Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC117

Epac activation, altered calcium homeostasis and ventricular arrhythmogenesis in Langendorff-perfused mouse hearts

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The recently described cAMP sensor, Exchange protein directly activated by cAMP (Epac), has been implicated in distinct cAMP-dependent, protein kinase A-independent cellular signalling pathways (Bos JL, 2006). We investigated effects of Epac activation in catecholamine-induced ventricular arrhythmogenesis. In contrast to control findings (n = 20), monophasic action potentials showed spontaneous triggered activity in 2 out of 10 intrinsically beating and 5 out of 20 extrinsically-paced Langendorff-perfused murine hearts perfused with the specific Epac activator 8-pCPT-2'-O-Me-cAMP (8-CPT, 1 μM) (Christensen AE *et al.* 2003). During steady extrinsic pacing at 8 Hz, 3 out of 20 such hearts showed spontaneous ventricular tachycardia (VT). Programmed electrical stimulation provoked VT in 10 of 20 similarly treated hearts ($P < 0.001$; $n = 20$, Fisher's Exact Test). However, no statistically significant changes ($P > 0.05$, ANOVA) in left ventricular epicardial (40.7 ± 1.2 versus 44.0 ± 1.7 ms; n = 10), or endocardial action potential durations (APD_{90}) (51.8 ± 2.3 versus 51.9 ± 2.2 ms; n = 10), transmural (ΔAPD_{90}) (11.1 ± 2.6 versus 7.9 ± 2.8 ms; n = 10) or apico-basal gradients of repolarization, ventricular effective refractory periods (29.1 ± 1.7 versus 31.2 ± 2.4 ms in control and 8-CPT-treated hearts, respectively; n = 10) and APD_{90} restitution characteristics accompanied these arrhythmogenic effects. However, fluo-3 fluorescence imaging of cytosolic Ca^{2+} demonstrated alterations in Ca^{2+} homeostasis in the form of increased Ca^{2+} wave generation in both paced and resting isolated 8-CPT-treated ventricular myocytes. An independent method of Epac activation that applied 100 nM isoproterenol to stimulate beta-adrenoreceptors in parallel with protein kinase A inhibition by 2 μM H-89, was also arrhythmogenic in the whole heart and similarly altered cytosolic Ca^{2+} homeostasis. The Epac-dependent effects at both the whole heart and cellular levels were reduced by inhibition of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) with 1 μM KN-93. These findings associate VT in an intact cardiac preparation with altered cellular Ca^{2+} homeostasis and Epac activation through a CaMKII-dependent mechanism for the first time, in the absence of the altered repolarization gradients previously implicated in re-entrant arrhythmogenesis (Killeen MJ *et al.* 2007; Thomas G *et al.* 2007).

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PC118

Is nitric oxide (NO) important in the adenosine A_{2A}-receptor-mediated vasodilatation of skeletal muscle contraction?

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During systemic hypoxia the contribution of adenosine to skeletal muscle vasodilatation is dependent on the presence of NO; NO is required for the release of adenosine from the endothelium (Edmunds *et al*. 2003) and mediates dilatation via endothelial A_1-receptors (Ray & Marshall, 2005). By contrast, skeletal muscle vasodilatation accompanying muscle contraction (exercise hyperaemia) is mediated by adenosine acting at A_{2A}-, but not A_1-receptors (Ray & Marshall, 2008). Adenosine can release NO from endothelium by acting at A_{2A}-receptors (Ray *et al*. 2002). Thus, we investigated the role of NO in exercise hyperaemia.

In three groups of rats, anaesthetized with Saffan (7-12 $mgkg^{-1}hr^{-1}$ I.V.), we recorded arterial blood pressure (ABP), femoral blood flow (FBF) and tension in the extensor digitorum longus. Isometric twitch contractions were evoked by stimulation of the sciatic nerve at 4Hz. Integral femoral vascular conductance (IntFVC) was calculated off-line. Group 1 (n=7) was the time control for, Group 2 (n=10), which received NOS inhibitor L-NAME before the third, and A_{2A}-receptor antagonist ZM241385, before the fourth contraction. Group 3 (n=12) received L-NAME before the third, the NO-donor SNAP to restore baseline FVC during the fourth and fifth contraction and ZM241385 before the fifth.

