remodelling[2]. This work aimed to determine the quantitative differences in transcription of smooth muscle cell and interstitial cell marker genes between immature and mature vascular myocytes. Genes investigated were: smooth muscle (SM) α-actin, β-actin, myosin light chain kinase (MLCK), desmin, smooth muscle myosin heavy chain (SM-MHC) isoforms SM1 and SM2 (smooth muscle marker genes) and c-kit and vimentin (markers of interstitial cells).

Investigations were carried out on freshly isolated myocytes from mesenteric arteries of guinea-pigs which were killed in accordance with national guidelines for humane killing of experimental animals (Schedule 1). Single mature and immature myocytes were obtained by enzymatic digestion and collected using a wide-bore pipette mounted on a micromanipulator. Cells were prepared for qRT-PCR using the TaqMan PreAmp Cells-to-Ct Kit (Applied Biosystems). This involved cell lysis, DNA removal, reverse transcription of RNA into cDNA and gene-specific preamplification using custom assays followed by analysis in a qRT-PCR analyser. Custom TaqMan gene expression assays were designed for each gene using available guinea-pig sequences and sequences determined by homology. CT values were compared directly between cell phenotypes and fold differences (ratio of mRNA quantity in mature cells versus immature cells) for each target gene were tested for statistical significance using one sample Student's t-test (p=0.05).

The results are shown in Table 1.

C-kit mRNA was not detected in either mature or immature myocytes (n=13, N=4), but was present in the wall of the small intestine (n=3, N=1), confirming the validity of the assay.

These data confirm common cell lineage for immature and mature vascular myocytes and identify subtle differences in gene expression pattern between them, which hint at different physiological roles of these two phenotypes.

Table 1. Relative differences in marker mRNA quantity between mature and immature myocytes (the statistically significant ones are shown in bold).

Gene	*Replicates (n)*	*Animals (N)*	*Fold Difference (±SEM)*	*Signif*
SM α-actin	10	3	2.68±1.08	p=0
β-actin	21	4	1.66±1.27	p=0
Desmin	6	2	2.48±1.65	p=0
Vimentin	9	3	1.07±0.45	p=0
SM-MHC SM1	10	3	8.85±8.36	p=0
***SM-MHC SM2**	10	3	>>33.36	
MLCK	11	3	0.37±0.10	p=0

Table 1. Relative differences in marker mRNA quantity between mature and immature myocytes (the statistically significant ones are shown in bold).

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PC6

Impaired antioxidant stress protein expression in fetal endothelial cells in pre-eclampsia: effects of oxygen tension

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Pre-eclampsia (PE) is a pregnancy-specific disease resulting in systemic maternal endothelial dysfunction, associated with placental hypoperfusion and increased generation of reactive oxygen species (ROS) and pro-inflammatory mediators (Noris, 2005). We previously identified phenotypic alterations in human umbilical vein endothelial cells (HUVEC) isolated from PE pregnancies (Steinert et al., 2002; Afzal-Ahmed et al., 2007). In the present study, we examined whether PE affects activation of the redox sensitive transcription factor Nrf2 and whether changes in oxygen tension (21% versus 5%) affect Nrf2 mediated HO-1 protein expression in normal and PE HUVEC.