C01 and PC01

AMP-activated protein kinase inhibits recombinant TASK-3 K^+ channels

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Hypoxia excites the carotid body by causing depolarization of type I cells, triggering voltage-gated Ca^{2+} influx and secretion of transmitters to excite afferent sensory neurons. In the rat, hypoxic depolarization of type I cells arises from inhibition of a 'leak' K current and BKCa channels [1]. The leak current has recently been proposed to be carried by TASK-1, TASK-3 and, predominantly, TASK-1/3 heterodimers [2]. We have previously proposed that hypoxia inhibits these currents via activation of AMP-activated protein kinase (AMPK) [3], and have also provided preliminary pharmacological evidence that heterologously expressed TASK-3 (but not TASK-1) channels are inhibited by AMPK activation. However, a recent study has suggested that neither channel type is regulated by AMPK activation [4]. Here we report new evidence to indicate that recombinant TASK-3 channels (stably expressed in HEK293 cells) are inhibited by AMPK. Data are expressed as mean ± s.e.m.

Whole-cell TASK-3 currents recorded at 37ºC were inhibited by bath application of 100μM A-769662, a specific AMPK activator (45.8 ± 6.6% inhibition, n=5, P<0.01, paired t-test). However, in the presence of the AMPK inhibitor compound C (20μM), A-769662 failed to inhibit TASK-3 currents (n=6). Over a 20 min period, TASK-3 currents were reduced in amplitude by 46.7 ± 2.7% (n=7) when a bacterially-derived, active (thiophosphorylated) recombinant AMPK heterotrimer ($\alpha 2\beta 2\gamma 1$) was included in the pipette solution. By contrast, inclusion of an inactive (α2D157A) AMPK heterotrimer led to a reduction in current amplitude of only 9.0 ± 4.1% (n=5) over the same time period.

These data indicate that TASK-3 is inhibited by activation by AMPK, and further support our suggestion that AMPK mediates hypoxic inhibition of leak K^+ channels in the carotid body.

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Supported by The Wellcome Trust

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

C02 and PC02

Modulation of Kv2.1 and Kv1.5 channels by AMP-activated protein kinase (AMPK)

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It has been suggested that the K^+ channels Kv2.1 and Kv1.5 are O_2 sensitive and play an important role in hypoxic pulmonary vasoconstriction (HPV; [1-3]). Relative expression levels of Kv2.1 and Kv1.5 in smooth muscle cells may determine, in part, differences in hypoxic sensitivity of conduit versus resistance arteries. Given that our previous studies suggested that HPV requires AMPK activation [4], we investigated whether AMPK regulates Kv2.1 and Kv1.5 stably expressed in HEK 293 cells.

From whole-cell patch clamp recordings we constructed conductance - voltage relationships for Kv2.1 as previously described [5]. Under control conditions, the half maximal conductance ($G_{0.5}$) was 10.9 ± 1.0mV (mean ± s.e.m., n=21 cells). This was shifted in the hyperpolarizing direction by pre-exposure (20 min, 37∞C) of cells to the AMPK activator A-769662 (100μM; $G_{0.5}$ -8.1 ± 2.1mV, n=10, P<0.01, unpaired t-test). When cells were pretreated with A-769662 in the presence of the AMPK inhibitor compound C (20μM), $G_{0.5}$ (12.4 ± 1.8mV) was similar to control, i.e. compound C prevented the hyperpolarizing shift in activation caused by AMPK. We identified S440 and S537 as potential phosphorylation sites in the Kv2.1 sequence. $G_{0.5}$ in S537A and S440A mutants were 6.0 ± 1.1mV (n=10) and 9.4 ± 0.9mV (n=10), respectively. Exposure to A-769662 caused a significant shift (P<0.05) in $G_{0.5}$ for the S537A mutant of -11.7 ± 1.3mV (n=10). By contrast, A-769662 caused only a modest shift of the $G_{0.5}$ for the S440A mutant of 3.8 ± 1.1mV (n=10). Parallel studies on phosphorylation (employing ^{32}P incorporation) indicated that the immunoprecipitated channel protein was a direct substrate for AMPK-dependent phosphorylation at these two sites.

In marked contrast to these findings for Kv2.1, AMPK markedly attenuated macroscopic Kv1.5 currents throughout their current-voltage relationship. Residual currents evoked at +30 mV measured 61.2 ± 0.2 % (n = 4, P<0.05) and 61 ± 0.1 % (n = 4, P<0.05) of control following intracellular dialysis of an active (thiophosphorylated) recombinant AMPK heterotrimer ($\alpha2\beta2\gamma1$) or extracellular application of 100μM A-769662, respectively. Current inhibition was not observed upon intracellular dialysis of an inactive (α2D157A) AMPK heterotrimer, or following application of A-769662 in the presence of compound C (40μM). Parallel studies on ^{32}P phosphorylation indicated that the immunoprecipitated channel protein was a direct substrate for AMPK-dependent phosphorylation.

Our data suggest that Kv2.1 and Kv1.5 are directly modulated by AMPK and thereby regulated in a manner consistent with the differential effects of both hypoxia and mitochondrial inhibitors on Kv currents recorded in arterial smooth muscle cells of conduit and resistance sized pulmonary arteries, respectively.

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Supported by The Wellcome Trust

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

C03

Hydrogen sulfide regulates Na^+/H^+ exchanger activity via stimulation of phosphoinositide 3-kinase/Akt and phosphoglycerate kinase-1 pathways

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Hydrogen sulfide (H2S) has recently been positioned as the third candidate of the newly emerging "gasotransmitters" family along with nitric oxide (NO) and carbon monoxide (CO) [1]. Intracellular pH (pHi) is an important modulator of cardiac function, influencing processes as varied as contraction. We previously reported that H2S regulates pHi in vascular smooth muscle and brain cells cells [2, 3]. The present study was therefore designed to investigate the pH regulatory effect of H2S in the rat cardiac myocytes and the contribution of this effect in the cardioprotective effects in rat isolated hearts. Intracellular pH was measured using a spectrofluorometer with the pH-sensitive dye, BCECF/AM. It was found that NaHS at a concentration range of 10-5-10-3 M (which yields approximately 3.3 x10-6~3.3x10-4 M H2S) produced sustained and obvious decreases in pHi in a concentration-dependent manner in the rat cardiac myocytes. NaHS also abolished the intracellular alkalinization caused by U50,488H, which activates Na^+/H^+ exchanger (NHE). Moreover, when measured with an NHCl4 prepulse method, we found that NaHS significantly suppressed the activities of NHE. Functional study shows that perfusion with NaHS significantly improved the post-ischemic contractile function in the isolated rat hearts subjected to ischemia/reperfusion. Blockade of phosphoinositide 3-kinase (PI3K) with LY294002 or phosphoglycerate kinase-1 with KT5823 significantly attenuated NaHS-induced suppression in NHE activity in the isolated cardiac myocytes and the cardioprotection in the isolated hearts. Western botting analysis shows that KT5823 failed to attenuate NaHS-stimulated Akt phosphorylation, suggesting that activation of phosphoglycerate kinase-1 may not be secondary to PI3K/At pathway. In conclusion, NaHS inhibited NHE activity via stimulation of PI3K/Akt and phosphoglycerate kinase-1 pathways, which may further protect heart against ischemia/reperfusion-induced injury.

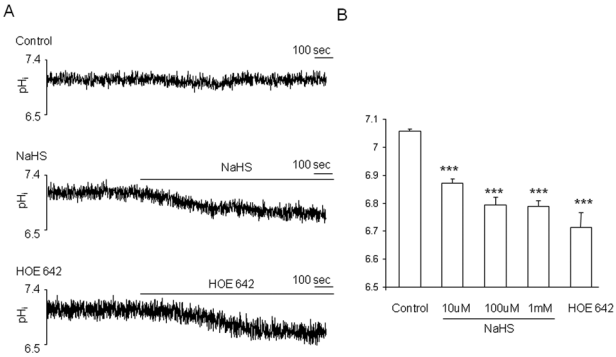

Figure 1. Hydrogen sulfide induces intracellular acidosis in single cardiac myocytes. (A) Typical tracing showing the effect of NaHS (100 μM) and HOE 642 (7 μM) on intracellular pH. (B) Concentration-dependent effect of NaHS. Mean±S.E.M., n=9-36, *** p<0.01.

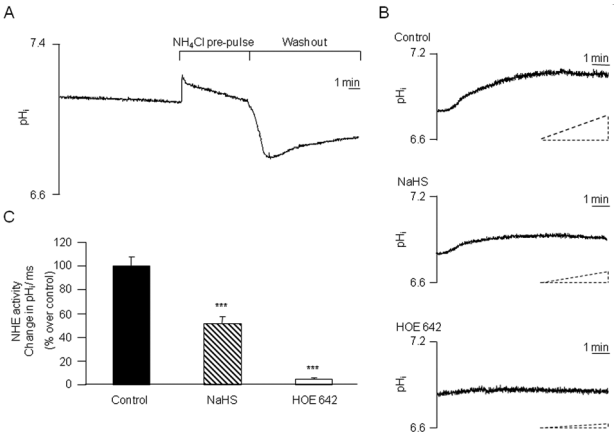

Figure 2 Effect of NaHS on NHE activity in the cardiac myocytes. (A) Representative tracing for NHE activity measurement. (B) Representative tracings for pHi recovery with and without NaHS or HOE 642 treatment. (C) Mean value showing that NaHS and HOE 642 significantly inhibited NHE activity. Mean±S.E.M. n = 22-30, ***p<0.001 vs control.

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This work was supported by Singapore Biomedical Research Council (BMRC) research fund (07/1/21/19/509).

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C04 and PC04

K_v1.5 channel expression in human placental resistance arteries of normal pregnancy and fetal growth restriction

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Fetal growth restriction (FGR), a serious pregnancy complication, is associated with raised placental vascular resistance and reduced blood flow which limits nutrient delivery to the fetus. The placenta functions as the fetal lung and, in common with pulmonary vascular disease, FGR is associated with tissue hypoxia. In lung, chronic hypoxia inhibits the expression of K_v1.5, an oxygen-sensitive K channel, which promotes smooth muscle cell (SMC) proliferation and reduces apoptosis, culminating in artery wall remodelling and reduced blood flow[1]. Placental chorionic plate artery SMCs express K_v1.5 [2] but expression in FGR in relation to SMC proliferation and apoptosis remains unexplored. We tested the hypothesis that in FGR chronic hypoxia reduces K_v1.5 expression in SMCs of placental resistance arteries promoting their proliferation and suppressing apoptosis.

Placentas were collected at term following normal pregnancy (NP) or FGR (individualised birthweight ratio <5th centile). Serial sections of chorionic plate with underlying villous tissue were examined by immunohistochemistry using antibodies to detect K_v1.5, SMCs (α-actin), endothelial cells (ECs: CD31), proliferation (Ki67) and apoptosis (TUNEL). To examine effects of oxygen on K_v1.5 expression, chorionic plate artery segments (n=83) from normal placentas (N=6) were maintained in culture at 21%, 6% or 1% oxygen (5% CO_2, 37∞C) for 24, 48 or 72 hr and immunostained for K_v1.5. K_v1.5 staining intensity was scored by 3 persons blinded to sample identity. Proliferation and apoptosis was expressed as the number of positively stained cells as a percent of total cells.

Immunostaining of positive control tissue and samples was absent when primary antibody was blocked by antigenic peptide (K_v1.5), replaced with non-immune IgG (α-actin, CD31, Ki67) or in the absence of TdT enzyme (TUNEL). Resistance artery SMCs and ECs showed positive staining for K_v1.5 (co-localised in serial sections). In both NP (N=10 placentas; 162 vessels) and FGR (N=8; 70 vessels), staining intensity was greater in ECs than SMCs ($p<0.02$; Wilcoxon signed rank) and SMC staining was stronger in large (>300μm) than small ($\leq$100μm) diameter vessels ($p<0.01$; Kruskal Wallis with Dunns post test). However, there were no differences in EC or SMC K_v1.5 expression, proliferation or apoptosis in FGR compared to NP. Furthermore, K_v1.5 expression in SMC and EC was not altered by exposure of arteries to 21%, 6% or 1% oxygen over 24-72 hr.

The similarity in K_v1.5 expression in NP and FGR implies that the channels are not down- regulated by placental hypoxia. This is supported by data showing that SMC K_v1.5 expression in arteries of NP was not suppressed by 72hr exposure to 1% oxygen. The expression of K_v1.5 in SMC and EC implies a functional role for the channel in regulating placental resistance artery tone in both NP and FGR.

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C05 and PC05

O_2-sensitive K^+ channels: role for $K_v1.5$ in human placental arteries?

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Hypoxic fetoplacental vasoconstriction (HFPV) is proposed to direct fetal blood to O_2-rich areas of the placenta to facilitate optimal transfer of O_2 and nutrients to the developing fetus[1]. A similar process in lung, ventilation-perfusion matching, is achieved by hypoxic vasoconstriction and mediated by $K_v1.5$ inhibition, an O_2-sensitive voltage-gated K^+ channel[2]. O_2-sensitive K^+ channels have been implicated in HFPV[3] but their molecular identity remains unknown. HFPV has been demonstrated experimentally in response to acute O_2 reductions over a range higher than the placenta encounters in vivo[1, 3]. Here we test the hypothesis that $K_v1.5$ contributes to agonist-induced chorionic plate artery constriction following acute and chronic exposures to pathophysiologically relevant O_2 tensions.