Time controls showed consistent tension and hyperaemic responses. In Group 2, baseline IntFVC was reduced by L-NAME (0.555±0.04 (mean±SEM) to 0.297±0.02CU*, ANOVA for repeated measures, p<0.001) but not by ZM241385. L-NAME reduced exercise hyperaemia (13.91±1.31 to 9.52±1.09CU*), and it was further attenuated by ZM241385 (to 5.46±1.12CU*). In Group 3, SNAP after L-NAME restored baseline IntFVC to control levels (Control: 0.702±0.09, L-NAME: 0.377±0.05*, L-NAME + SNAP: 0.616±0.09CU); ZM241385 had no further effect. Exercise hyperaemia was also restored to control levels after L-NAME by SNAP (Control: 17.10±1.18, L-NAME: 10.87±1.09*, L-NAME + SNAP: 16.99±1.38CU), and this response was further attenuated by ZM241385 (12.75±0.98CU*).

These results confirm that adenosine acting via A_{2A}-receptors contributes to exercise hyperaemia. However, they indicate

level. Overall our data suggest that either the sparse labeling of PSD does not reflect the presence of functional receptors, or the level of synaptic NMDAR expression is below that which can be resolved during our recordings. Alternatively, NMDARs are present but not activated during the brief glutamate transient arising from the release of single quanta. Future modeling studies will address this question.

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC81

Sheep RyR2 channel activity is regulated by an endogenous kinase other than PKA or CaMKII; an effect not mediated by S2809

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Previously we have demonstrated that PKA-dependent phosphorylation at S2809 is associated with a significant increase in RyR2 channel open probability (Po), and characteristic changes in gating[1]. To further investigate RyR2 phosphorylation, sarcoplasmic reticulum vesicles were isolated[2] from sheep hearts obtained from an abattoir and were either incorporated into artificial membranes for single-channel studies or used for Western blot[1]. In the presence of 50μM cytosolic free Ca^{2+}, 5 min incubation with 5mM Mg^{2+} and 1mM ATP, followed by wash out to control conditions, significantly increased channel Po (from 0.071±0.023 to 0.334±0.076 (SEM; *n*=17, p<0.01, Student's t-test)). The increase in Po however, was smaller than that observed when exogenous PKA was included in the incubation medium (from 0.126±0.035 to 0.574±0.106 (SEM; *n*=10). Closer examination of the individual Mg^{2+}ATP treated channels suggested the presence of two different sub-populations of RyR2. In 10 of 17 channels, Mg^{2+}ATP treatment resulted in an increase in channel Po and a change in channel gating similar to that observed after PKA-dependent phosphorylation (Po rose from 0.033±0.011 to 0.534±0.079 (SEM; *n*=10). In the remaining channels, Mg^{2+}ATP had no sustained effect (*n*=7). ATP alone, in the absence of Mg^{2+}, produced a fully reversible increase in Po in all channels treated (*n*=12). It is possible that the heterogeneous response of the channels to Mg^{2+}ATP is due to the close association of an endogenous kinase with some of the RyR2 channels reconstituted into bilayers. Use of the PKA inhibitor, PKI (10 μM), did not prevent the Mg^{2+}ATP dependent increase in channel Po although it does prevent the effects of exogenously added PKA. Similarly the Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) inhibitor, autocamtide-2 related inhibitory peptide II (AIPII) (50 nM), did not prevent the increase in Po at concentrations expected to inhibit CaMKII. Increasing the concentration of AIPII to a level reported to inhibit the action of PKC (1 μM) however, prevented the irreversible Mg^{2+}ATP-induced changes. Further, use of the PKC specific inhibitor, chelerythrine chloride, also prevented the Mg^{2+}ATP related change in channel activity. In the presence of 1μM chelerythrine chloride, Po was 0.120±0.039 before and 0.044±0.021 after treatment with Mg^{2+}ATP (SEM; *n*=7). Chelerythrine chloride alone had no effect on channel Po. Although chelerythrine chloride inhibited the Mg^{2+}ATP-dependent increase in channel Po, it did not prevent phosphorylation of RyR2 at S2809. Western blot analysis demonstrated that under lipid bilayer comparable conditions, only PKI prevented Mg^{2+}ATP phosphorylation of S2809. We conclude that RyR2 channels may be associated with and phosphorylated by an endogenous kinase, possibly PKC, which does not appear to phosphorylate S2809.

