C43

HYPOXIC PULMONARY VASOCONSTRICTION: IS AMP-ACTIVATED PROTEIN KINASE THE PRIMARY METABOLIC SENSOR AND EFFECTOR?

A.M. Evans[1], K.J. Mustard[2], C.N. Wyatt[1], M. Dipp[1], N.P. Kinnear[1] and D.G. Hardie[2]

[1]School of Biology, St Andrews University, St-andrews, UK and [2]Division of Molecular Physiology, University of Dundee, Dundee, UK

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

C44

THE POTENTIAL ROLE FOR AMP-KINASE IN HYPOXIC CHEMOTRANSDUCTION OF RAT CAROTID BODY.

C.N. Wyatt[1], P. Kumar[2], C. Peers[3], P. Kang[3], D.G. Hardie[4] and A.M. Evans[1]

[1]School of Biology, St Andrews University, St-andrews, UK, [2]Department of Physiology, University of Birmingham, Birmingham, UK, [3]Institute of Cardiovascular Research, University of Leeds, Leeds, UK and [4]Division of Molecular Physiology, University of Dundee, Dundee, UK

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

C45

EFFECTS OF ACUTE HYPOXIA ON CALCIUM HOMEOSTASIS IN PRIMARY CULTURES OF HUMAN SAPHENOUS VEIN ENDOTHELIAL CELLS.

P. Kang[1], K.E. Porter[1], P.J. Kemp[2] and C. Peers[1]

[1]Medicine, University of Leeds, Leeds, UK and [2]School of Biomedical Sciences, University of Leeds, Leeds, UK

Acute hypoxia is well known to regulate $[Ca^{2+}]_i$ indirectly by altering the activity of plasmalemmal ion channels (e.g. Peers & Kemp, 2001). However, its direct effects on intracellular Ca^{2+} homeostasis are less well studied. Here, we have investigated the effects of acute hypoxia on agonist-evoked changes of $[Ca^{2+}]_i$ in human saphenous vein endothelial cells. Cells were isolated and maintained in primary culture as previously described (Budd et al., 1991) and $[Ca^{2+}]_i$ was monitored in Fura-2 loaded cells as previously described (Smith et al., 2003).
Bath application of 10μM ATP in the absence of external Ca^{2+} evoked a transient rise in cytosolic $[Ca^{2+}]$ (peak = 0.211 ±0.008 ratio units (ru; n = 118 cells). Exposure of cells to hypoxia (pO2 ~ 25mmHg) for 150s caused a small but distinct increase in $[Ca^{2+}]_i$ (peak 0.012 ± 0.002 ru; n = 46). Following this exposure, subsequent application of 10μM ATP evoked a transient rise of $[Ca^{2+}]_i$ (peak, 0.134 ± 0.007 ru; n=37) which was significantly smaller than that observed in cells not exposed to hypoxia ($p<0.001$, unpaired Student's t-test). Following exposure to either ATP or to acute hypoxia, re-introduction of Ca^{2+} to the perfusate evoked a similar degree of capacitative Ca^{2+} entry in both cases, peaking at 0.080 ± 0.006 ru following ATP exposure and 0.077 ± 0.009 ru following exposure to hypoxia (n=36 in each case). Our data indicate that acute hypoxia evokes Ca^{2+} release from an ATP-sensitive intracellular pool in human venous endothelial cells, and such release is sufficient to trigger capacitative Ca^{2+} entry.

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Smith, I.F. et al. (2003) J. Biol. Chem. 278, 4875-4881.

Supported by the British Heart Foundation and a Pfizer CASE studentship to PK.