Chorionic plate arteries were dissected from normal term placentas (N=14), bathed in physiological salt solution (PSS) and immediately prepared for isometric tension studies. Vasoconstriction was assessed with U46619 (thromboxane-mimetic 10^{-10}–$2x10^{-6}$M) in the presence and absence of 4-aminopyridine (4-AP; 1mM) and DPO-1 (3μm), blockers of voltage-gated (K_v) and $K_v1.5$ channels respectively. These acute experiments were performed in 5% O_2/ 5% CO_2 (N=8; pO_2=6%; normoxia) or 5% CO_2 in N_2 (N=6; pO_2=2%; hypoxia) gassed PSS. Vessels from the same placentas were isolated and cultured for 48hr at 6% O_2 (N=8) or 2% O_2 (N=6) and experiments repeated to assess effects of chronic oxygenation. Significant differences ($P<0.05$) were assessed by Two-Way ANOVA and Mann-Whitney U Test.

Chronic exposure/ culture at 6% and 2% O_2 did not affect agonist-induced (U46619 dose-response curves) or depolarisation-induced (120mM KPSS) constrictions compared to acute exposure. Enhanced U46619 constriction was not observed following acute or chronic exposure to 2% O_2 compared to 6% O_2. In acute experiments, pre-incubation with 4-AP or DPO-1 caused a significant upward shift in the U46619 dose-response curve at 6% and 2% O_2. However, this effect of K_v channel blockade on U46619-induced contraction was abolished in cultured vessels at either O_2 tension compared to the respective time-matched controls.

U46619-induced constriction of chorionic plate arteries was not increased in acute hypoxia or following culture in hypoxic conditions. In acute experiments, K_v and $K_v1.5$ channel inhibition increased U46619-induced constriction at both O_2 tensions. Loss of K_v channel inhibition following culture at either O_2 tension suggests $K_v1.5$ channel expression/function is not regulated by oxygenation over this range but may be an intrinsic characteristic of the culture process. In line with our previous reports of $K_v1.5$ expression in isolated smooth muscle cells[4], this study provides the first evidence for functional $K_v1.5$ channels in chorionic plate arteries over a pathophysiologically relevant oxygenation gradient for the placenta.

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C06 and PC06

Hypoxic upregulation of the bone morphogenetic protein antagonist gremlin1 contributes to the development of pulmonary hypertension

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Pulmonary hypertension is a common complication of chronic hypoxic lung diseases. Recently we reported that gremlin, a BMP antagonist, was selectively upregulated in hypoxic human pulmonary microvascular endothelial cells in vitro (Costello et al., 2008). Given the important role of bone morphogenetic proteins (BMP) in normal pulmonary vascular homeostasis, we postulated that upregulation of the BMP antagonist gremlin1 was an important pathogenic mechanism contributing to the development of hypoxic pulmonary hypertension.

To test this hypothesis we exposed groups of wild type mice (N=10) and mice heterozygous (N=9) for a mutation in the gremlin1 gene (grem1+/-) to hypoxia (FIO2= 0.10) for a period of three weeks. Mice were then anaesthetised (sodium pentobarbitone 70mg.kg-1), anticoagulated, killed by exsanguination and the lungs immediately isolated, ventilated and perfused at constant flow with standard venous out-

flow and airway pressures such the pulmonary arterial pressure measurements were made in zone 3 conditions. Further lungs were fixed in paraformaldehyde at standard airway pressure and stained using a goat anti-gremlin antibody.
Gremlin protein was expressed in the pulmonary vascular endothelium of normoxic and chronically hypoxic mice. Haematocrit was similarly elevated in the wild type and grem1+/- groups following hypoxic exposure. The mean (±SEM) ratio of right ventricular to left ventricular plus septal weight was significantly lower ($P<0.05$) in the chronically hypoxic grem1+/- mice (0.364±0.016) than in the wild types (0.432±0.022). Mean pulmonary arterial pressure in the grem1+/- mice (8.1±0.2 mmHg) was significantly lower ($P<0.01$) than that in the wild type mice (9.1±0.2 mmHg) following three weeks of hypoxia.
These data show that heterozygous loss of gremlin attenuates the pulmonary vascular response to chronic hypoxia in mice and suggest a key role for this BMP antagonist in the development of hypoxic pulmonary hypertension similar to that found at high altitude and in chronic lung disease.

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Funded by the Health Research Board and the Programme for Research in Third Level Institutes (HEA), Ireland.

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C07

Cardiac adaptations to hypoxia in the high-altitude bar-headed goose

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Bar-headed geese fly at up to 9000m elevation during their migration over the Himalayas, sustaining high metabolic rates in the severe hypoxia at these altitudes. Previous work suggests that adaptations to enhance pulmonary O_2 loading and peripheral O_2 diffusion exist in bar-headed geese [1,2], but very little is otherwise known about the mechanisms for this physiological feat. We used a comparative approach to investigate whether specializations in cardiac O_2 transport and energy metabolism contribute to hypoxia adaptation in this species. Bar-headed geese had higher capillary densities in the left ventricle of the heart than low-altitude geese (bar-headed geese: 2795 ± 125 mm^{-2}, N=6; pink-footed geese: 2070 ± 38, N=8; barnacle geese: 2228 ± 109, N=8; significance tested with ANOVA and Holm-Sidak

post-hoc tests), which should improve O_2 diffusion during hypoxia. While myoglobin abundance and the activities of many metabolic enzymes showed only minor variation between species, bar-headed geese had a striking alteration in the kinetics of cytochrome c oxidase (COX), the heteromeric enzyme that catalyzes O_2 reduction in oxidative phosphorylation. This was reflected by a lower maximum catalytic activity (V_{max}; bar-headed geese: 2.04 ± 0.24 μmol/mg protein/min; pink-footed geese: 3.85 ± 0.36; barnacle geese: 4.37 ± 0.38) and a higher affinity for reduced cytochrome c (K_m; bar-headed geese: 39.8 ± 7.7 μmol; pink-footed geese: 179.8 ± 28.6; barnacle geese: 179.1 ± 18.1). There were small differences between species in mRNA and protein expression of COX subunits 3 and 4, but these were inconsistent with the divergence in enzyme kinetics. However, the *COX3* gene of bar-headed geese had an amino acid substitution at a site that is otherwise conserved across vertebrates and resulted in a major functional change of amino acid class (Trp-116→Arg; the former was present in all 606 vertebrate species available in Genbank). This mutation was predicted by structural modelling to alter the interaction between COX3 and COX1. Adaptations in mitochondrial enzyme kinetics and O_2 transport capacity may therefore contribute to the exceptional ability of bar-headed geese to fly high.

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Supported by NSERC of Canada.

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C08 and PC08

Autonomic control of the heart and the distribution of vagal preganglionic neurones in the bullfrog compared with the African clawed toad

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In heterothermic animals heart rate and the degree of autonomic tone on the heart typically varies with temperature. A predominant inhibitory tone is exerted by the parasympathetic system via the vagus nerve that is supplied by vagal preganglionic neurones (VPN) in the brainstem (Taylor et al 1999). This study measured autonomic tone on the heart of the bullfrog (*Rana catesbeiana*) at 3 temperatures and correlated the results with a neuranatomical study of the distribution of VPN in the brainstem. The results are compared with an earlier study on the toad, *Xenopus laevis* (Taylor and Ihmied 1995; Wang et al 1999). Bullfrogs (158 ± 30.9 g; n = 37) were kept at 25∞C with free access to water. Food was withheld 3 days before experiments, performed in accordance with ethical guidance at UNESP. Frogs (n = 5) were anaes-

thetized in a solution of MS 222 (500 mg l-1). The cervical vagus was exposed for injection of 6-8 μL of a 2% solution of the neural tracer True Blue (Sigma). The animals were held at 20∞C for 5 to 15 days then terminally anaesthetised in MS 222 and perfused with heparinised 9% saline then a 4% solution of formaldehyde buffered to pH 7.3. The brain was sectioned and fluorescing neurone cell bodies were counted and mapped according to their proximity to the 4th ventricle and their rostro-caudal distance from obex. VPN were located in a single nucleus within the central grey distributed over a rostro-caudal extent of 5mm and appeared to be of similar size. In contrast, *Xenopus* had a distinct lateral group of larger VPN located in the white matter outside of the central grey, constituting about 30% of total VPN.
For measurement of heart rate frogs (n= 48) were anaesthetised in benzocaine (1 g L–1). The femoral artery was occlusively cannulated, with lidocaine applied to the incision. The frogs were held for 24 hours in climatic chambers at 10, 20 or 30∞C. Blood pressures were recorded via a data acquisition system sampling at 100Hz. The β-adrenergic antagonist propranolol or the cholinergic muscarinic antagonist atropine were injected intra-arterially (3 mg kg-1 each), with the order of injection alternated and the efficacy of the autonomic blockade verified by injection of agonists. The adrenergic and cholinergic tones on the heart were calculated from cardiac intervals (1/fH). Data were evaluated using a paired t-test. Differences in heart rate were considered statistically significant at a 95% level of confidence ($P < 0.05$). Although heart rate increased with temperature, cardiac vagal tone in *Bufo* was low at all 3 temperatures, varying between -5% and +10% between 10 and 30∞C. Studies on *Xenopus* revealed that vagal tone varied from 75% at 5∞C to 185% at 15∞C and 329% at 25∞C. It seems possible that high levels of cardiac vagal tone are associated with dual origins for VPN in these amphibians.

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C09 and PC09

Interaction between hypoxia and glucose in the rat carotid body, *in vitro*

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The *in vitro* response of the carotid body to low or zero glucose appears equivocal, being robust in co-cultures of Type I cells with petrosal neurons or in carotid body thin slices (1, 2) but absent in whole carotid body preparations (3). As this difference may be due to the metabolic status of the various preparations or to an O_2-dependence, we measured chemoafferent responses to zero glucose at varying levels of PO_2 and following prior exposure to either hypoxia or hyperoxia.

Pairs of carotid bodies were isolated from adult (> 30 days), male Wistar rats during isoflurane inhalation anaesthesia (2.5% in O_2) and single fibre recordings of chemoafferents were made from the sinus nerve as described previously (4). Whilst recordings were made from the first of each pair of carotid bodies the second organ was incubated in a gassed (95% O_2/5% CO_2) ice-cold HCO_3^- -buffered solution before it also was recorded from. The time of incubation was between 4-5 hrs. Superfusate glucose was either 11mM or 0mM. Superfusate PO_2 was adjusted to set basal discharge at an *in vivo*, normoxic value of 0.5-1.0 Hz. Data is expressed as means ± S.E.M and significance measured using t-tests (Statview, Abacus Concepts) and taken as $P<0.05$.

On removal of glucose from the superfusate, chemodischarge in the first carotid body remained unchanged at a basal frequency of 0.68±0.14 Hz for 32.8±1.9 min, at which time discharge frequency increased significantly to 2.70±0.19 Hz ($P < 0.01$), before failing irreversibly. This time was significantly reduced to 15.4±2.8 min ($P < 0.001$) by either 2 or 3 prior exposures of the carotid body to severe hypoxia. In the second, hyperoxia-incubated, carotid body, the time taken to respond to zero glucose was also significantly reduced to 19.8±1.4 min ($P < 0.001$). All preparations responded to reducing PO_2 with exponential increases in discharge both in the presence and absence of glucose. However, in the first carotid body of each pair, the PO_2 response curves in the absence of glucose were significantly left shifted relative to control but were right shifted in the second of each pair, with the PO_2 needed to achieve a discharge rate of 5Hz being decreased significantly by 8.39±0.68 mmHg ($P < 0.05$) in the first and increased by 13.3±3.89 mmHg ($P < 0.05$) in the second preparation.

Our data confirms previous findings that show that the freshly-isolated, intact carotid body has a naturally poor acute sensitivity to reduced glucose and shows that this is not a PO_2 dependent effect. We suggest, however, that the direct glucose sensitivity of *in vitro* chemoreceptor tissue may be increased by prior exposure to repeated bouts of natural hypoxic stimulation or by incubation in high PO_2 media.

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

C10 and PC10

Investigation of timing, cell location and mechanical effects of calcium waves in cardiac myocytes

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Calcium waves (CaWs) in cardiac myocytes arise from abnormal sarcoplasmic reticulum (SR) calcium release. CaWs are more common in cells isolated from failing than normal hearts. Extrusion of calcium by the sodium calcium exchanger induces afterdepolarisations and may trigger action potentials (AP) and potentially initiate arrhythmias. We investigated the timing, cell location and mechanical effects of calcium waves in cardiac myocytes acutely isolated from rat hearts and loaded with fluo4AM. The occurrence of CaW was observed using line scanning confocal microscopy in 10 cells. Trains of normal calcium transients were induced by action potential voltage clamp utilising the permeabilised patch technique (8 animals, 10 cells). Calcium waves were studied during brief pauses (2.5s) between the APs (n=82) and between regular (1Hz) APs (n=12). Contraction during APs was measured from the observed shortening in cell length.

The temporal distribution of CaWs during pauses was non-random (Runs test, $P < 0.00001$), early CaWs being less common than late CaWs (14 of 166 vs. 54 of 166; Fisher's exact $P < 0.0001$). When waves occurred in both of two temporally adjacent pauses the two CaWs were more likely to originate in the same cell area than other areas of the cell (chi-squared, $P < 0.0001$). CaWs occurring between AP associated calcium transients merged with the subsequent calcium transient and were associated with a large reduction in AP induced contraction (mean 29.5%, 95%CI 22.5-36.6%).