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PC82

Post-natal developmental changes in ion channel and Ca^{2+} handling protein expression in the ventricle

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Transmural gradients in action potential duration (APD) and Ca^{2+} handling proteins are important for both the normal functioning of the ventricle and arrhythmogenesis. In the rabbit, the transmural gradient in APD is minimal in the neonate. During post-natal development, APD increases both in the epicardium and the endocardium, but the prolongation is more substantial in the endocardium leading to a significant transmural gradient. We have investigated changes in ion channel expression in the subepicardial and subendocardial layers of the left ventricular free wall in neonatal (2-7 days of age; n=11) and adult male (~6 months of age; n=11) New Zealand White rabbits using quantitative PCR (qPCR), *in situ* hybridisation (ISH) and immunohistochemistry. The rabbits were killed humanely in accordance with the regulations of the United Kingdom Animals (Scientific Procedures) Act 1986. qPCR revealed that in the neonate, Nav1.5 (responsible for I_{Na}), ERG (responsible for $I_{K,r}$) and minK (responsible for $I_{K,s}$) mRNAs were more abundant in the endocardium than the epicardium, whereas the reverse was true for KChIP2 (in part responsible for I_{to}) mRNA. Moreover, in the adult, Cav1.2 (responsible for $I_{Ca,L}$), SERCA2a, RyR2, ERG, KvLQT1 (responsible for $I_{K,s}$) and KChIP2 mRNAs were more abundant in the epicardium than the endocardium. Consistent with this, ISH

showed that KChIP2 mRNA was more abundant in the epicardium than the endocardium both in the neonate and adult. However, in the neonate, whereas Nav1.5 mRNA was more abundant in the endocardium than the epicardium, it was uniformly distributed across the ventricle in adult. Cav1.2 mRNA was uniformly distributed across the neonatal ventricle, while in the adult ventricle, Cav1.2 mRNA was significantly more abundant in the epicardium than the endocardium. Immunohistochemistry confirmed that NCX1, SERCA2a and RyR2 proteins were more abundant in the epicardium than the endocardium in the adult, but not in the neonate. To conclude, there are complex developmental changes in ion channel and Ca^{2+} handling protein expression across the ventricle that may have implications for the treatment of arrhythmias.

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PC83

Regulation of plasma membrane expression of P2X4 receptors in immune cells

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P2X4 receptors are one of the predominant subtypes of purinergic receptors expressed in macrophages and microglia and their up-regulation has been shown to contribute to neuropathic pain. P2X4 receptors are prominently localized to lysosomes and resist degradation by virtue of N-linked glycans decorating the intra-luminal loop of the receptor. In order to understand how the expression of these receptors at the plasma membrane is regulated, we compared the proportion of receptors expressed at the cell surface in cultured microglia and macrophages following exposure to modulators of microglial/macrophage activation. Surface expression was analysed by biotinylation of exposed proteins and by cross-linking proteins with membrane impermeant cross-linkers, followed by SDS-PAGE and western blotting. The modulators included lipopolysaccharide (LPS), ATP and phorbol esters. A 24h incubation with LPS (500ng/ml) resulted in an up-regulation of P2X4 surface expression in cultured microglia without an evident increase in total expression of the receptor, indicating redistribution from intracellular compartments to the plasma membrane. In contrast, similar exposure of cultured astrocytes to LPS had no effect on the surface expression of P2X4 receptors. Exposure of microglia to LPS was sufficient to inhibit proliferation and we have compared the involvement of the P2X7 receptor in both the anti-proliferative effects of LPS and the up-regulation of P2X4 receptors. Brief incubations with phorbol esters produced a similar up-regulation of surface P2X4 receptors in bone marrow derived macrophages (BMDMs) and we are examining the underlying mechanisms involved.