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

C46

NAADP MEDIATES CALCIUM SIGNALS BY ENDOTHELIN-1 IN ISOLATED RAT PULMONARY ARTERY SMOOTH MUSCLE CELLS

N.P. Kinnear[1], J.M. Thomas[2], A. Galione[2] and A.M. Evans[1]

[1]Division of Biomedical Sciences, School of Biology, University of St Andrews, St Andrews, Fife, UK and [2]University Department of Pharmacology, University of Oxford, Oxford, Oxon, UK

Our previous studies on isolated pulmonary artery smooth muscle cells (PASMC) have shown that nicotinic acid dinucleotide phosphate (NAADP) triggers Ca^{2+} release from intracellular stores (Boittin *et al.* 2002) and that these stores are lysosome-related (Kinnear *et al.* 2003). Pulmonary vasoconstrictors mediate their actions, in part, by altering intracellular Ca^{2+}. In this study we have investigated the effect of selectively depleting the lysosomal Ca^{2+}store on the actions of two vasoconstrictors: prostaglandin-F2 alpha ($PGF_{2\alpha}$) and endothelin-1 (ET-1) and the effect these vasoconstrictors have on NAADP levels.
PASMC were isolated from adult male Wistar rats (150-300g) that had been killed by cervical dislocation. Changes in intracellular Ca^{2+} were detected using Fura-2 fluorescence imaging, as described previously (Boittin *et al.* 2002). $PGF_{2\alpha}$(2 μM)caused an increase in the f340/f380 fluorescence ratio from 0.54±0.04 to 1.29±0.18 (n=5, mean±S.E.M.). The vacuolar proton pump inhibitor bafilomycin-A1 (100nM, 50min) had no effect on this rise in Ca^{2+} (n=5).In contrast to this, the Ca^{2+} signals observed with ET-1 were abolished by incubation with bafilomycin A1. ET-1 (100 nM) caused an increase in f340/f380 fluorescence ratio from 0.56±0.09 to 1.17±0.02 (n=4) in the absence of bafilomycin A1. This increase was inhibited by 98.4% after incubation with bafilomycin A1 (100nM, 50min). Incubation of the cells with thapsigargin (1μM)prevented ET-1 (100nM) from inducing global Ca^{2+} waves and contraction. However, small spatially restricted Ca^{2+} bursts were still observed in 5 out of 9 cells causing an increase in f340/f380 ratio of 17±4% within a given region of interest in the cell. Similarly, after incubation of cells with ryanodine (20μM), spatially restricted Ca^{2+} bursts were also detected in 3 out of 8 cells with the increase in f340/f380 ratio measuring 24±0.1% within a region of interest.

Using a competitive radioreceptor binding assay (Masgrau *et al.* 2003) the effect of ET-1 on NAADP levels in endothelium denuded pulmonary arteries was investigated. A 30s exposure of ET-1 (1μM) increased levels of NAADP from 0.21±0.04 to 1.33±0.2 pmol/mg protein (n=12). Exposure of arteries without endothelium to
$PGF_{2\alpha}$ (2 μM) caused no significant increase in NAADP levels (n=3).
These data suggest that ET-1 but not $PGF_{2\alpha}$ mobilises Ca^{2+} from a thapsigargin and ryanodine-insensitive store via NAADP.

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Kinnear et al (2003). J. Physiol (Lond). **552p**, C70

Masgrau et al (2003). Current Biology. **13**, 247-251

This work was supported by the Wellcome Trust and the BBSRC.

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

C47

SUB-SECOND CHANGES IN F-ACTIN LEVELS COMPATIBLE WITH ACTIVATION OF STORE-OPERATED CA^{2+} ENTRY BY *DE NOVO* CONFORMATIONAL COUPLING IN HUMAN PLATELETS

P.C. Redondo[1], M.T. Harper[2], J.A. Rosado[1] and S. Sage[2]

[1]*Physiology, University of Extremadura, Caceres, Spain and* [2]*Physiology, University of Cambridge, Cambridge, UK*