In conclusion, CaWs exhibit tendencies to arise in particular cell areas, their timing is strongly related to the timing of neighboring calcium transients and the following AP induced contractions are significantly reduced. These findings suggest a novel potential pathological consequence of CaWs and emphasises the importance of the timing of CaW initiation.

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This research was funded by the British Heart Foundation.

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

C11 and PC11

Effects of exercise training upon cardiac function in women with and without type 2 diabetes

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Introduction
Individuals with type 2 diabetes have a risk of heart failure 2-5 times greater than age matched controls, and the risk is greater for women compared to men. Prior to the onset of heart failure, individuals with type 2 diabetes exhibit structural and functional abnormalities of the myocardium. Although exercise training improves myocardial function in animal models of type 2 diabetes, data is in humans is limited. This study aimed to investigate the effect of moderate intensity exercise training upon cardiac function in overweight and obese postmenopausal women with type 2 diabetes (T2) compared to BMI matched, healthy controls (ND).
Methods
Seven T2 and 8 ND women (BMI > 25 kg/m^2) volunteered for the study; all were postmenopausal (mean age 57 ± 4.9 years). Prior to and following 6 months of moderate intensity exercise training (55-75% heart rate reserve), participants completed a modified Bruce cardiopulmonary exercise test to assess VO_2peak. Cardiac function (resting and peak cardiac power output; pkCPO, and cardiac reserve; C-res) were calculated from cardiac output, determined by non -invasive inert gas re-breathing, and mean arterial pressure (MAP) determined by manual sphygmomanometry. Anthropometry (body mass, height, waist and hip circumferences) and a fasting venous blood sample (for insulin, glucose, HbA1c) were also assessed. One-way mixed mode ANOVA assessed group differences at baseline. Two-way mixed mode ANOVA with repeated measures tested the interaction between time (0 Vs 6 months) and group (T2 Vs ND).
Results
At baseline ND and T2 groups were similar for VO_2 peak, pkCPO, C-res and anthropometry (P>0.05). T2 women had greater insulin resistance (P<0.05 for glucose, insulin, HbA1c, HOMA). Following training changes in body mass (-3.3 ±3.5 kg, ND; -1.9 ±2.4 kg, T2) and BMI (-1.2 ± 1.4 kg/m^2, ND; -0.9 ± 0.8 kg/m^2, T2) were similar for both groups (P>0.05). Change in VO_2 peak was not significantly different between the groups although ND women showed a trend for greater increases (+349.8 ± 308.6 ml.min^{-1}, ND; +143.3 ± 157.4 ml.min^{-1}, T2). T2 women improved waist (-4 ±2 cm, P=0.03) and hip circumferences (-3.5 ±2 cm, P=0.05) whereas ND women did not. The exercise training significantly improved PkCPO and C-res in the ND but not in the T2 women (P<0.05; Figure 1). Peak cardiac output increased in the ND (+3 ± 1.7 L.min^{-1}) but not in the T2 women (-0.63 ±2.3 L.min^{-1}; P<0.05) and peak MAP (+2.4 ± 9.9mmHg ND; -0.9 ± 12.3mmHg, T2) was no different between the groups.

Conclusion

Compared to ND women, the cardiac function of T2 women in response to moderate intensity exercise training is impaired. This appears to be a function of a lack of plasticity of the flow generating capacity of the heart.

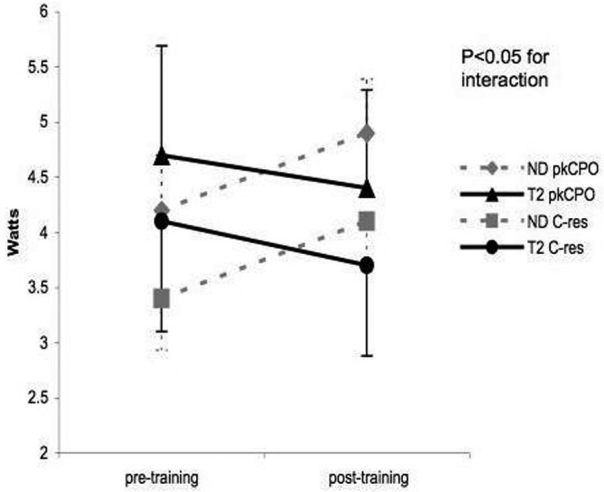

Figure 1.

Between-group difference in peak cardiac power output

(pkCPO) and cardiac reserve (C-res) pre and

post exercise training

We are grateful for the funding of this study which was provided by Heart Research UK (2508/06/08).

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C12

Effect of Intermittent Hypoxia on Erythropoietin, Soluble Erythropoietin Receptors and Ventilation in Healthy Humans

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Upon exposure to acute hypoxia, the body responds almost instantly by an increase in ventilation due to the stimulation of the carotid bodies. A more efficient mechanism of adaptation, as experienced during chronic exposure to hypoxia, is an improvement in the oxygen carrying capacity following hypoxia-induced erythropoietin (Epo) secretion and accelerated erythropoiesis. During the last decade, extensive research on the mechanisms of action of Epo led to the discovery of potential new roles beyond the aforementioned hematopoietic effects. For instance, it has recently been demonstrated that Epo is involved in the ventilatory acclimatization to hypoxia in mice (Soliz et al., 2005). Subsequently, the same group also demonstrated this response is mediated by a decrease in soluble Epo receptors (sEpoR) (Soliz et al., 2007). We hypothesized that i) sEpoR would be downregulated by exposure to intermittent hypoxia (IHx), thereby allowing Epo concentration to rise; ii) these modifications would be correlated with the alteration in the acute hypoxic ventilatory response (AHVR) and breathing pattern (tidal volume – VT, breathing frequency – fR).

Nine healthy male subjects (age: 29±5 yr, height: 173.8±9.5 cm and weight: 76.2±10.6 kg) were exposed to 6h of daytime IHx [2min normoxia (peak end-tidal PO2 (PETO2) = 88.0 Torr) and 2min hypoxia (nadir PETO2 = 45.0 Torr)] for four consecutive days (Days 1-4), preceded by two normoxic control days (4 days apart; combined as Baseline during analyses), and followed by one recovery day (4 days after the ned of IHx; Day 8). Epo and sEpoR concentrations and AHVR were measured on Baseline, Days 1, 2, 4 and 8.

We observed a nadir in sEpoR on Day 2 (-70% vs. Baseline; $p<0.01$), concomitant with the peak in Epo secretion (+50% vs. Baseline; $p<0.01$) (Figure 1). Following each daily exposure to IHx, ventilation (VE) as measured during the AHVR test rose because of the increase in VT while fR remained unchanged. Similarly, AHVR increased progressively along with IHx ($p=0.008$). There was an overall negative correlation between Epo and sEpoR ($r=-0.261$, $p=0.05$), and between sEpoR and VT ($r=-0.331$,

p=0.02). Epo was positively correlated with AHVR (r=0.475, p=0.001) and VE (r=0.458, p=0.001) (Figure 2).
We conclude that sEpoR is downregulated upon exposure to IHx and thereby modulates the Epo response. Furthermore, the alterations in AHVR and breathing pattern following IHx seem at least partially mediated by the increase in Epo.

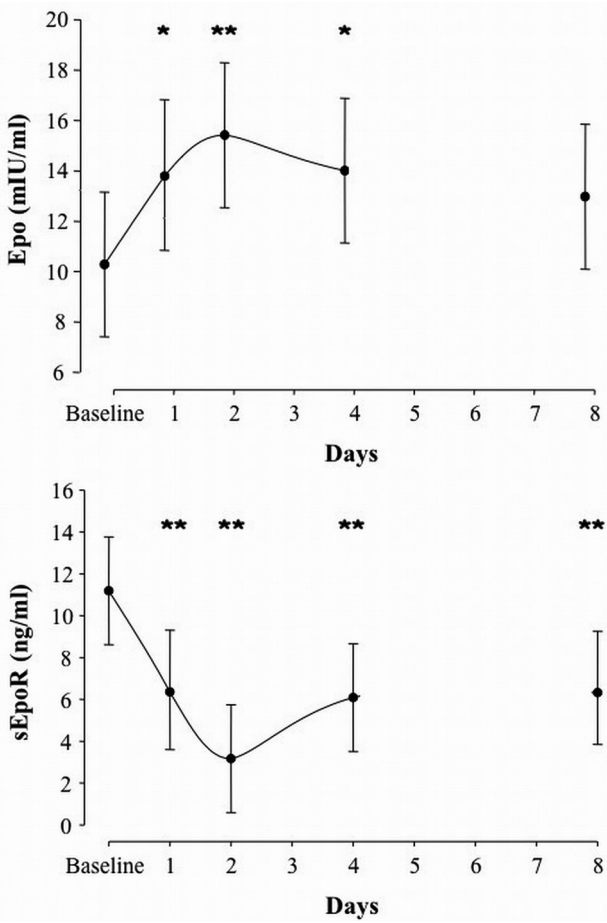

Figure 1. Serum Epo and plasma sEpoR concentrations over 4 days of intermittent hypoxia.

* P ≤ 0.05 and ** P ≤ 0.01 vs. Baseline.

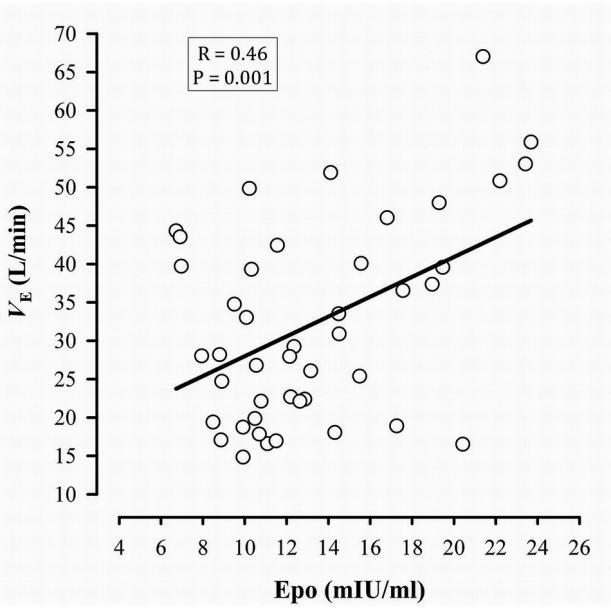

Figure 2. Correlation between Epo and minute ventilation (VE) measured during the AHVR test.

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Funding: AHFMR, HSFC and Hotchkiss Brain Institute

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C13 and PC13

Effects of chronic intermittent hypoxia on rat respiratory muscle structure and function

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Chronic intermittent hypoxia (CIH) is a dominant feature of sleep-disordered breathing (SDB) due to recurrent apnoea (respiratory pauses). It is recognized that respiratory muscles have enormous capacity for remodelling. Respiratory muscle weak-

ness and fatigue contributes to impaired respiratory homeostasis. Despite the clinical relevance, the effects of CIH on respiratory muscle function are relatively underexplored. Therefore, this study was designed to investigate the effect CIH on rat respiratory muscle structure and function.
Adult male Wistar rats were exposed to CIH (90s normoxia/ 90s hypoxia [5% oxygen at the nadir; SaO_2 ~ 80%], 8h/day) for two weeks. Sham treatments were conducted in separate animals in parallel as controls. Following exposure, the animals were anaesthetized with 5% isoflurane and killed by spinal transection. Sternohyoid and diaphragm muscle contractile and endurance properties were examined in vitro at 35∞C under hyperoxic (95% O_2/ 5% CO_2) and anoxic (95% N_2/ 5% CO_2) conditions. Additionally, muscle was snap frozen and stored for structural analysis (immunofluorescence and enzymatic histochemistry).
CIH decreased diaphragm force (18.1±1.7 vs. 14.5±1.9 N/cm2, sham (n=8) vs. treated (n=7) at 100 Hz, mean±SEM, P=0.0034, ANOVA) and decreased diaphragm endurance (52±5% vs 38±3%, sham (n=8) vs CIH (n=6), mean±SEM, % of initial force at 2 mins, P<0.0001, ANOVA). Anoxic tolerance was increased in CIH diaphragm. Areal density of fibres expressing SERCA 1 and SERCA 2 was similar in sham and CIH muscle. There was an increase in type 2B fibre density (5.4±1.5% vs. 13.7±3.8% areal density, sham (n=5) vs. treated (n=5), P=0.08). CIH did not alter the SDH or GPDH activity of the diaphragm. Sternohyoid muscle force was increased (11.8±1.3 vs. 15.3±1.5 N/cm2, sham (n=8) vs. treated (n=7) at 100 Hz, P<0.001, ANOVA) following CIH treatment, while sternohyoid muscle endurance was decreased (16.5±1.3% vs 11.1±1.6%, sham (n=8) vs CIH (n=7), P=0.08, ANOVA). Under anoxic conditions, CIH reduced muscle force but did not affect muscle endurance. There was no significant change in sternohyoid fibre-type, SDH or GPDH activity.
We conclude that 2 weeks of CIH induces respiratory muscle remodelling. CIH had a differential effect on diaphragm and sternohyoid muscle force. We speculate that these opposing effects are due to the stark contrast in muscle composition, as diaphragm is predominantly a slow oxidative muscle, while sternohyoid is primarily a fast glycolytic muscle. CIH caused both respiratory muscles to become more fatigable. Respiratory muscle fatigue may have deleterious consequences for respiratory homeostasis in vivo. Our results may be relevant to respiratory disorders characterized by recurrent hypoxaemia such as the sleep apnoea syndrome.