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PC84

Channel blocking properties of a partial agonist at the human muscle acetylcholine receptor

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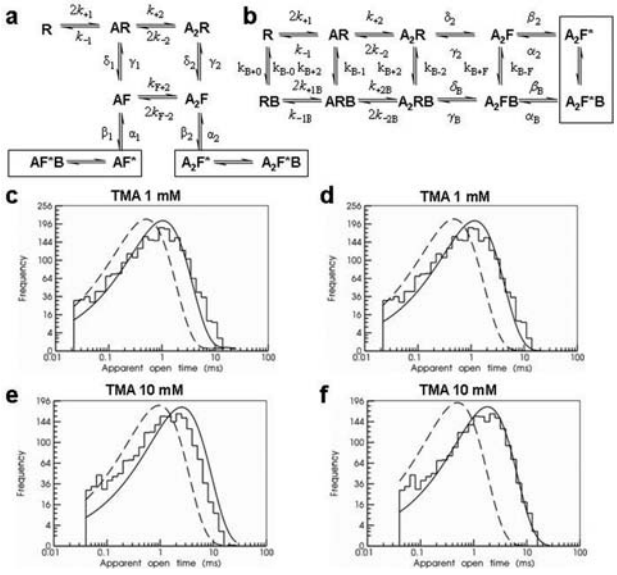
Agonists at the muscle acetylcholine receptor (AChR) can all block the channel as well as activate it. For many partial agonists e.g. choline or tetramethylammonium (TMA), concentrations for activation and block are similar.

We recorded cell-attached TMA-activated single-channel currents from HEK293 cells transfected with human nicotinic AChRs ($\alpha\beta\delta\varepsilon$, transfection ratio 2:1:1:1). In single-channel records at -80 mV, the amplitude of the openings appears to decrease progressively with agonist concentration because of fast channel block.

Several records obtained at different TMA concentrations were fitted simultaneously with HJCFIT[1]. For TMA the equilibrium constant, K_B, for open channel block was 8.9 ± 0.6 mM, as estimated from the reduction of apparent single-channel amplitude, cf EC_{50} of 2.2 ± 0.5 mM.

If essentially no blockages are detected, the open state and the open-blocked state can be treated as a single compound open state for the purpose of analysing kinetics. Such compound states are indicated by the boxes in Fig 1a and 1b. During fitting, the exit from the compound open state in Fig 1a is given by a transition rate that is not α_2 but rather $\alpha_2/(1 + c_B)$, where $c_B = [B]/K_B$ and [B] is the blocker (agonist) concentration. This reflects the fact that the compound state spends only a fraction of time $1/(1 + c_B)$ in the state from which exit can occur. Similarly, for the mechanism in Fig 1b, the transition rate for leaving the compound open state via the blocked state (A2F*B) is taken not as α_B but rather $\alpha_B c_B/(1 + c_B)$, i.e. α_B is multiplied by the fraction of time which the compound open state spends in the blocked state. The left column shows a fit with a mechanism that allows block of channels only when they are open. The predicted distribution of apparent open times at the lower concentration (1 mM, Fig 1c) of TMA superimposes on the observations quite well, but at the higher concentration (10 mM, Fig 1e) the prediction is poor. The predominant mean apparent open time is about 1.5 times smaller than is predicted.

The right column in Fig 1 shows fit of a mechanism (Fig 1b) in which the block is not selective for the open state, but can occur from any state. In this case the distribution of apparent open times is predicted accurately at both low and high concentrations of TMA. The mean values were $\alpha_2 = 2370\ s^{-1} \pm 12\%$ (CVM, $n = 4$ fits) and $\alpha_B = 1550\ s^{-1} \pm 19\%$, so the channel shuts almost as fast when it is blocked as when it is not. The mean opening transition rate for the unblocked channel was $\beta_2 = 71300\ s^{-1} \pm 9\%$, similar to that for acetylcholine[2], and for the blocked channel, the mean opening rate was almost as fast, $\beta_B = 49000\ s^{-1} \pm 9\%$. Thus TMA seems not act as a pure open channel blocker, but AChRs blocked by TMA can close and return to their resting state without re-opening.