Ca^{2+} entry in human platelets can be activated by depletion of intracellular Ca^{2+} stores. This store-operated Ca^{2+} entry (SOCE) is reduced if remodelling of the actin cytoskeleton is inhibited (Rosado *et al.*, 2000), suggesting platelet SOCE is activated by secretion-like coupling, where trafficking of endoplasmic reticulum towards the plasma membrane allows coupling between proteins in these two membranes. Platelet Ca^{2+} store depletion results in *de novo* coupling of the type II inositol 1,4,5-trisphosphate receptor (IP_3RII) to the Ca^{2+} entry channel hTRPC1 (Rosado & Sage, 2000). Coupling is reduced if cytoskeletal remodelling is inhibited. An essential requirement of the secretion-like coupling hypothesis is that remodelling of the cortical F-actin layer occurs rapidly enough to allow membrane trafficking and protein coupling to account for the observed Ca^{2+} entry. Here we have used a quenched-flow approach to examine thrombin-evoked changes in platelet F-actin content on a sub-second timescale.
The latency of thrombin-evoked Ca^{2+} release was determined using stopped-flow fluorimetry with fura-2-loaded platelets (Brownlow & Sage, 2003). For determination of platelet F-actin content, cells were mixed with thrombin (or buffer as control) for various time intervals before fixing with 3% (w/v) paraformaldehyde in phosphate buffered saline using a rapid quench flow system. Platelet F-actin content was then determined as previously described (Rosado *et al.*, 2000). All experiments were conducted at 37 °C.
Thrombin (0.1 unit ml^{-1}) evoked Ca^{2+} release from intracellular stores (in the presence of 1 mM EGTA) with a delay of 1.42 ± 0.15 s (mean ± S.E.M., n = 15). Thrombin (0.1 unit ml^{-1}) evoked an initial decrease in platelet F-actin that commenced within 0.1 s and reached a minimum 1 s after stimulation. The F-actin content then increased, exceeding basal levels again approximately 3 s after stimulation. Mixing cells with agonist free buffer did not evoke significant changes in F-actin content. Our results indicate that thrombin stimulates rapid actin depolymerisation in platelets before F-actin increases above resting levels. The actin depolymersation precedes release of Ca^{2+} from intracellular stores, indicating that it occurs via a Ca^{2+}-independent pathway. Rapid actin depolymerisation is compatible with membrane trafficking underlying the *de novo* coupling of IP_3RII to hTRPC1, which occurs with minimal temporal separation from thrombin-evoked Ca^{2+} release and Ca^{2+} entry (Brownlow & Sage, 2003).

Brownlow SL & Sage SO (2003). *Biochem J* **375**, 697-704.

Rosado JA *et al.* (2000). *J Biol Chem* **275**, 7527-7533.

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

C48

A ROLE FOR CALPAIN IN STORE-OPERATED CALCIUM ENTRY IN HUMAN PLATELETS.

A.G. Harper and S.O. Sage

Physiology, University of Cambridge, Cambridge, UK

Store-operated calcium entry (SOCE) is an important pathway for calcium influx in human platelets. Previous studies have demonstrated a role for extracellular signal-regulated kinase (ERK) in the activation of SOCE in these cells (Rosado & Sage, 2001). Components of the ERK cascade involved in SOCE are uncharacterised. One candidate is m-calpain which has been shown in other cell types to be activated, at least in part, by ERK phosphorylation (Glading *et al.*, 2004). Calpain has a role in many aspects of platelet activation including secretion, spreading and aggregation (Croce *et al.*, 1999). Here we have studied the effects of calpain inhibition on SOCE in human platelets.
Fura-2-loaded platelets were preincubated at 37°C for 10 minutes with various concentrations of Calpain Inhibitor III (C3) and then stimulated using 200nM thapsigargin (TG) in the presence of 100μM EGTA for 3 minutes before addition of 1.6mM $SrCl_2$. Sr^{2+} is a poor substrate for the plasma membrane Ca^{2+}-ATPase (PMCA) and so was used to estimate unidirectional divalent cation influx. C3 caused a dose-dependent inhibition of Sr^{2+} entry with 300μM C3 reducing entry to 16 ± 2 % of control (mean ± S.E.M., n=7; $p<0.005$). There was also an apparent potentiation of TG-evoked Ca^{2+} release, probably attributable to an inhibition of the PMCA. Similar results were obtained with the mechanistically different calpain inhibitor, PD150606. Neither inhibitor acted as a store-operated channel blocker. Thrombin-evoked Ca^{2+} signalling was also significantly inhibited by C3 preincubation.
Platelet calpain activity was monitored by loading washed cells with the cell permeable, fluorogenic calpain substrate *t*-BOC-Leu-Met CMAC (100μM for 40 minutes at 37°C). Cells were then preincubated with 300μM C3 or the vehicle and the rate of calpain activity was measured for 20 seconds after stimulation

with 200nM TG or vehicle in the presence of 200μM EGTA in a stopped-flow fluorimeter. TG increased calpain activity to 143 ± 13 % of basal (mean ± S.E.M., n=7; p<0.01). The TG-evoked increase was reduced to 124 ± 10 % of basal after preincubation with C3 and was closely temporally matched to the onset of TG-evoked Ca^{2+} release.