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C14 and PC14

Respiratory muscle weakness following chronic intermittent hypoxia during early development in the rat

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It is known that the developing respiratory system is subject to considerable developmental plasticity and certain insults during vulnerable periods of development can induce persistent maladaptive changes. The effects of chronic intermittent hypoxia (CIH) on respiratory muscle function during early development are not known but we hypothesize, based on observations in adult animal models of OSA, that CIH during early life may perturb respiratory muscle function, perhaps permanently. Therefore, we sought to investigate the effects of CIH on respiratory muscle force production during early development.

Litters of Wistar rats together with their dams were placed from birth in hypoxia chambers. The CIH litters received alternating cycles of 90 sec hypoxia (reaching 5% O_2 at the nadir) and 210 sec normoxia (21% O_2) for 8hr/day for 7 days. The control litters were exposed to circulating normoxic gas for 7 days. After 1 week, half of the control and CIH litters were killed humanely under 5% isoflurane. The diaphragm and sternohyoid (pharyngeal dilator) muscles were removed and contractile and endurance properties were examined in tissue baths containing physiological salt solution (PSS) at 35ºC. The remaining half of both sets of litters were left to recover in normoxia for a further week, before functional studies were performed.

CIH-treated rats had a significantly reduced body mass and increased right ventricular mass. CIH caused a significant decrease in sternohyoid and diaphragm isometric twitch force and contraction time (n=8, $P<0.05$), but had no effect on half-relaxation time. CIH caused a significant depression in the sternohyoid force-frequency relationship; peak force was 4.3±0.8 N/cm^2 and 1.6±0.3 N/cm^2 in control and CIH-treated muscles, respectively (n=8, $P<0.0001$). CIH caused a significant decrease in the diaphragm force-frequency relationship; peak force was 13.8±1.0 N/cm^2 and 8.6±1.1 N/cm^2. There was no significant change in the sternohyoid EF_{50} values (i.e. stimulus frequency producing 50% of peak force), however CIH significantly increased diaphragm EF_{50} values; 26±1 Hz and 31±1 Hz in control and CIH-treated rats respectively (n=8, $P<0.01$). Following 1 week recovery in normoxia, the negative inotropic effect of CIH on sternohyoid and diaphragm force persisted (n=8, $P<0.001$).

In summary, CIH during the first week of life had a substantial negative inotropic effect on rat respiratory muscles, which persisted after a recovery period. CIH-induced respiratory muscle weakness could have detrimental effects on respiratory performance and homeostasis. This may have implications for neonatal respiratory disorders associated with CIH.

Supported by the Health Research Board (RP/2008/159) and IRCSET.

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C15

Pulmonary oxidative-nitrosative stress in high-altitude pulmonary hypertension

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When excessive, the increase in pulmonary artery systolic pressure (PASP) at high-altitude (HA) can lead to high-altitude pulmonary oedema (HAPE) with potentially fatal consequences. However, to what extent the pulmonary circulation contributes to the intravascular accumulation of free radicals and subsequent implications for pulmonary hypertension and susceptibility to altitude illness remains to be examined. Therefore, the current study examined if the increase in PASP at HA would be associated with a net trans-pulmonary output of free radicals and corresponding loss of nitric oxide (NO).

Twenty six mountaineers provided central venous and radial arterial samples at low-altitude (LA) and within 20h following active ascent to the Capanna Regina Margherita located at 4559m (HA). PASP was determined by Doppler echocardiography, pulmonary blood flow by inert gas re-breathing and exchange via the Fick principle. Acute mountain sickness (AMS) and high-altitude pulmonary oedema (HAPE) were diagnosed using clinical questionnaires and chest radiography. Electron paramagnetic resonance spectroscopy, ozone-based chemiluminescence and high-sensitivity ELISA were employed for plasma detection of the ascorbate free radical ($A^{\bullet-}$), NO and 3-nitrotyrosine (3-NT) respectively.

Ascent to HA increased PASP from 23 ± 4 to 38 ± 10mmHg ($P < 0.05$) which was more pronounced in subjects diagnosed with (severe) AMS (n = 11) and HAPE (n = 3) compared to subjects who remained free of illness (n = 8). HA increased the arterio-central venous concentration difference resulting in a net trans-pulmonary output or gain of $A^{\bullet-}$ [HA: +715 ± 852 vs. LA: +137 ± 398 arbitrary units÷Gauss/min, $P < 0.05$] and net uptake or loss of (total) NO (HA: -684 ± 661 vs. LA: +621 ± 778 nmol/min, $P < 0.05$). Exchange was more pronounced in AMS and HAPE ($P < 0.05$ vs. Healthy) and correlated against the rise in PASP ($r = 0.69$, $P < 0.05$) and arterial 3-NT ($r = 0.46$, $P < 0.05$).

In conclusion, these findings are the first to suggest that a free radical-mediated reduction in pulmonary NO bioavailability (oxidative-nitrosative stress) may prove the unifying mechanism underlying HA-induced pulmonary hypertension and susceptibility to (AMS and) HAPE. These findings have broader implications for other human pulmonary diseases characterised by arterial hypoxaemia notably COPD.

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C16 and PC16

Pre- and post-ischaemic Apelin-13 effect on cardiac function and APJ receptor expression in rat hearts

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Apelin is the endogenous ligand for the G-protein-coupled APJ receptor. Its gene encodes a 77 aminoacid preprotein with the active sequence in the C-terminal region. Various fragments of apelin have been isolated and classified according to the aminoacid number. Apelin-13 is reported to be the most active fragment. Apelin and APJ have been found in vascular smooth muscle, endothelial cells and cardiomyocytes. Apelin-13 protects rat hearts against ischaemia-reperfusion injury if given at the concentration of 500 nM after ischaemia, but not before. We investigated: a) whether the protection includes an improved mechanical recovery after ischaemia; b) whether increased Apelin-13 concentrations, given before ischaemia, could induce protection; c) whether the protective effect of Apelin-13 given after ischaemia is due to an ischaemia-induced APJ overexpression.

Rats were anaesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) and were killed by decapitation. The hearts were excised and perfused with oxygenated buffer. They were divided in 5 groups: i (control): a 30 min global ischaemia was followed by a 120 min reperfusion. ii: Apelin-13 was given after ischaemia at 500 nM concentration. iii and iv: Apelin-13 was given before ischaemia at 500 and 1000 nM concentration respectively. v: Apelin-13 was not given and reperfusion was stopped at 0, 7, 15, 30 or 120 min. APJ expression was evaluated by RT-PCR and Western blot analyses.

In Groups i-iv left ventricular pressure (LVP) was measured and infarct size was assessed by nitro-blue tetrazolium. Lactate-dehydrogenase (LDH) was tested in the effluent at fixed intervals during reperfusion. Data are expressed as mean±sem and were statistical analyzed by 1-way ANOVA. RT-PCR and Western blot analyses were performed in triplicate for each sample.

In the control group, global ischaemia and reperfusion caused an increase ($p<0.001$) in diastolic LVP 6±1 to 40±9 mmHg without any change in systolic LVP and with a reduction by about 70% of developed pressure. After ischaemia, Apelin-13 reduced infarct size from 54±3% to 26±4% ($p<0.001$) and abolished the increase in diastolic LVP. No effect was obtained when Apelin-13 was infused before ischaemia even at 1000 nM. Total LDH released during reperfusion was proportional to the infarct size. Ischaemia increased both APJ transcription and translation within the first 15 min after ischaemia, i.e. after most of reperfusion injury has taken place.

It is confirmed that apelin-induced protection is not achieved if the compound is given before ischaemia, even at increased concentration. The protection includes an improved post-ischaemic mechanical recovery consisting in the suppression of the contracture due to ischaemia and reperfusion. Finally, the timing of injury and APJ protein expression does not explain why the compound is effective only if given after ischaemia.

This work was supported by Compagnia di San Paolo, Torino.

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C17 and PC17

A sympathetic view of fetal programming of cardiovascular disease

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The link between being small for gestational age and increased risk of adult cardiovascular disease is well established (1). It has been shown that chronic hypoxia *in utero* (CHU) and chronic maternal nutrient deficiencies have distinct effects on vascular function (3). However, it is known that both insults can lead to hypertension, although the origin/s of this are not understood. Endothelial dysfunction has been found in mesenteric arteries of rats subjected to CHU (4). However, equally importantly, increased sympathetic nerve density has been shown in femoral arteries of neonatal chicks subjected to CH in the egg (2), suggesting that neurogenic, as well as endothelial factors may contribute to the increased rates of cardiovascular disease seen in the adult animals.

To address this, we have compared *in vivo* recordings from single sympathetic neurones supplying the Spinotrapezius muscle vasculature in 10 week old male Wistar rats subjected to CHU (12% O_2 from gestational d11-21) to those made in normal (N) rats. Under anaesthesia (Alfaxan, 3-6mg.kg^{-1}.hr^{-1} I.V.), an *in vivo* preparation of the Spinotrapezius muscle was made as described by Marshall (1982, 5). Arterial blood pressure (ABP) and tracheal pressure were recorded via pressure transducers. Nerve recordings were made by applying light suction via a glass microelectrode (~30μm tip) to the surface of an arteriole supplying the spinotrapezius. They were analysed using Spike2 software. Single units were discriminated from multi unit recordings (generally 2-3 units/recording), by using spike shape templates, followed by analysis of principle components.

When breathing 21% O_2 in N_2. mean ABP was similar in N and CHU rats (N: 121±3, CHU: 119±5, mmHg, mean±SEM). 43 single units were discriminated (N: 17 units, N=7, CHU: 26 units, N=6). All units had both cardiac and respiratory rhythm (as assessed by stimulus triggered histogram analysis using the ABP and tracheal pressure traces), confirming they were sympathetic neurones. Mean firing frequency in individual units was similar in N and CHU (0.48±0.09,vs 0.52±0.1, Hz; mean±SEM)

These preliminary results suggest that resting sympathetic nerve activity in individual fibres supplying skeletal muscle vasculature is not affected by CHU. However, if there is increased sympathetic nerve density in CHU, the overall sympathetic tone reaching the resistance arterioles may be increased. Work is ongoing to determine the sympathetic nerve density and to compare responses evoked in MSNA by stimuli such as acute hypoxia and baroreceptor challenge in CHU and N rats.
These new findings have the potential to make a major contribution to our understanding of how adult cardiovascular disease is programmed during development.

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C18 and PC18

Classical model of ventilation applied to exercise in occupational lung disorders

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Introduction. The classical model portrays ventilation as the sum of alveolar and airway deadspace components [1]. The latter is the product of anatomical deadspace and breathing frequency. It is commonly overlooked, yet in healthy shipyard workers the term was significant [2]. Here the model is used with an additional disease-specific term to examine ventilation in men referred for assessment for suspected occupational lung disorders (OLD); they comprised 54 men with coalworkers' pneumoconiosis (CWP), 26 with work-related wheeze (WRW), 11 with diffuse pleural thickening (DPT) and raised ventilation, 14 with diffuse pulmonary fibrosis and 9 with presumed centriacinar emphysema. 44 referred men who were found to have normal function formed a comparison group (CG).

Methods. The subjects completed a respiratory questionnaire (MRC) and lung function assessment by standard methods. Progressive treadmill exercise was used to derive ventilation ($V'exp_{st}$) and respiratory exchange ratio (RER_{st}) at an O_2 uptake of 1.0 l min^{-1} (45 mmol min^{-1}), and tidal volume and respiratory frequency (designated Vt_{30} and fR_{30}) at a ventilation of 30 l min^{-1}. Special care was taken over the derivation of RER_{st} because the relationship was non-linear. The model of $V'exp_{st}$ on RER_{st} and fR_{30} [2] was extended by the inclusion of a disease-specific constant term. Outliers with valid data were not excluded. Significance (*) was at the 5% level.
Results. Mean $V'exp_{st}$ in OLD was 33.1 (range 36.7 to 26.9) l min^{-1} and exceeded that in CG (23.4 l min^{-1})*. In CWP mean values for $V'exp_{st}$, RER_{st} and fR_{30} were respectively 34.6 l min^{-1}, 0.83 and 26 min^{-1}. The corresponding means for the coefficients in CG were 0.77 and 20.3 (min^{-1})*. For these two groups together the extended model yielded:
$V'exp_{st} = 32.2\ RER_{st}^{*} + 0.42\ fR_{30}^{*} + 6.6$ CWP (yes or no)* -9.84 ($R^2 = 0.67$).
The constant, interactions between the variables, also terms for age and smoking were not significant. From the model the factors contributing to increased $V'exp_{st}$ in CWP were alveolar drive (17%), shallow breathing (24%) and deadspace ventilation (59%).
In DPT restriction to lung expansion was not a consistent feature, yet mean fR_{30} was 33.3 min^{-1}, an increase compared with CG *. By contrast in WRW, fR_{30} (mean 17.1 min^{-1}) was reduced*.
Conclusion. Breathing frequency contributes to ventilation under standard conditions in chest disorders as well as in health. Thus it should be taken into account whenever ventilation is an issue. In DPT, unexplained tachypnoea is often assumed to be functional. The present results suggest it could be of reflex origin. This possibility might usefully be explored.

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Medical Research Council

Health and Safety Executive

Dr DJ Chinn provided technical support

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

C19 and PC19

The Cardiorespiratory Reflex Effects of Stimulation of Superior Laryngeal Nerve Paraganglia in the Urethane Anaesthetised Rat

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The cardio-respiratory effects of carotid body (CB) and aortic body (AB) stimulation are well described[1]. Superior laryngeal nerve (SLN) paraganglia have been shown to be structurally similar to the glomus cells of the CB and are excited by both cyanide and hypoxia *in vitro*[2]. The aim of the present study was to elucidate the reflex action of these paraganglia on breathing and the circulation.