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PC85

The Na^+/H^+ exchanger isoform 3 is the key proton recycling mechanism to drive PepT1-mediated dipeptide transport in native murine intestine

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Backround: Inhibition of the apical Na^+/H^+ exchanger isoform 3 (NHE3) activity interferes with dipeptide uptake in intestinal cell lines. Aim: This study investigates how dipeptide transport affects murine jejunal fluid absorption and enterocyte pHi in vivo, whether NHE3 function is mandatory for PepT1 function in the native intestine, and whether the reason for its importance lies in its ability to regulate enterocyte pHi during H+/dipeptide uptake. Methods and Results: The luminal application of Gly-Sar resulted in a strong, PepT1- as well as NHE3-dependent increase in intestinal fluid secretion, measured by single-pass perfusion, and a decrease in villous enterocyte pHi, measured by two-photon microscopy in the anesthetized mouse (administration of 10 μL/g intraperitoneal (IP) haloperidol/midazolam/fentanyl cocktail (haloperidol 12.5 mg/Kg, fentanyl 0.325 mg/Kg and midazolam 5 mg/Kg body weight)). It also caused a strong short circuit current (Isc) response in chambered jejunal mucosa in vitro, which was not influenced by the absence or presence of the luminal Cl^-/HCO_3^- exchanger SLC26a6, was absent in the absence of PepT1 expression, and was virtually abolished in the absence of NHE3 expression. Villous enterocyte pHi decreased significantly stronger upon the luminal application of Gly-Sar in the absence of Slc26a6, as well as in the absence of NHE3. The absence or inhibition of NHE1 and NHE2, which are also expressed in murine jejunum, did not affect the Gly-Sar mediated pHi-decrease. Conclusions: NHE3 and Slc26a6 are equally involved in pHi regulation after PepT1-mediated uptake of H+/dipeptide over the apical BBM, but only NHE3 is essential for both PepT1-mediated dipeptide as well as salt and fluid absorption. The requirement of NHE3 for PepT1-mediated transport may be explained by a unique H^+-recycling function of NHE3 in conjunction with PepT1.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC86

Effects of phenylephrine on spontaneous electrical waveforms in the guinea-pig prostate

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Introduction: Two distinct types of spontaneous electrical activity can be recorded from the guinea-pig prostate, slow waves and pacemaker potentials. Slow waves arise from smooth muscle cells while pacemaker potentials are believed to arise from prostatic interstitial cells (PICs). These c-kit positive cells are mainly located between the glandular epithelial and smooth muscle layers of the guinea-pig prostate and provide the depolarising pulse to neighbouring smooth muscle cells to initiate slow waves.
Aim and Methods: In this study the effects of phenylephrine on the spontaneous electrical activity in the guinea-pig prostate was investigated using intracellular microelectrodes to record changes in membrane potentials. Paired Student's t-test was used for tests of significance, values are expressed as mean ± SEM and $p<0.05$ was considered to be significant.
Results: Phenylephrine (1μM) increased the frequency of slow wave activity from 3.9 ± 0.9 min^{-1} to 9.4 ± 2.2 min^{-1} (n=6, $p<0.05$) and pacemaker activity from 6.8 ± 1.0 min^{-1} to 9.0 ± 0.9 min^{-1} (n=3, $p<0.05$) without affecting other measured parameters. In the presence of nifedipine (1μM), phenylephrine also increased the frequency of both waveforms and in addition caused a membrane depolarisation from -53.4 ± 1.7 mV to -51.0 ± -1.9 mV in slow waves (n=8, $p<0.05$) and -51.4 ± 2.8 mV to -47.6 ± 2.5 mV in pacemaker potentials (n=3, $p<0.05$). In cells where nifedipine abolished the spontaneous electrical activity, phenylephrine was able to restore activity which was associated with a resting membrane depolarisation of 2-4mV (n=7, $p<0.05$). In the presence of nifedipine, cyclopiazonic acid (CPA, 10μM), carbonyl cyanide 3-chlorophenylhydrazone (CCCP, 1-10μM) or niflumic acid (10-100μM) abolished electrical activity. In the presence of CPA or CCCP, phenylephrine

that NO is not required for adenosine to be released during contraction, as adenosine acting on A_{2A}-receptors still contributed to exercise hyperaemia when NOS was inhibited. Further, as the contribution of adenosine acting via A_{2A}-receptors to exercise hyperaemia was fully restored when tested against a background of NOS inhibition and tonic NO-induced dilatation, it seems that adenosine released during contraction does not depend on new synthesis of NO to produce vasodilatation. We therefore propose that, during muscle contraction adenosine is released from the skeletal muscle fibres independently of NO and acts directly on A_{2A}-receptors on the vascular smooth muscle to cause vasodilatation.