We have demonstrated that TG-evoked Ca^{2+} store depletion rapidly induces an increase in calpain activity that plays a role in the activation of SOCE in human platelets. This is the first report of the intracellular measurement of calpain activity in human platelets and is also the first demonstration of a role of a protease in SOCE.

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Glading A *et al.* (2004). *Mol Cell Biol* **24**, 2499-2512.

Rosado JA & Sage SO (2001). *J Biol Chem* **276**, 15659-15665.

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

C49

TETRACINE INHIBITS BOTH THE INITIATION AND PROPAGATION OF SPONTANEOUS CA2+-TRANSIENTS IN THE MICROVASCULAR SMOOTH MUSCLE OF INTACT RAT RETINAL ARTERIOLES

J. McGeown[1], J. Tumelty[1], C. Scholfield[1] and T. Curtis[2]

[1]Smooth Muscle Group, Queen's University of Belfast, Belfast, UK and [2]Dept of Ophthalmology, Queen's University of Belfast, Belfast, UK

Intracellular [Ca2+] within the smooth muscle of blood vessels is an important determinant of cell contraction and, therefore, vascular resistance. Ca2+-imaging of smooth muscle cells in the walls of intact retinal arterioles reveals both brief, spatially localised events resembling Ca2+-sparks, and prolonged Ca2+-oscillations (McGeown et al, 2004). Here we report results from experiments testing the role of ryanodine-receptor (RyR) linked Ca2+-release in these events.

Sprague Dawley rats (200-300g) were anaesthetised and killed by cervical dislocation. Arterioles were dispersed from retinae by trituration, and incubated with 10μM Fluo-4AM. Changes in [Ca2+]i were confocally imaged in smooth muscle cell arrays (9-17 cells). Arterioles were superfused with saline at 37oC and line-scanned at 500 scans s-1. Fluorescence data (F) were extracted, background-corrected and normalized to resting fluorescence (F0). Changes in [Ca2+]i were analysed in terms of their amplitude (ΔF/F0), full duration at half maximal fluorescence rise (FDHM), rise time to peak fluorescence, and full width at half maximal fluorescence (FWHM). The average speed of propagation was calculated using the average slope of the half-maximal fluorescence contour. Differences in means were assessed statistically using paired or unpaired Student's t-tests.

In 26 cells from 6 arterioles, the frequency of brief, spark-like events was decreased from 0.54±0.07 s-1 (mean±SEM) under control conditions to 0.07±0.04 s-1 during 30s superfusion with tetracaine (100μM; P<0.0001). Although tetracaine abolished spark activity in over 70% of cells, it persisted in 7 cells. Mean spark amplitude was not altered in these cases but their duration (FDHM) was increased from 41.0±5.3 ms to 175.4±21.6 ms (P<0.00001). This prolongation largely reflected slowing of the rising phase, total rise time being increased from a control value of 29.9±2.8 ms (n=44 events) to 117.6±17.6 ms (n=36 events) during tetracaine superfusion (P<0.0001). The speed of spread of sparks also fell from a control value of 71.2±11.6 μms-1, to 15.8±2.0 μms-1 for events initiated at the same sites in the presence of tetracaine (P<0.0001), while FWHM fell from 1.59±0.20 μm to 0.99±0.11 μm (P<0.02). These results suggest that RyR-gated Ca2+-release channels are important both for the initiation and propagation of spontaneous transients in retinal arteriolar vascular smooth muscle.