Experiments were carried out on Wistar rats (body mass: 272 ± 38 g, n=6) anaesthetised with 20% urethane (1.5g kg^{-1} i.p.). The right external jugular vein was cannulated for administration of sodium cyanide (NaCN) in normal saline. Both SLNs were cut close to the larynx but preserving the region of bifurcation, an area that reliably contains glomus tissue, with the free ends placed in small 20μl tissue baths containing Tyrode's solution. The CB and AB were first stimulated by injecting 20-40μl NaCN through the pre-filled jugular vein cannula. SLN paraganglia were then selectively stimulated by adding 10μl of 0.1mg ml^{-1} NaCN to the tissue baths. These two tests were then carried out simultaneously to assess potential interaction between the reflex responses. At the end of the experiment both SLNs were cut and the tests repeated to ensure that responses were due to activation of the SLNs and not NaCN leakage from the bath.

The initial mean cardiorespiratory values were: respiratory frequency, 86 ± 23 breaths min^{-1}; heart rate, 415 ± 40 beats min^{-1}; mean arterial pressure, 93 ± 14 mmHg; hindlimb conductance 0.012 ± 0.007 ml $min^{-1}mmHg^{-1}$; arterial blood pH 7.41 ± 0.02; P_{O2}, 95 ± 13 mmHg; P_{CO2}, 38 ± 2 mmHg; haematocrit, 50 ± 4%.

Intra-cardiac injection of NaCN caused tachypnoea (increasing respiratory frequency by 48 ± 29%) and hindlimb vasodilatation. The response resembled that previously reported in the saffan anaesthetised rat[3]. Of interest, the SLN paraganglia had no significant effect on breathing or heart rate but caused a significant hindlimb conductance increase and a greater vasodepressor response (-10 ± 6%) compared to stimulation of all the other chemoreceptor sites. The effect on hindlimb conductance was additive when SLN stimulation was conducted at the same time as systemic cyanide injection. Further experiments are required to determine whether the SLN chemoreflex evokes a pattern consistent with a hypothalamic defence response, Sherrington's pseudoaffective response or a thermoregulatory response. All values reported are mean ± S.D.

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

PC20

Investigation of strain response of cardiac muscle by fluorescence lifetime imaging microscopy of the myosin essential light chain

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We applied fluorescence lifetime imaging microscopy (FLIM) to map the microenvironment of the myosin essential light chain (ELC) in cardiac and skeletal muscle fibres. Four ELC mutants containing single cysteine residue at different positions in the C-terminal half were labeled with 7-diethylamino-3-((((2-iodoacetamido)ethyl)amino)carbonyl)coumarin. First, the mutants were introduced into permeabilized rabbit psoas muscle fibres under conditions that favour exchange with the native light chain. The fibres were examined under Leica SP5 microscope equipped with a time-correlated single photon counting module. The fluorescence decay in each pixel of FLIM images was fitted with a single exponential and the mean fluorescence lifetime in the A-band regions was found. The mean lifetimes in relaxed fibres were 1.44, 1.64, 1.73 and 1.83 ns, for ELC-142, ELC-127, ELC-160 and ELC-180 respectively. When in rigor, lifetime increased significantly for all label positions, which may be related to a change in conformation of ELC with respect to the heavy chain. However, when 1% stretch was applied to the rigor fibres, the lifetime of ELC-127 and ELC-180 decreased, but did not change in cases of ELC-142 and ELC-160, where the labels are located at the opposite ends of helix F. We applied a similar protocol to exchange ELC mutants in the isolated rat trabeculae. Confocal microscopy confirmed high efficiency and specificity of exchange in the cardiac muscle. The fluorescence lifetime measurements of ELC-180 in relaxed and rigor trabeculae gave similar values to those in psoas fibres, demonstrating the viability of FLIM approach to study the strain response of the cardiac muscle myosin.

Supported by BBSRC.

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PC21

AMPK-dependent regulation of BKCa channels is splice variant specific

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Large conductance voltage- and calcium-activated potassium (BKCa) channels are phosphorylated and inhibited by AMP-activated protein kinase (AMPK), which mediates, in part, the response to hypoxia of the oxygen-sensing carotid body type I cells [1]. However, hypoxia-response coupling is tissue specific, despite the fact that BKCa channels are widely expressed across a variety of cell types that do not respond to hypoxia. We therefore investigated the possibility that AMPK-dependent regulation of BKCa may be conferred by the selective expression of splice variants of the pore-forming α-subunits of BKCa channels [2]. All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986; carotid bodies were isolated as previously described [1]. Bacterially-derived recombinant AMPK phosphorylated and inhibited BKCa channels (transiently expressed in HEK-293 cells) in a manner that was splice variant-specific. Current inhibition was observed in the ZERO variant during intracellular dialysis with thiophosphorylated AMPK which peaked at 36 ± 7 % inhibition ($P<0.01$) after 5 ± 0.4 min (n = 8). The inclusion of the stress-regulated exon (STREX) increased the stoichiometry of AMPK-dependent phosphorylation of the BKCa α-subunit from 1 to 2 moles/mole when compared with the ZERO variant. This increased phosphorylation of STREX prevented AMPK-dependent inhibition of the STREX variant BKCa current. Point mutation of a single serine (Ser-657) to alanine within the STREX insert reversed the increase in phosphorylation stoichiometry to 1 and recovered channel inhibition by AMPK, which peaked at 30 ± 9 % ($P<0.01$) after 6 ± 0.8 min (n=3). RT-PCR showed that carotid body type I cells express only the ZERO, but not STREX, variant, and intracellular dialysis of recombinant AMPK attenuated BKCa currents in these cells (35% ± 2; n = 6). Such conditional regulation of BKCa channel splice variants by AMPK may therefore contribute to the cell-specific nature of hypoxia-response coupling observed in carotid body type I cells and other oxygen-sensing cells.

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This work was funded by a Wellcome Trust Programme Grant (WT081195AIA)

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PC22

The effects of Des-Arg^9-bradykinin on isolated rat hearts

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It is possible that Des-Arg^9-bradykinin, the active metabolite of bradykinin and selective bradykinin B1 receptor agonist contributes to the regulation of cardiovascular functions, but little is known about the role of this peptide on coronary vascular tone and heart rate. Furthermore, the possible action of Des-Arg^9-bradykinin on contractile force, +dP/dtmax and -dP/dtmin has not been investigated. Therefore, I studied the probable effects of the peptide on coronary perfusion pressure, heart rate, left ventricular developed pressure (the index of cardiac contractility), +dP/dtmax (the maximal rate of pressure development) and the -dP/dtmin (the maximal rate of pressure fall) in rat hearts. The hearts were isolated under light ether anesthesia and perfused under constant flow conditions (12 ml/min) with modified Krebs-Hanseleit solution and 10, 100 and 1000 nM Des-Arg^9-bradykinin infused to the hearts for 30 min. Ten and 100 nM Des-Arg^9-bradykinin did not change the heart rate but, 1000 nM increased significantly ($p<0.01$). All the doses of Des-Arg9-bradykinin caused a significant reduction in perfusion pressure, developed pressure, +dP/dtmax and -dP/dtmin ($p<0.001$ for all). There is sufficient evidence from this study that Des-Arg^9-bradykinin possesses vasodilatory efficacy with a modest negative inotropic action. These results might suggest that Des-Arg9-bradykinin can also decrease contraction and relaxation rate. Furthermore, higher dose of the peptide may exert tachycardic action.

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PC23

The role of superoxide (O_2^-) in muscle vasodilatation in chronically hypoxic (CH) rats

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Previously we showed in normoxic (N) rats that vasodilatation evoked by 5 min acute hypoxia (8%O_2) is reduced by the xanthine oxidase (XO) inhibitor oxypurinol and potentiated by infusion of superoxide dismutase (SOD)[1]. 50% of the vasodilatation evoked by 8%O_2 is mediated by adenosine[2] so we proposed that during hypoxia,

H_2O_2 produced by SOD from O_2^- generated from the breakdown of adenosine by XO, contributes to vasodilatation. As chronic hypoxia (CH) increases oxidative stress we investigated the role of this pathway in CH rats.

CH male Wistar rats breathed 12%O_2 for 1, 3 and 7 days prior to acute experiments. In anaesthesized (Alfaxan 12mg.kg^{-1}.hr^{-1}) normoxic (N) and 1, 3 and 7CH rats, integrated femoral vascular conductance (IntFVC) was measured 5 min before and during 8%O_2. In Group 1 (n=10, 7, 10 & 9) the response to 8%O_2 was measured before and after the XO inhibitor oxypurinol and in Group 2 (n=9 each) the SOD inhibitor DETC. In a further group (n=6 each) of N, 1, 3 and 7CH rats XO and SOD activity was assayed in Tibialis Anterior (TA) muscle.

In N rats, XO inhibition increased baseline IntFVC (3.0±0.2 vs 3.3±0.2 CU*; mean±SEM; *P<0.05 Student's paired t test) but decreased the response to 8%O_2 (3.4±0.4 vs 2.6±0.3 CU*). In contrast, in CH rats, oxypurinol had no effect on baseline IntFVC or the response to 8%O_2. XO activity was comparable in CH and N TA. In N rats, SOD inhibition increased baseline IntFVC (1.9±0.2 vs 2.5±0.2 CU*) but had no effect on the response to 8%O_2. However, in CH rats, DETC had no effect on baselines but reduced the IntFVC response to 8%O_2 (1CH 1.9±0.2 vs 0.9±0.2*; 3CH 1.8±0.2 vs 0.9±0.3*; 7CH 1.9±0.2 vs 0.9±0.4 CU*). Further, in TA, SOD activity was decreased by CH (N 669±24 vs 1CH 454± 18*; 3CH 390±28*; 7CH 535±16* U.ml^{-1}.mg protein^{-1}).

In contrast to N rats, XO inhibition with oxypurinol had no effect on the vasodilatation evoked by 8%O_2 in CH rats suggesting that O_2^- produced from the breakdown of adenosine no longer contributes to vasodilatation via the production of H_2O_2 by SOD. However, inhibition of SOD reduced the vasodilatation evoked by 8%O_2 in CH rats suggesting that O_2^-, from sources other than adenosine breakdown by XO, can be dismuted to H_2O_2. Other sources of O_2^-, which may be important in hypoxia, include Complexes I and III of the mitochondrial respiratory chain, NADPH oxidase and metabolism of arachidonic acid by cyclooxygenase. In addition, the change in the contribution of H_2O_2 to vasodilatation evoked by 8%O_2 in CH rats cannot be explained by a change in SOD activity. It may be that measuring activity in whole muscle homogenates masks any changes in SOD activity in the vasculature. Further studies will look at the effect of CH on SOD expression and activity specifically in the vasculature.

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This work was supported by the British Heart Foundation.

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

PC24

Proliposome technology as an approach to generating anticancer liposomes

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Cancer is one of the major causes of death in spite of a substantial increase in understanding of the molecular mechanism behind its occurrence. Glioma is the type of brain tumour which arises in the glial cells of the brain. Glioma is categorized into three areas namely: astrocytoma, oligodendroglioma and astro-oligodendroglioma (mixture of both). Every year in UK approximately 2% of the brain tumour patient are diagnosed with glioma therefore, it is very important to find a remedy for treatment of this cancer. One of the interesting properties of liposomes is their natural ability to target tumour. It is the most extensively used biodegradable carriers and regarded as the most promising ones in drug delivery (Katare et al., 1990; Crommelin and Sindelar, 2002). Liposomes are very safe adjuvants as they are manufactured from phospholipids that are similar to the phospholipids present in the biological membranes. Unfortunately, liposomes are chemically and physically unstable and are difficult to manufacture on a large scale using the conventional method of preparation (thin film method). However, these stability problems can be avoided by formulating liposomes using the proliposome method. Proliposomes are of two types, namely, particulate-based proliposomes and solvent-based proliposomes. Particulate proliposomes are dry, free-flowing carrier particles coated with phospholipids that generate liposomes on addition of aqueous phase (Payne et al 1986). Secondly, a solvent based proliposome method offers a relatively simple means of generating liposomes with a high entrapment of hydrophilic agents, by the addition of aqueous phase to a concentrated alcoholic solution of phospholipids (Perrett et al 1991). The aim of our project is to use phospholipids to manufacture solvent-based proliposomes which can be used to generate phospholipid vesicles (liposomes) when aqueous phase (e.g. water) is added. Various phospholipids will be investigated and the resultant size of the vesicles will be compared with the conventional method of producing liposomes. The model anticancer drugs will be entrapped in liposomes and the efficacy of anticancer-liposome formulations on the viability of glioma cell lines and molecular mechanism of the cell death will be investigated.

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PC25

The actomyosin ATPase response to stretches and releases in rat cardiac trabeculae

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Cross-bridges exist in a number of transient states, each differing in their biochemical and structural properties, during each cycle of ATP hydrolysis. Techniques pioneered in our laboratory allow measurement, in real time, of changes in the distribution of cross-bridge states as a function of physiological (e.g. cross-bridge strain) and physical (e.g. temperature) determinants of muscle function (1, 2). Our aim here was to use these methods to characterize the mechanoenergetic properties of rat cardiac trabeculae.