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BHF

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PC119

Hypotensive effect of the nitrosyl ruthenium complex nitric oxide donor in renal hypertensive rats

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Nitrosyl ruthenium complexes have been studied as a new class of NO donors. We have described a new compound (trans-[RuCl([15]aneN4)NO]2+), which in vitro releases NO by the action of a reducing agent such as catecholamines. The NO released from this compound induces vasorelaxation and cytosolic calcium decrease in the vascular smooth muscle cells. The renal hypertension induced by one kidney clipping (2K-1C; performed under tribromoethanol (0.25g/kg i.p.) anaesthesia) presents high sympathetic activity. Therefore, the present study aimed to investigate the effect of this NO donor on the arterial pressure in severe and moderate renal hypertensive 2K-1C rats. We also evaluated the toxicity of the complex in the vascular smooth muscle cells in the concentration used to induce the maximum vasodilatation. The mean arterial pressure (MAP) was measured before and up to six hours after intravenous in bolus injection of trans-[RuCl([15]aneN4)NO]2+ (10 mM/kg) in conscious hypertensive and normotensive (2K) rats. In the hypertensive rats (basal 196.70 ± 8.70 mmHg, n=5), the MAP was reduced in -34.25 ± 13.50 mmHg (P<0.05) 6 hours after the compound injection. In 2K rats the compound had no hypotensive effect. We have also studied the effect of injection of 0.1 mM/kg in normotensive (basal 118.20 ± 11.25 mmHg, n= 4), moderate (basal 160.90 ± 2.30 mmHg, n= 6), and severe hypertensive rats (basal 202.46 ± 16.74 mmHg, n= 6). The compound at the dose of 0.1 mmol/L/kg did not have effect (P>0.05) on MAP of normotensive and moderate hypertensive rats. However, in the severe hypertensive rats there was a significant reduction on the MAP of -28.64 ± 12.45 mmHg, that corresponds to 13.72 ± 5.04% of reduction of the basal MAP. The cell viability after the incubation of vascular smooth muscle cells with 0.1 mM trans-[RuCl([15]aneN4)NO]2+ for 3 h was not toxic as revealed by the MTT assay. The cells viability was 100% in the absence (control) and 97.0 ± 0.5% in the presence of the NO donor. In conclusion, the NO donor reduced the MAP of all hypertensive rats in the dose of 10mM/kg and in the severe hypertensive rats at the dose of 0.1mM/kg. The present investigation showed the hypotensive effect of the new NO donor reinforcing the idea that the use of this compound could be useful in different degrees of hypertension.

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PC120

Respiratory sinus arrhythmia and the distribution of heartbeats throughout the respiratory cycle

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It has been suggested that respiratory sinus arrhythmia (RSA) contributes to the optimisation of pulmonary gas exchange efficiency by clustering heartbeats in the inspiratory phase of the respiratory cycle (Hayano's hypothesis). This hypothesis is supported by animal and human studies, which show a significant correlation between RSA magnitude and indices of gas exchange efficiency (1, 2). However, recent studies indicate that RSA may play a limited role in clustering heartbeats (3, 4) and that changes in the proportion of heartbeats in inspiration (HB_{insp}) are more closely related to changes in the inspiratory period to breath period ratio (IE/II ratio) than RSA magnitude per se. In this study we sought to examine the pattern of heartbeat distribution throughout the respiratory cycle across a range of RSA magnitudes likely to be observed physiologically in man.