McGeown, JG et al. (2004). J Physiol (in press)

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

C50

CALCIUM SIGNALLING, CAVEOLAE AND HUMAN MYOMETRIAL CONTRACTILITY

A.J. Kendrick[1], J. Zhang[1], M. Tattersall[1], L. Bricker[2], S. Quenby[2] and S. Wray[1]

[1]Department of Physiology, The University of Liverpool, Liverpool, UK and [2]Department of Obstetrics and Gynaecology, University of Liverpool, Liverpool, UK

Several signalling components, including Ca channels are enriched in plasma membrane domains high in cholesterol; lipid rafts and caveolae. Signalling cascades maintain uterine quiescence during pregnancy until the onset of labour. Myometrial cholesterol is higher in pregnant that non-pregnant women and may be further elevated in obese mothers. Moreover, cholesterol manipulation in rat uterus destabilises lipid rafts and disrupts caveolae. We hypothesised that disruption of lipid domains following cholesterol manipulation may be detrimental to Ca signalling and reduce myometrial contractility, particularly in the obese mothers. Following ethical approval, uterine biopsies were obtained with consent from pregnant women undergoing Caesarean sections and non-pregnant women having hysterectomies. Cholesterol was loaded and extracted with water-soluble cholesterol (5mg/ml) and 2% methyl-B-cyclodextrin, respectively. The impact on force and intracellular [Ca] (Indo-1) were examined. Statistical significance (P<0.5) was determined using ANOVA and Tukeys test. The contractility of 9 non-pregnant samples significantly declined or was abolished by cholesterol. Significant decline in total force fell to a mean value of 29.0 (± 12.7, P<0.0011, Tukeys, n=4) and amplitude to a mean value of 43.9 (± 11.3, P<0.0016, Tukeys, n=4). Ca changes paralleled these changes. Similarly, cholesterol was associated with a significant decrease in agonist-stimulated contractions. Significant decline in total force fell to a mean value of 28.7 (± 13.9, P<0.0036, Tukeys, n=3) and duration to a mean value of 40.1 (± 10.1, P<0.03, Tukeys, n=3). Cholesterol-induced changes were not reversed by cholesterol extraction. Moreover, in 7 pregnant samples, even in the presence of oxytocin, cholesterol reduced con-

tractility and Ca transients. In vivo evidence for the deleterious effect of cholesterol on uterine contractility is provided by a retrospective study of 3913 births at the Liverpool Women's Hospital. We found a higher rate of emergency caesarean section among obese (BMI >30) (19%, odds ratio 1.5, 1.22-1.85 95% CI), frequently due to inadequate uterine contractions (5.7%, odds ratio 3.54, 2.17-5.78 95% confidence interval), compared with normal women. Thus Ca signalling, which is central to myometrial contractions is significantly influenced by cholesterol. The deleteriously effect of cholesterol loading on membrane microdomains may contribute to uterine quiescence and labour complications in obese women.

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

C51

TUMOUR NECROSIS FACTOR-α DOES NOT AFFECT EVOKED DOPAMINE RELEASE AND REUPTAKE IN THE RAT STRIATUM IN VITRO

C. O'Neill and J.J. O'Connor

Human Anatomy & Physiology, Conway Institute, UCD, Belfield, Dublin 4, Ireland

Neurodegenerative processes are likely to be due at least in part by the release of pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumour necrosis factor (TNF)α. Evidence is now emerging that these cytokines may act as immuno-transmitters affecting dopamine and serotonin transmission (Allan & Rothwell, 2001). Application of IL-1 can modulate K -and NMDA-evoked dopamine release in the nucleus accumbens (Song et al. 1999). Also TNFα has been shown to potentiate striatal dopamine uptake into synaptosomes (Cho et al. 1999). However there are conflicting reports for the effects of TNFα on regional brain neurotransmitters.

Using the technique of fast cyclic voltammetry (FCV; Kruk & O'Connor, 1995), we have investigated in real time the release and reuptake kinetics of dopamine in the rat striatum in the presence and absence of TNFα. Rats were humanely killed and changes in the extracellular concentration of dopamine evoked by electrical stimulation of rat brain slices containing the caudate putamen were monitored using FCV. In FCV, a triphasic voltage ramp is applied to the slice, and the resultant current is measured using a carbon fibre electrode. Dopamine is known to oxidise at 610 mV and the current measured is proportional to the concentration of dopamine present in the extracellular fluid. Single pulse stimulation (0.1 ms; 10 V) and 4 pulses at 0.5 Hz were applied once every 5 min over a 3 hr period. Data are expressed as mean±sem and analysed using Students unpaired t-test.