Inorganic phosphate (P_i) release, and therefore actomyosin (AM) ATPase rate, was determined during contractions of Triton-permeabilized trabeculae at 20∞C. Female Sprague-Dawley rats were killed by cervical dislocation and thin, unbranched, uniform trabeculae dissected from the left ventricle. Initial sarcomere length (SL) was set to 1.9μm by laser diffraction, contraction was elicited by laser-flash photolysis of NPE-caged ATP (P^3-l(2-nitrophenyl)ethyladenosine-5'-triphosphate) and time-resolved P_i release monitored using the fluorescent protein MDCC-PBP, (N-(2[1-maleimidyl]ethyl)-7-diethylamino-coumarin-3-carboxamide phosphate binding protein.

Isometric force produced during maximal activation (32μM Ca^{2+}; n=5) was 50.8±6.4 kNm^{-2}. The ATPase rate during the first AM turnover (assuming 150 μM myosin heads) was 12.1±1.0s^{-1}, which decreased to a steady state of 6.5±0.4s^{-1} once the isometric plateau had been reached. This is comparable to previous steady state measurements, using an NADH-linked enzyme assay for the rate of ADP appearance, of 10s^{-1} (3) and 3.3s^{-1} (4). At submaximal activation (3μM Ca^{2+}; n=6) isometric force was reduced to 43.2±5.2 kNm^{-2}, initial ATPase rate was 13.2±1.5s^{-1} and steady state ATPase rate was 6.72±0.4s^{-1}.

During lengthening (5% of muscle length, L_0; 0.5 L_0s^{-1}; SL=2.1μm) the rate of P_i release, and therefore AM ATPase rate decreased to 1.6±0.9s^{-1} at maximal activation (n=5) and 3.0±0.7s^{-1} at submaximal activation (n=6). After stretch, the ATPase rate at SL 2.1μm was 4.0±0.3s^{-1} at maximal activation and 3.6±0.2s^{-1} at submaximal activation. Shortening (5% L_0; 0.5 L_0s^{-1}) caused a transient increase in P_i release rate to 7.5±1.7s^{-1} at maximal activation and 7.0±0.4s^{-1} at submaximal activation. ATPase rate declines slowly throughout the experiment due to ADP accumulation

and ATP depletion, explaining only a small increase in ATPase rate during shortening compared with steady state.
Our previous work has shown similar responses to lengthening and shortening of permeabilized psoas fibres. The slower P_i release during the stretch is due to the forcible detachment of cross-bridges through a reversal of the power stroke, resulting in less ATP being hydrolysed. The faster P_i release rate during shortening is brought about by acceleration of the rate of ADP release from AM at low strain, resulting in faster cross-bridge cycling.

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PC26

Prenatal hypoxia alters NO function in skeletal muscle of anaesthetised adult female rats

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The prenatal environment has been shown to cause a variety of effects in the offspring that persist into adulthood[1]. This phenomenon of developmental programming often results in altered vascular function leading to hypertension. We have previously demonstrated that chronic hypoxia in utero (CHU) induces changes in vascular regulation in male offspring (CHUM) by altering the functional role of NO[2], however, there is no information on how female offspring (CHUF) are affected. It is also generally accepted that females have a lower risk of developing cardiovascular disease than males[3] but it is not known how this is affected by developmental programming. Pregnant female Wistar rats were housed in a hypoxic chamber (maintained at 12% O_2) during days 10-20 of pregnancy. Dams were then moved into air for the remainder of pregnancy and the offspring were reared in air. Acute experiments were performed, under anaesthesia (Alfaxan 12mg.kg^{-1}.hr^{-1}), on normal (N) and CHUF rats (9-10 weeks old) to assess the cardiovascular responses to acute hypoxia (breathing 8% O_2) under 3 conditions: Control, after blocking NO synthase (L-NAME) and after restoring baseline vascular tone with NO donor (SNP). Vasodilator responses were assessed in the hindlimb by changes in integrated femoral vascular conductance (IntFVC). Samples of Tibialis Anterior (TA) were taken for eNOS expression by Western blotting.
Arterial blood pressure (ABP) in normoxia was higher in CHUF than N rats (127±3 vs 112±3 mmHg; mean±SEM; $P<0.01$). Breathing 8% O_2 induced a fall in ABP and skeletal muscle dilatation in both groups, but the increase in IntFVC was significantly

smaller in CHUF (0.8±0.2 vs 2.2±0.6 CU in N rats; P<0.05). L-NAME induced a significant increase in ABP only in N rats, and attenuated the hypoxia-induced increase in IntFVC in N (0.7±0.1 CU; P<0.05) but not CHUF rats (0.6±0.1 CU). Infusion of SNP restored baseline IntFVC in both groups. Moreover, the hypoxia-induced increase in IntFVC was restored in N (2.2±0.3 CU). but augmented in CHUF (2.7±0.6 CU; P<0.001). No differences were found between N and CHUF in eNOS expression in TA muscle.

The higher baseline ABP and lack of effect of L-NAME in CHUF suggests they exhibit endothelial dysfunction resulting from decreased NO bioavailability; our data suggest this does not result from reduced eNOS protein expression. Importantly, L-NAME did affect baseline ABP in CHUM[2]. That exogenous NO during NOS inhibition augmented the hypoxic dilatation in CHUF raises the possibility that their decreased NO availability results from O_2^- generated by eNOS which attenuates the role of NO in hypoxia-induced muscle vasodilatation. In conclusion, developmental hypoxia provides a programming stimulus that affects endothelial regulation of vascular tone in ways that are gender specific.

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

PC27

Adenosine potentiates human mast cell fibrinolytic activity

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Mast cells are mostly known for their important role in the development of inflammation and allergies. They also contribute to the development of cardiovascular diseases including thrombosis and arthrosclerosis. For example, by secreting tissue-type plasminogen activator (tPA), mast cell can initiate fibrinolysis.

The purpose of this study was to determine whether adenosine, a potent mast cell activator, can modulate mast cell fibrinolytic activity. HLMC cells (human mast cell line) were dispersed from macroscopically normal lung obtained within 1 h of resection for lung cancer. All patients gave written informed consent, and the study was approved by the Leicestershire Research Ethics Committee. We found that 100 μM adenosine (2h at 37C) significantly increased mast cell tPA activity (by 58.6±3.5% for HMC-1 and 55±6.8% for HLMC) and tPA transcript expression level (by 6.4 folds for HMC-1 and 3.6 folds for HLMC). Adenosine deaminase abolished this potentiation. Real-time PCR revealed that HMC-1 predominantly expressed A2A and A2B receptors while HLMC abundantly expressed A2A, A2B and A3 subtypes. ZM241385

(1 μM), an A2A antagonist, significantly reduced adenosine induced mast cell tPA activity potentiation by 86.2% in HMC-1 (65% in HLMC); an inhibition similar (87.4% in HMC-1) to which obtained with a cocktail of antagonists for A1, A2A, A2B and A3 receptors (each at 1 μM). We also assessed mast cells fibrinolytic properties using a clot lysis assay (measuring fibrin clot lysis 10 hours after the full formation of a tissue factor-induced fibrin clot). Supernatants of HMC-1 treated for 24h with adenosine (100 μM) significantly increased clot lysis by 92.6%. This effect was abolished by ZM241385.
In conclusion, our results show that adenosine potentiates mast cell fibrinolytic activity mainly through the A2A adenosine receptor.

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

PC28

Respiratory modulation of muscle sympathetic nerve activity in patients with hypertension

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Since the earliest direct recordings, it has been known that sympathetic nerve activity (SNA) shows respiratory modulation. During normal breathing in healthy humans, sympathetic vasoconstrictor outflow to the vasculature of the skeletal muscle reaches a nadir at peak inspiration and peaks during expiration (Dempsey et al. 2002). Notably, recent work has suggested that altered respiratory–sympathetic coupling is a causal factor in producing the increased vascular resistance and blood pressure (BP) in the spontaneously hypertensive rat (Simms et al. 2009). The aim of the present study was to begin to elucidate the respiratory modulation of muscle SNA in human hypertension.
Ten hypertensive patients (62±2 years; body mass index [BMI] 27±1 kg/m^2; mean ± S.E.M.; 5 men) and six age and BMI matched normotensive control subjects (59±2 years; BMI 27±2 kg/m^2; 2 men) rested in a supine position, breathing at a eupnoeic frequency, while heart rate (HR; ECG), arterial BP (automated sphygmomanometry and finger photoplethysmography) and respiratory movements (strain gauge pneumobelt) were continuously monitored. Multiunit recordings of postganglionic muscle SNA were obtained from the peroneal nerve using the microneurography technique. To examine respiratory–sympathetic modulation, respiratory and muscle SNA signals were first normalised to unit variance and mean-removed, followed by calculation of the cross-correlation function. This resulted in a correlation coefficient (i.e., r value) varying between 1 and -1. A peak (or trough) at positive time lag indi-

cates respiration leading the muscle SNA signal. Cross correlations were averaged across subjects, allowing for calculation of S.E.M. and 95% confidence intervals.
As expected, mean BP was higher in hypertensive group (114±4 vs. 83±3 mmHg, hypertensives vs. control; P<0.05). In contrast, HR (62±3 vs. 67±7 beats/min^{-1}) and muscle SNA (57±4 vs. 54±7 bursts/100 heartbeats^{-1}) were similar in the hypertensive and control groups (P>0.05). In the control group, the cross correlation function displayed a significant negative r value of -0.16±0.02 at positive lag of 538 ms, indicative of an inhibitory effect of respiration on muscle SNA. In the hypertensive group, this inhibitory relationship was significantly attenuated (P<0.05; r value 0.01±0.03 at positive lag of 636 ms).
These preliminary data indicate that the inhibitory effect of respiration on muscle SNA is suppressed in older patients with hypertension. Further studies are required to elucidate the underlying mechanisms and in particular the potential changes in peripheral afferent feedback versus central modulation.

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

PC29

Anandamide-induced currents in rat cultured primary sensory neurons

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Anandamide (AEA) produces dual effect on nociceptive primary sensor neurons; at 10-30nM a cannabinoid (CB) 1 receptor-mediated inhibitory, while above 1μM a transient receptor potential vanilloid type 1 ion channel (TRPV1)-mediated excitatory effect. Here, we characterised currents evoked by 1-30μM AEA in rat cultured primary sensory neurons.
Conventional whole-cell recordings were done at 37oC from neurons cultured for 2-5 days in the presence of 50ng/ml nerve growth factor. The bath and pipette solutions contained (in mM): NaCl 150; KCl 5; HEPES 10; glucose 10; MgCl2 2; CaCl2 2; and NaCl 5; KCl 150; HEPES 10; MgCl2 2, EDTA 1, respectively. The holding potential was -60mV. Statistical analysis was done by the Student t-test or ANOVA, as appropriate. Following ANOVA, the significance of the differences was studied by the Fisher test. Differences were regarded as significant at p>0.05. Results are expressed as mean±SEM, n refers to the number of neurons.
AEA induced concentration-dependent inward currents in a sub-population of primary sensory neurons with an EC50 of 6.7μM. All neurons, which were responsive to 10μM AEA (n=14), were also responsive to 500nM capsaicin that selectively and specifically activates TRPV1. However, only ~3/4 of the cells, which responded to 500nM capsaicin responded also to 10μM AEA. The amplitudes of the AEA- and cap-

saicin-evoked responses showed only a weak correlation (R2=0.28; p=0.048). Further, the amplitude of the capsaicin-induced responses was significantly larger in the dual-responsive (-3.5±0.5nA, n=14) than in the capsaicin-only-responsive neurons (-1±0.3nA, n=4).
The 10μM AEA-evoked currents were inhibited by the TRPV1 antagonist capsazepine (10μM) in all the 3 cells we tested. Further, the reversal potential of the AEA-evoked currents, were similar to that of the capsaicin-evoked responses (-0.64±3.5mV (n=5) and -0.82±2.8mV (n=9) for capsaicin and AEA, respectively).
Addition of the CB1 receptor antagonist rimonabant (200nM) to the bath solution resulted in a significant, ~30%-50% reduction in the efficacy of AEA at 3μM-30μM. However, superfusion of cells with rimonabant (200nM) for 2 minutes between two consecutive AEA (10μM) applications resulted in a significant increase (~50%) in the AEA-evoked response. Repeated application of 10μM AEA in drug-free bath solution, unlike that of 500nM capsaicin, produced no tachyphylaxis.
These data suggest that AEA- and capsaicin-evoked activations of TRPV1 are different. Further, these data also suggest that the CB1 receptor and TRPV1 are engaged in a complex cross-talk in primary sensory neurons. AEA through that corsstalk may play a significant role in regulating the activity and excitability of nociceptive primary sensory neurons and nociceptive input into the central nervous system.