In 12 healthy male volunteers (aged 20-25) we recorded ECG, respiratory flow and continuous BP measurements in the supine position and modified RSA magnitude by fixed paced breathing at 6, 9 and 12 breaths per minute. RSA pattern and magnitude were obtained by cubic spline interpolation of cardiac cycle intervals as a function of the respiratory cycle. One-way repeated measures ANOVA showed that reductions in breathing frequency resulted in a significant increase in RSA magnitude ($p < 0.01$), but this was not associated with significant changes in the proportion of heartbeats in inspiration, HB_{insp} ($p = 0.33$). Although IE/II ratio did not differ significantly with breathing frequency ($p = 0.069$), linear regression analysis

showed a strong relationship between HB_{insp} and changes in IE/II ratio ($r = 0.85$, $p < 0.01$) but not with changes in RSA magnitude ($r = 0.19$, $p = 0.55$).
These results suggest that, contrary to the common view, RSA magnitude does not cause significant heartbeat clustering into inspiration in humans. The mechanism behind associations between RSA and indices of gas exchange efficiency and the underlying function of RSA remain unclear.

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PC121

Virally-mediated expression of ATP degrading enzymes as a new tool to study ATP-mediated signalling *in vivo* and *in vitro*

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Recent data suggest that ATP is released in the medulla in response to hypoxia and hypercapnia and may mediate excitation of presympathetic rostral ventro-lateral medulla (RVLM) neurones. Thus, during hypoxia or hypercapnia ATP is released within the areas where these neurones are located (Gourine et al. 2005b). Activation of P2 receptors in the RVLM evokes marked increases in blood pressure and renal sympathetic nerve activity (Horiuchi et al. 1999;Ralevic et al. 1999; Thomas et al. 2001) while exogenous ATP excites bulbospinal presympathetic RVLM neurones (Ralevic et al., 1999). However, further studies into the role of ATP in central respiratory and sympathetic chemosensitivity are hampered by the lack of selective pharmacological tools. The existing P2 antagonists (e.g. PPADS and others) are notorious for their lack of specificity and are not suitable for chronic in vivo studies. Here we validate a novel strategy to study the role of ATP-mediated signalling in the CNS based on a viral gene transfer of ATP-degrading enzymes. Extracellular ATP is broken down in several steps and this is catalyzed by ectonucleoside triphosphate diphosphohydrolases. Another family of enzymes, the alkaline phosphatases convert a variety of substrates, including ATP to adenosine. Here we have successfully used one such enzyme, the placental alkaline phosphatase (PLAP, the human gene) in a proof-of-principle experiment using a lentiviral vector LVV-EL1α-hPLAP. This LVV expresses PLAP under control of a non-specific EL1α promoter leading to the gene expression in neurones, glia and other cells. In rats (n=8) four unilateral injections of LVV-EL1α-hPLAP were made unilaterally into the right RVLM (under ketamine (60 mg/kg) and medetomidine (250 μg/kg) i.m. anaesthesia). Seven days later horizontal slices containing the ventral medullary surface were prepared and used for an in vitro experiments as described in (Gourine et al. 2005a; Gourine et al. 2005b). ATP microelectrode biosensors were used to determine release of ATP from the ventral surface chemosensitive areas in response to isohydric hypercapnia using aCSF solution in which NaHCO3 was increased to 50 mM (isosmotically replacing NaCl) and equilibrated with 10% CO2/90% O2 (pH ~ 7.45, pCO2 ~65 mmHg at 37°C). It was found that the amount of ATP released in response to isohydric hypercapnia was significantly (by ~50%; P=0.008) smaller on the transduced side of the medulla. Thus, expression of PLAP in the RVLM can be used as a highly effective approach for rapid degradation of ATP released into the extracellular space during chemosensory stimulation. We conclude that virally-mediated expression of ATP degrading ecto-enzymes can be used as a novel tool to study the multiple functional roles of ATP-mediated signalling in the central nervous system.

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Effects of chronic hypoxia on diaphragm function in juvenile and adult rats

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Chronic hypoxia occurs in normal individuals at altitude and also in patients suffering from respiratory disease. Skeletal muscle structure and oxidative capacity are age dependent are known to be affected by chronic hypoxia The aim of this study was to examine the age-dependent effects of chronic hypoxia on diaphragm muscle contractile and endurance properties.
Adult (12 week old) and juvenile (3 week old) Wistar rats were exposed to either hypobaric hypoxia (barometric pressure 380mmHg) (n=12) or normobaric normoxia (n=12) for 6 weeks. At the end of the treatment periods, isometric contractile and endurance properties of isolated strips of diaphragm muscle were measured in tissue baths under hyperoxic