Perfusion of TNFα (5.0 ng/ml; 380 pM) for 2 hr had no significant effect on single pulse release or reuptake kinetics (control single pulse peak release was 0.30±0.03 μM versus 0.37±0.04 μM 2 hr post TNFα; P>0.05, n=4). During the 0.5 Hz stimulation protocol, no effect on the ratio of the 3rd stimulation to the 1st stimulation was observed (0.53±0.08 control, versus 0.49±0.03 in TNFα treated slices; P>0.05; n=4). This indicates that D_2 autoreceptors were not affected by TNFα. These results show that acute exogenous application of pathophysiological concentrations of TNFα does not affect dopamine release, reuptake, or D_2 autoreceptors kinetics. These studies will provide valuable information towards our understanding of the mechanisms of action of TNFα on dopamine release.

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

C52

ROLE OF THE ACTIN CYTOSKELETON IN THROMBIN-EVOKED CALCIUM SIGNALLING IN HUMAN PLATELETS.

M.T. Harper and S.O. Sage

Physiology, University of Cambridge, Cambridge, UK

Store-operated calcium entry (SOCE) is believed to contribute a major component of the thrombin-evoked increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]i$) in human platelets. We have previously shown that SOCE, artificially induced by the sarco/endoplasmic reticulum Ca^{2+}-ATPase inhibitor thapsigargin, is dependent on the remodelling of the actin cytoskeleton (Rosado *et al.* 2000). Here we have investigated the role of remodelling of the actin cytoskeleton in Ca^{2+} signalling evoked by thrombin, a physiological agonist that activates protease-activated receptors (PAR) 1 and 4 in human platelets.

Fura-2-loaded human platelets were incubated at 37 °C with cytochalasin D (CytD; 10 μM) or the vehicle (DMSO) for 40 min, then stimulated by thrombin (1 U/ml), the PAR-1 agonist SFLLRN (3 μM), or the PAR-4 agonist AYPGKF (300 μM) in the presence or absence of extracellular Ca^{2+}. Ca^{2+} release was estimated as the integral of the $[Ca^{2+}]i$ increase recorded for 3 min after the addition of the thrombin or AYPGKF, or 90 seconds after addition of SFLLRN, in the presence of 1 mM EGTA. Ca^{2+} entry was estimated as the integral of the Ca^{2+} response in the presence of 1 mM $CaCl_2$, corrected for the Ca^{2+} release over the same period. Sr^{2+} entry was used as a measure of unidirectional divalent cation flux as Sr^{2+} is reported to be a poor substrate for the PMCA, and was estimated as the integral of the 340nm/380nm fluorescence ratio over 3 min after thrombin addition in the presence of 1mM $SrCl_2$, corrected by subtraction of the signal due to Ca^{2+} release over the same time period. Statistical significance ($p<0.5$) was determined using Student's paired *t* tests.

Inhibition of actin polymerisation with CytD increased thrombin-evoked Ca2+ entry to 148±9.2% of control ($n = 12$; $p < 0.01$). Thrombin-evoked Sr^{2+} entry was increased to 134±6.4% ($n = 8$; $p < 0.005$). Thrombin-evoked Ca^{2+} release was not significantly affected.

In addition to SOCE, thrombin is reported to activate a non-capacitative pathway that is dependent on protein kinase C (PKC)

(Rosado & Sage, 2000). However, inhibition of PKC with Ro-31-8220 (5 μM) did not prevent the potentiation of thrombin-evoked Ca^{2+} entry by CytD (n = 6).
Neither SFLLRN-evoked Ca^{2+} release nor entry was significantly affected by CytD treatment (n = 7). AYPGKF-evoked entry was increased by 134±12.8% (n = 4; $p < 0.05$) by CytD, whereas AYPGKF-evoked release was not significantly affected.
These data indicate that, in contrast to thapsigargin-evoked SOCE, thrombin-evoked Ca^{2+} entry is potentiated when actin polymerisation is inhibited with CytD. This effect is restricted to the PAR-4 component of thrombin signalling. CytD may prevent the internalisation of PAR-4, which is important in PAR-4 desensitisation.