Jie Chen and Marta Mlynarczyk contributed equally to this work

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PC30

The effects of hypoxia on ATP release from Human Umbilical Artery and Vein endothelial cells (HUAEC, HUVEC)

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ATP can act in a paracrin/autocrine fashion on P2Y receptors on endothelial cells (ECs) to elicit release of nitric oxide and prostaglandins, which cause dilatation of the vascular smooth muscle (VSM). However, ATP can also act directly on P2X receptors on VSM to cause contraction (Burnstock, 1990) and in human umbilical artery VSM cells, ATP can induce $[Ca^{2+}]_i$ oscillations that are generally considered to induce vasomotion (Meng et al., 2007). A hypoxic intrauterine environment is associated with poor fetal O_2 and nutrient delivery and leads to disorders such as intrauterine growth retardation and pre-eclampsia. Further, such conditions are associated with increased vasomotion (Hempstock et al., 2003). Thus, the question arises as to whether hypoxia can induce release of ATP from the EC of umbilical vessels.
In the present study, EC were freshly isolated from human umbilical arteries (HUAEC) and veins (HUVEC). (Luu et al., 2010). These were separately grown on culture inserts with pore size of 0.4 μm and pore density of 2.0 ± 0.2 x 106 / cm^2. After rinsing with

sterile PBS, they were placed in fresh culture medium and then exposed to 1% O_2, 5% CO_2, balanced with N_2 at 37 ∞C for 30 minutes (hypoxia), or kept in normal culture conditions (normoxia). Triplicates of cells from three individual donors were used for each series of experiments. Samples of 50 or 100 μl of medium were taken from the apical and the basolateral side of each culture insert. These were then assayed for ATP by using the highly sensitive luciferin-luciferance reaction (CellTiter-Glo® Reagent from Promega). The strength of luminescence signal was recorded and ATP concentration was calculated from a standard curve generated for each series of experiments. Statistical analysis was carried out using Student's t-test.
There was no difference in ATP released in normoxia and hypoxia from HUAEC (Apical: 1.0% O_2: 21% O_2: 11.0 ± 3.33 nM; 14.6 ± 3.33 nM; P= 0.18. N=6, where N = number of cords). Basolateral: 21% O_2: 0.37 ± 0.033 nM; 1.0% O_2: 0.42 ± 0.075 nM. P= 0.20. N=6). However, hypoxia for 30 mins significantly increased ATP release from both the apical (21% O_2: 0.435 ± 0.08 nM; 1.0% O_2: 3.04 ± 0.91 nM. P= 0.01. N=3) and basolateral (21% O_2: 0.04 ± 0.001 nM; 1.0% O_2: 0.11 ± 0.01 nM. P< 0.001. N=3) side of HUVEC.
These results indicate that acute hypoxia can induce ATP release from the basolateral and especially from the apical surface of HUVEC, but not from HUAEC. This leads us to propose that a fall in pO_2 in the umbilical vein that carries blood to the fetus from the placenta may be a mechanism by which ATP can be released and act in a paracrine way to induce vasomotion.

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British Heart Foundation

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PC31

Cellular mechanism of action of α-momorcharin, β-momorcharin and α,β-momorcharin in cell viability of cancer cell lines

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A multitude of plants have been used extensively for the treatment of cancers throughout the world. In fact, in many parts of the world especially in poor countries, this may be the only form of cancer therapy. Much research has been focused on the scientific evaluation of traditional anti-cancer drug from the tropical plant, Momordica charantia (MC) which has been used frequently as an anti-cancer agent

1. The green leaves and fruits, the seeds and stems of MC composed of many different proteins and steroids that are chemically active. One such protein is α,β-momorcharin which possesses anti-cancer and anti-HIV properties 2,3. This study investigated the anti-cancer effects and the cellular mechanisms of action of different concentrations of α,β-momocharin (200 μM - 800 μM) on the viability of 1321N1, Gos-3, U87-MG and Weri Rb1, Sk Mel, Corl -23 cancer cell lines compared to normal healthy L6 muscle cell line following incubation for 24 hr using 2500 cells in each 200 μl 96-well plates. The effect of different concentrations (100 μM - 400 μM) of the crude water and methanol soluble extract of MC was also investigated for comparison. The cell viability was measured using MTS assay kit. Caspase–3 activity was measured by caspase kit (Sigma) while cytochrome –c release was measured by cytochrome–c kit (Sigma), respectively for every 8 hr, 16 hr and 24 hr. Initial results have shown that either α, β momocharin or the crude water and methanol soluble extract of MC can evoke significant dose- dependent ($P<0.05$; Student's t-test) decreases in the viability of each cell line tested compared to untreated cells of each cancer cell line. In 1321N1, Gos-3, U87-MG and Weri Rd1, Sk Mel, Corl -23 cell lines, α,β-momorcharin (800 μM) evoked significant ($P<0.05$) increases in caspase–3 activity compared to untreated cell lines. However, these values were significantly ($P<0.05$) less than the caspase control value. Similarly, α,β-momorcharin elicited significant ($P<0.05$) increases in cytochrome–c activity and in intra cellular free calcium concentrations [Ca2+]i in Fura-2 AM loaded cells in the six different cancer cell lines compared to the positive control (normal level). Similar effects were obtained with the crude water and methanol soluble extract employing a dose of (400 μM). In contrast, either α,β-momorcharin or crude extract had no significant effect on cell viability or on the activity of either caspase–3 or cytochrome–c in normal L6 muscle cell line although there was a small increase in [Ca2+]i. Together, the results have indicated that both α,β-momorcharin and crude extract of MC can exert their anti-cancer effect on cancer cell lines by increasing cytochrome –c activity and [Ca2+]i suggesting the involvement of apoptosis in glioma cell death.

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PC33

Sternohyoid muscle function and MHC isoform expression in normoxic and chronically hypoxic rats during postnatal development

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The upper airway muscles play a pivotal role in preventing airway collapse. Postnatal maturation is associated with major histochemical and biochemical changes in skeletal muscle which correlate with changes in muscle contractility. Perturbations to respiratory muscle during development such as exposure to chronic hypoxia (CH) may interfere with the normal maturational process. The functional/structural phenotype of the sternohyoid (SH) muscle during the neonatal period has not yet been classified and the effects of chronic hypoxia on respiratory muscles are largely unknown. Rat pups were raised under normoxic or hypoxic conditions at various postnatal ages during development (P9-P60). Animals were killed humanely by cervical dislocation and the paired SH muscles were surgically removed and cut into strips. SH contractile and endurance properties were assessed in vitro. Muscle fibre oxidative capacity was assessed by measurement of succinate dehydrogenase (SDH) activity. Myosin heavy chain (MHC) isoform expression was determined using monoclonal antibodies that identify MHC slow, 2a, 2x and 2b fibres. SH had significantly faster contractile kinetics and increased specific tension with advancing age (2.3 ± 0.4 vs. 11.7 ± 1.3 N/cm^2, (mean ± SEM) peak tetanic force P9 (n=5) vs. P60 (n=8), $P<0.05$, one-way ANOVA. We observed dramatic age-related increases in muscle fatiguability. SH 5 minute fatigue index was 105 ± 5% vs. 24 ± 2 %, (% of initial force) P9 vs. P60, $P<0.05$. There was no age-related change in SH oxidative capacity. Muscle contractility was largely determined by developmental changes in MHC isoform expression. Areal density of MHC slow fibres was 10 ± 2 vs. 2 ± 1 %, P9 vs. P60 ($P<0.05$) whereas areal density of MHC 2b fibres was 1 ± 1 vs. 59 ± 3 %, P9 (n=6) vs. P60 (n=9), $P<0.05$. CH (450mmHg ambient pressure) increased SH muscle force in animals exposed to hypoxia for one week in early but not late development (e.g. peak force was 4.6 ± 1.1 vs. 8.2 ± 1.2 N/cm^2 control (n=6) vs. CH (n=8), $P<0.05$ at P19 and 8.2 ± 1.3 vs. 10.6 ± 1.6 N/cm^2 at P39 control (n=8) vs. CH (n=6). Functional remodelling in SH following CH was not attributable to changes in muscle oxidative capacity or MHC isoform expression. This study characterises the phenotypic profile of the SH from the neonatal period into adulthood. The results indicate that in rats the SH undergoes dramatic structural and functional remodelling in the first 2 months of postnatal life. Slow to fast fibre type transitions and decreased muscle endurance increases the vulnerability of the upper airway to collapse. Hypoxia-induced muscle plasticity may have implications for the control of airway patency in vivo.

Supported by the Health Research Board of Ireland and University College Dublin

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PC34

Ventilatory effects of chronic intermittent hypoxia in the rat

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Sleep-disordered breathing (SDB) is prevalent and is associated with significant cardiovascular, metabolic and neurocognitive morbidities. Chronic intermittent hypoxia (CIH) is a hallmark feature of SDB due to recurrent pauses in ventilation (apnoea). CIH has been shown to elicit plasticity at multiple levels of the respiratory control system, with reports of both adaptive and maladaptive consequences for respiratory homeostasis. We sought to further characterise the effect of chronic intermittent hypoxia on the control of breathing in unanaesthetised freely-behaving male rats using whole-body plethysmography.

Adult male (n=14) Wistar rats were exposed to alternating periods of N_2 and air for 90s each, for 8hrs a day for 3 days (CIH) group (5% O_2 at nadir; $SaO_2 \tilde{} 80\%$). The sham group were subject to alternating cycles of air/air, under identical experimental conditions.

Following treatment, baseline normoxic ventilation and ventilatory responses to acute hypoxia ($F_iO_2 = 0.10$, 20 mins) and hypercapnia ($F_iCO_2 = 0.05$, 10 mins) were assessed. Ventilation following exposure to acute intermittent hypoxia (AIH), consisting of 5 mins hypoxia ($F_iO_2 = 0.10$) and 7 mins normoxia (breathing room air) for 10 cycles, was also assessed in both groups.

Baseline normoxic ventilation was significantly increased in CIH-treated rats compared to sham animals [57.3 ± 1.7 vs. 71.8 ± 1.8, mean±SEM, V_E; ml/min/100g body weight, sham (n=7) vs. CIH (n=7), Student's t test, $p<0.0001$]. Neither hypoxic [60 ± 7% vs.45 ± 11%, % change from baseline for sham vs. CIH] nor hypercapnic [73 ± 8% vs.89 ± 15%, % change from baseline, sham vs. CIH] ventilatory responses were affected by 3 days of CIH. Sham animals displayed facilitated breathing immediately following exposure to AIH [57.3 ± 1.7 vs. 61.3 ± 1.5 ml/min/100g before vs. immediately after AIH, Student's paired t test $p = 0.0466$]; this persisted for up to one hour post-AIH [66 ± 3.7 ml/min/100g]. Unlike sham animals, CIH-treated animals did not display further enhanced breathing following AIH [71.8 ± 1.8 vs. 70.2 ± 2.3 ml/min/100g]. There was no evidence of enhanced breathing 60min post-AIH in CIH-treated animals [70 ± 4.0 ml/min/100g].

We have shown that CIH – a dominant feature of SDB in humans – significantly affects the respiratory control system increasing normoxic breathing in unanaesthetised rats during quiet rest. CIH did alter hypoxic or hypercapnic ventilatory sensitivity. Long-lasting facilitation of breathing may have maladaptive consequences for respiratory homeostasis.

Funded by the Health Research Board

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PC36

Early cross-talk between dental pulp stem cell and ischemic cardiomyocytes in an *ex vivo* model

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In the last years, mesenchymal stem cells (MSC) have been extracted from adult tissues other than bone marrow (e.g. muscle, skin, adipose tissue, dental pulp). In vitro characterization revealed that MSC from dental pulp (DP-MSC) are multipotent and show excellent differentiation potentiality in response to specific stimuli. In spite of their multipotency DP-MSC are characterized by an early compartmentalization which protects them against differentiating stimuli from the environment. Since in cocolture with neonatal cardiomyocytes DP-MSC have been seen to differentiate into cardiomyocytes, the present investigation aims at characterizing them and studying their early homing in normal and infarcted isolated rat hearts.
Dental pulp-mesenchimal stem cells were obtained from the teeth of anaesthetized (ketamine (90 mg/kg) and xylazine (10 mg/kg)) and decapitated adult Wistar rats. The cells were characterized with RT-PCR and immunofluorescence. 10^6 DP-MSC marked with carboxyfluorescin were implanted in the ventricular apex of Langendorff isolated and perfused hearts rapidly excised from syngenic rats killed as above. The hearts were randomly assigned to two groups: in the Control group the cells were implanted immediately after stabilization and no other maneuver was performed. In another group the cells were implanted after 30 min of ligature of the left anterior descending coronary artery and 5 min of reperfusion (Ischaemia/reperfusion = I/R group). After four hours from the implantation, the location of the cells in both groups was assessed in both groups and the injured area in I/R group was highlighted with trypan blue staining. Characterization indicated that DP-MSC express some myocardial precursor markers, like NKX2.5, GATA-4, and MEF2C. In addition, RT-PCR showed the transcription of β2-adrenergic receptors. Carboxyfluorescin allowed to recognize DP-MSC in the host tissue by their green color. In Control group DP-MSCs remained in the site of injection as round-shaped cell clusters, while in I/R group they migrated towards the injured areas, as evidenced by trypan blue staining. Yet, in I/R group some DP-MSCs were elongated in parallel with cardiomyocytes and showed the presence of connexin-43 on the membrane. This observation is in contrast with that of a previous investigation where bone marrow MSCs maintained their round shape when injected near the infarcted area after 30 min reperfusion.

The results indicate that in infarcted hearts DP-MSCs can migrate and integrate within the peri-infarcted area in already 4 hours. In addition the characterization suggests that they can differentiate into either cardiomyocytes or vascular smooth muscle fibers. Further studies should identify factors that influence the apparently different responses of these cells.

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PC37

Involvement in NO-cGMP pathway in cardiac protection by Apelin-13 in the rat

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Apelin is an endogenous peptide found in various tissues and organs included the cardiovascular system, where it displays inotropic and vasodilator effects and protects myocardium against ischaemia-reperfusion injury. Depending on the number of aminoacids, different forms of Apelin have been classified. Out of the various forms, apelin-13 has been found to be the most active on the cardiovascular system. The mechanism of the myocardial protection exerted by Apelin is still controversial. An intervention of a phosphatidylinositol 3-kinase (PI3K) as a signal transducer has not yet been fully confirmed. The present research aims at investigating whether PI3K leads to protection via nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) pathway.