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

C53

17β-OESTRADIOL ACTIVATES PROTEIN KINASE COMPLEXES IN A GENDER-SPECIFIC MANNER IN RAT DISTAL COLONIC CRYPTS.

F. O'Mahony, V. Betts, R. Alzamora, W. Thomas and B.J. Harvey

Charitable Infirmary Trust Molecular Medicine Laboratories, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin 9, Dublin, Ireland

The steroid hormone, 17β-Oestradiol (E2), has been shown to modulate electrolyte balance in the distal colon via a non-genomic mechanism (McNamara et al, 1995). We have previously reported the rapid E2 regulation of Na^+/H^+ exchange and K^+ channels, which requires the upstream activation of complex kinase pathways including Protein Kinase C (PKC) (Clondiffe et al, 2001) and Mitogen Activated Protein Kinases (MAPK). Recently we reported the activation of Protein Kinase D (PKD) a known downstream target of PKC (O'Mahony et al, 2003). This study sought to identify the signalling molecules stimulated rapidly in response to E2 (10nM) and resulting protein-protein kinase interactions. Sprague-Dawley rats (3 months old) were sacrificed by cervical dislocation and distal colonic crypts were isolated. The oestrous cycle stage was determined by cervical smear. PKC phosphorylation was determined by western blotting using specific phospho-antibodies. Protein complexes were detected by co-immunoprecipitation. PKD autophosphorylation was detected by an in vitro kinase assay (IVKA). Protein Kinase A (PKA) activation was investigated using a non-radioactive PepTag Assay (Promega). We report for the first time a gender specific activation of PKCα (2 fold, 5min) and PKCε (2 fold, 3min) in female distal colonic crypts with no activation noted in male rat colonic crypts. We demonstrated PKA activation (4 fold, 5min) in the female with no activation noted in male tissue. IVKA analysis of E2-induced autophosphorylation of PKD showed an increase in activity at the oestrous stage (1 fold, 15min) with a decrease at the dioestrous stage (2 fold, 5min). This differential PKD regulation may be due to the oestrogen background of the female rat. Co-immunoprecipitation of PKD complexes showed an association with PKCδ (5 min) and P38 MAPK (15 min) with no association noted in the male tissue. PKCδ also associated with P38 MAPK (2 fold, 15min) specific to female tissue indicating a complex between PKCδ/PKD/P38 MAPK. PKD did not associate with PKCε or PKA in the female or male distal colonic crypt. In conclusion, we provide the first evidence for the differential activation of PKC and the oestrous cycle dependent activation of PKD in the female distal colonic crypt. This study demonstrated the formation of multi-protein complexes in response to E2 specific to the female rat colon. The downstream ion channels targets of these complexes remains to be elucidated.

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

PC33

RAPID NON-GENOMIC INCREASE IN INTRACELLULAR CALCIUM ION CONCENTRATION INDUCED BY ENVIRONMENTAL ESTROGENS IN HUMAN BREAST CANCER CELLS.

D. Walsh[1], P. Dockery[2] and C. Doolan[1]

[1]Physiology, Biosciences Institute, University College Cork, Cork, Ireland and [2]Anatomy, Biosciences Institute, University College Cork, Cork, Ireland

In this study, we wish to identify and characterise the alternative pathway through which environmental estrogens may mediate their intracellular effects. Three human breast cancer cells were employed including MCF-7 cells, which express both ERα and ERβ; MDA-MB-231 cells, which express ERβ but not ERα; and SKBR-3 cells, which express neither ERα nor ERβ. The effect of environmental estrogenic compounds on intracellular calcium ion concentration ($[Ca^{2+}]_i$, was measured by fura-2 fluorescence and compared to that of 17β-estradiol (E2). A rapid and maintained increase in $[Ca^{2+}]_i$ was observed following the application of nanomolar concentrations (0.1-100 nM) of E2 and the environmental estrogens bisphenol-A, o.p'-DDT, diethylstilbestrol and 4-*tert*-octylphenol regardless of ERα and ERβ expression. The steroid-induced $[Ca^{2+}]_i$ response was completely abolished by incubating the cells in Ca^{2+}-free medium, suggesting that the source of Ca^{2+} increase is extracellular. Pre-treating the cells with the ER antagonist ICI 182,780 had no effect on either basal nor the steroid-triggered $[Ca^{2+}]_i$ response.

In summary, we have demonstrated ER independent acute non-genomic effects of environmental estrogenic compounds at nanomolar concentrations on $[Ca^{2+}]_i$. The results of this study demonstrate an alternative to the classical genomic pathway to explain the potent effects of these endocrine disruptors.

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

PC34

ROLE OF NA^+-CA^{2+} EXCHANGE IN HYPOXIC PULMONARY VASOCONSTRICTION

S. Becker, L. Moir and P.I. Aaronson

King's College, London, UK

Hypoxic pulmonary vasoconstriction (HPV) is a lung-specific mechanism which serves to match pulmonary ventilation and perfusion under physiological conditions, thus optimising gas exchange. However, with global pulmonary hypoxia, *e.g.,* during chronic obstructive pulmonary disease, HPV causes pulmonary hypertension, vascular remodelling and heart failure. The cause of HPV is most likely multifactorial and Ca^{2+} influx, Ca^{2+} release and Ca^{2+} sensitisation have been implicated with its development. Recently, Ca^{2+} extrusion *via* Na^+-Ca^{2+} exchange (NCX) was reported to be abolished in single cells under hypoxic conditions, leading Wang et al. (2000) to propose that HPV might be due to an inhibition of NCX by hypoxia. We have therefore investigated whether NXC plays a role in HPV in intact rat small pulmonary arteries (PA).

Isolated PA (200-600 μm i.d.) from humanely killed rats were mounted on an isometric myograph, preconstricted with $PGF_{2\alpha}$ and exposed to hypoxia (0% oxygen) for 40 min. This evoked a biphasic contractile response, which consisted of a transient contraction (phase 1) followed by a progressively increasing sustained contraction (phase 2). Application of inhibitors of the NCX and Na^+ substitutes were used to assess the role of NCX activity during HPV. PA were pretreated with the Ca^{2+} antagonist diltiazem ($10 * 10^{-6}$M) in order to prevent effects on contraction which might be caused by membrane depolarisation. All values represent mean contraction relative to high K^+ contraction ± SEM in control and in the presence of drugs, respectively. Student's T-Test and the Mann-Whitney Rank Sum Test were used to determine statistical significance.

The NCX inhibitor KB-R7943 ($30 *10^{-6}$ M) almost abolished phase 1 (67 ± 5% vs. 17 ± 5%) and significantly reduced phase 2 (33 ± 5% vs. 20 ± 6%), whereas amiodarone ($50 *10^{-6}$ M), which also inhibits NCX, significantly blocked phase 1 (38 ± 2% vs. 25 ± 3%), but not phase 2 (15 ± 2% vs. 12 ± 1%). Substitution of Na^+ with Li^+ resulted in an inhibition of phase 1 (48 ± 4% vs. 32 ± 4%); phase 2, however, was strongly increased (10 ± 2% vs. 18 ± 4%). A similar result was observed when Na^+ was substituted with NMDG. The ability of PA to relax following re-oxygenation was variably inhibited by Na^+ substitution, but was not affected by the NCX inhibitors.

These results suggest that the phase 1 contraction of HPV involves Ca^{2+} influx *via* reverse-mode NCX. The involvement of NCX during phase 2 appears to be more complex, but the results are consistent with the possibility that NCX is primarily involved in Ca^{2+} extrusion during phase 2.

Wang, Y. X., Dhulipala, P. K., & Kotlikoff, M. I. (2000). Hypoxia inhibits the Na(+)/Ca(2+) exchanger in pulmonary artery smooth muscle cells. FASEB J. 14, 1731-1740

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