Twenty eight anaesthetised (ketamine (90 mg/kg) and xylazine (10 mg/kg)) rats were killed by decapitation. The hearts were excised and perfused with oxygenated buffer. A 30 min global ischaemia was followed by a 120 min reperfusion. Left ventricular pressure was measured and infarct size was assessed by nitro-blue tetrazolium test (Group I; n=5). Group II hearts were perfused with 0.5 μM apelin-13 during the first 20 min of reperfusion (n=5); in addition to Apelin, Group III-VI hearts received the following compounds 5 min before and 25 min after ischaemia: the PI3K inhibitor LY 294002 (15 μM) (n=4) or the NO-synthase inhibitor L-nitro-n-arginine (L-NNA) (100 μM) (n=5), the guanylyl-cyclase (GC) inhibitor 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (10 μM) (n=4) or the blocker of mitochondrial ATP sensitive K+ channels (mitoATP K+) 5-hydroxydecanoate (5-HD) (100 μM) (n=5), respectively. Data are expressed as mean±sem and were statistically analyzed by 1-way ANOVA.

After ischaemia and reperfusion, the infarction was extended to the 54±3% of the left ventricular mass. Moreover, starting from the late phase of ischaemia, the left ventricular end diastolic pressure (LVEDP) increased from 6±1 to 40±9 mmHg ($p<0.001$), without any significant change of the left ventricular systolic pressure (LVSP). The increase of LVEDP, which was taken as an index of myocardial contracture, was responsible for about 70% reduction of the left ventricular developed pres-

sure (LVDevP). Apelin-13 significantly ($p<0.001$) reduced the infarct size to 26±4%. It also reduced LVEDP by about 50% ($p<0.001$) thus limiting the fall of LVDevP to about 20% ($p<0.001$). The inhibition of PI3K, NO-synthase and GC and the blockade of mitoATP K+ channels prevented the effect of Apelin-13 on infarct size and contracture.
Our results indicate that Apelin-induced protection is mediated by NO-cGMP cascade which is likely to be elicited by PI3K-AKT pathway as suggested by its suppression by PI3K inhibition. The protection includes not only a limitation of the infarct size, but also a reduction of myocardial contracture.

This work was supported by Compagnia di San Paolo, Torino.

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PC38

Ventilatory long-term facilitation following a bout of intermittent hypoxia: the role of carotid chemoreceptors and ventilatory drift due to carbon dioxide

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A sustained elevation in ventilation upon recovery from a bout of intermittent hypoxia, known as ventilatory long-term facilitation (vLTF) has previously been shown in a range of animal species and humans during sleep 1,2,5,6,7. However, it has only recently been possible to reproduce vLTF in awake humans. Harris et al. 2006 were the first to elicit vLTF in awake humans, but at the same time demonstrating that there was a requirement for a concomitant elevation in CO_2 3. Although this finding has been reproduced in humans under conditions of sustained elevation in CO_2 4,8, it remains to be determined which location(s) in the human body intermittent hypoxia affects in mediating vLTF. The logical location would be the carotid chemoreceptors as they are the primary oxygen sensing sites in the human body but currently no evidence to support this exists. Participants completed two trials in a randomized cross over design. During trial 1 participants were exposed to an intermittent hypoxia protocol almost identical to that of Harris et al. 2006. End-tidal partial pressure of CO_2 ($PETCO_2$) was elevated 4 mmHg above each participant's normal levels and this was sustained throughout the study. The intermittent hypoxia protocol consisted of 8 episodes of 4 minutes hypoxia ($PETO_2$= 50mmHg) interspersed with 4 minutes of euoxia ($PETO_2$= 100mmHg). During the periods preceding and following intermittent hypoxia (baseline and recovery phases, respectively) participants were exposed to 100% O_2 for 1-minute periods, in order to silence the carotid chemoreceptors and thus allowing their contribution in mediating vLTF to be evaluated. Trial 2 served as a control trial and it was identical to trial 1 except that participants were not exposed to intermittent hypoxia but breathed a gas mixture that

resulted in elevating each participant's $PETCO_2$ by 4 mmHg and maintaining $PETO_2$ at 100 mmHg. In trial 1 minute ventilation increased from a baseline value before intermittent hypoxia of 22.34 ± 2.76 l/min to 29.79 ± 2.79 l/min at the end of recovery. The same comparison in trial 2 revealed a similar response with minute ventilation increasing from a baseline value of 23.87 ± 1.82 l/min to 29.94 ± 3.98 at the end of recovery. The influence of exposing participants to 100% inspired O_2 was also similar between the two trials. In trial 1 minute ventilation decreased at baseline by 1.75 ± 0.45 l/min due to inspiration of 100% O_2, and by 1.99 ± 0.4 l/min following intermittent hypoxia. In trial 2 the respective values were 2.20 ± 1.53 vs. 3.41 ± 1.76 l/min, respectively. These results suggest that intermittent hypoxia-induced vLTF in awake humans as previously shown under hypercapnic conditions may not be mediated by the intermittent hypoxia, but may in fact be a product of ventilatory drift due to sustained hypercapnia.

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PC39

Hypoxia-induced angiogenesis

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Hypoxia upregulates a number of genes, regulated by hypoxia-inducible factor (HIF), involved in erythropoiesis, iron metabolism, apoptosis & metastasis, and glycolysis that are important for organ survival under conditions of low PO_2. Others control growth and adaptive remodelling of the existing vasculature (angiogenesis). In response to local hypoxia, using limb ischaemia as a surrogate model of peripheral vascular disease (PVD), HIF signalling may be enhanced by preventing its in situ degradation using the peptide dimethyloxalylglycine (DMOG), promoting skeletal muscle angiogenesis [1]. However, when the proximate stimulus is systemic hypoxia, useful for studying diseases such as chronic obstructive pulmonary disease (COPD), angiogenesis is at best modest and unresponsive to DMOG treatment in mice [2].

Other species appear to be pre-adapted to maintain high aerobic activity under hypoxic conditions [3]. Normobaric hypoxia induces angiogenesis on a regional basis, even within apparently homogeneous muscles, such that it can be easily missed [4]. Interestingly, HIF expression may also be increased by mechanical deformation of cells, as seen in muscle overload [5], and an alternate way of overcoming the usually poor angiogenic response in ischaemic muscle is to apply stretch [6]. Thus, systemic and local hypoxia may act differently, or have different thresholds for inducing capillary growth, suggesting alternate therapeutic strategies may be effective according to the origin of the hypoxic challenge.

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This work is supported by the British Heart Foundation and the Rowbotham Bequest.

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SA01

The role of potassium channels in oxygen sensing

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The study of the molecular mechanisms involved in low-oxygen chemotransduction has provided a large number of oxygen-sensitive ion channels, both in chemoreceptor and non-chemoreceptor cells. The focus in the last years has been placed in the molecular characterization of these channels, the identity of the oxygen sensor, and its link with the functional response of the cell. This search has been most extensively prosecuted in chemosensory systems involved in acute oxygen sensing such as pulmonary artery smooth muscle cells (PASMCs) carotid bodies (CBs), and airway neuroepithelial bodies (NEBs). The large amount of data regarding the molecular nature of the oxygen-sensitive ion channels and the oxygen-sensors in these preparations has modified substantially the initial hypothesis of a conserved O2-sensing mechanism. Experimental findings in these chemosensory systems cannot be reconciled into a unifying theory of a single oxygen-sensitive ion channel and a universal O2 sensor, and this fact has stimulated the building of an emerging paradigm of acute oxygen sensing that opens new and interesting theories from a physiological and pathophysiological standpoint. This new model tries to explain several well established facts, such as the heterogeneity of oxygen-sensitive ion channels within the same preparation (as there are species-related differences, and even more than one type of oxygen-modulated channel), the presence of these same channels of channel subunit combinations in oxygen-insensitive preparations and the diversity of O2-sensing mechanisms described. Altogether, these facts heighten the concept that several different mechanism have developed to ensure adequate hypoxic responses. Moreover, this concept could not only imply that chemoreceptor cells posses some “backup” or second choice mechanism to respond to hypoxia, but may also reflect the fact that the integrated cellular response to hypoxia requires the concerted action of several O2 sensing mechanism and/or several effectors. The current picture regarding the molecular nature and the role of oxygen-sensitive ion channels in carotid body chemoreceptor cells physiology perfectly exemplify these new concepts.

Supported by Heracles network (grant R006/009), Spanish Department of Science grant BFU2007-61524 and Junta de Castilla y León grant GR242.

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SA02

Ion channel regulation by AMP-activated protein kinase: the key to hypoxia-response coupling in the carotid body and pulmonary artery

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Vital homeostatic mechanisms monitor O_2 supply and adjust respiratory and circulatory function to meet demand. The pulmonary arteries and carotid bodies are key systems in this respect. Hypoxic pulmonary vasoconstriction aids ventilation-perfusion matching in the lung by diverting blood flow from areas with an O_2 deficit to those rich in O_2, while a fall in arterial pO_2 increases sensory afferent discharge from the carotid body to elicit corrective changes in breathing patterns. Considered here is the new concept that hypoxia, by inhibiting oxidative phosphorylation, activates AMP-activated protein kinase (AMPK) leading to consequent phosphorylation of target proteins such as ion channels, which mediate pulmonary artery constriction and carotid body activation.

With respect to the role of AMPK in O_2 sensing, investigations on pulmonary arterial smooth muscle have perhaps provided the strongest support [1]. Thus, exposure of pulmonary arterial smooth muscle to hypoxia precipitated an increase in the AMP/ATP ratio, and concomitant activation of AMPK and phosphorylation of acetyl CoA carboxylase (ACC), an established marker of AMPK action. Moreover, AMPK activation and ACC phosphorylation was induced in pulmonary arterial smooth muscle both by the mitochondrial inhibitor phenformin and by AICAR, which activates AMPK without affecting the cellular AMP:ATP ratio, and each agent induced an increase in the intracellular Ca^{2+} concentration by mobilizing intracellular stores in acutely isolated pulmonary arterial smooth muscle cells as does hypoxia. Most significantly, however, AMPK activation by AICAR evoked a slow, sustained and reversible constriction of pulmonary artery rings that exhibits all the primary characteristics of hypoxic pulmonary vasoconstriction, which may be blocked by the non-selective AMPK antagonist compound C. New data obtained using both AMPK activators and intracellular dialysis of an active (thiophosphorylated) recombinant AMPK heterotrimer ($\alpha 2\beta 2\gamma 1$) now demonstrate that recombinant Kv2.1 and Kv1.5 are directly phosphorylated and modulated by AMPK, and in a manner consistent with the differential effects of both hypoxia, mitochondrial inhibitors and AMPK on Kv currents recorded in arterial smooth muscle cells of conduit and resistance sized pulmonary arteries, respectively.

Consistent with a general role for AMPK in hypoxia-response coupling, we have previously shown that AMPK activation, like hypoxia, also activates the carotid body by causing depolarization of type I cells, triggering voltage-gated Ca^{2+} influx and consequent secretion of transmitters to excite afferent sensory neurons [2]. Furthermore, we showed that carotid body activation by hypoxia is blocked by the non-selective AMPK antagonist compound C. Our most recent investigations now provide further evidence in support of a role for AMPK. In the rat, depolarization of type

I cells arises from inhibition of a TASK-like, leak K current and BKCa channels. We have now demonstrated that intracellular dialysis from a patch pipette of an active (thiophosphorylated) recombinant AMPK heterotrimer ($\alpha 2\beta 2\gamma 1$) inhibits recombinant TASK-3 (but not TASK-1) and BKCa channels expressed in HEK293 cells. With respect to BKCa channels parallel phosphorylation studies have confirmed that the immunoprecipitated channel protein is a direct substrate for AMPK, that channel inhibition by AMPK is splice variant specific and in a manner consistent with the BKCa splice variant expressed in type I cells. Perhaps most significantly, however, in AMPK knockout mice the ventilatory response to hypoxia is attenuated.
In conclusion, AMPK may be sufficient and necessary for hypoxia-response coupling and may regulate O_2 and thereby energy (ATP) supply at the whole body as well as the cellular level [3].

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This work is supported by a Programme Grant from the Wellcome Trust

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SA03

The effect of oxidative stress and hypoxia on glucocorticoid responsiveness

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Glucocorticoids are the most effective and widely used anti-inflammatory treatments but in some diseases they are ineffective. We have shown that many of the anti-inflammatory actions of glucocorticoids may be explained by ligand-activated glucocorticoid receptors switching off activated inflammatory genes that are associated with acetylated core histones through the recruitment of the nuclear enzyme histone deacetylase-2 (HDAC2), which reverses histone acetylation leading to gene suppression. In several inflammatory diseases, such as chronic obstructive pulmonary disease (COPD) and severe asthma, glucocorticoids are far less effective in suppressing inflammation. This appears to be due to a reduction in the expression and activity of HDAC2. Oxidative stress and cigarette smoke induce steroid resistance

through reducing HDAC2 activity and expression through the activation of phosphoinositide-3-kinase-delta (PI3Kd), thus leading to phosphorylation and subsequent ubiquitination and proteolytic degradation of HDAC2. Hypoxia also induces steroid resistance through reducing the expression of HDAC2 and this is mediated through the activation of HIF-1α, which directly binds to the promoter region of HDAC2 to inhibit its gene transcription. Oxidative stress is increased in several severe inflammatory diseases and in severe COPD there is also hypoxia, which together lead to profound steroid resistance. The steroid resistance secondary to oxidative stress is reversible with low concentrations of theophylline and nortriptyline, which have long been used in clinical practice. We have shown that both drugs work by selectively inhibiting activated PI3Kd and that their effects are mimicked by knockdown of PI3Kd by interference RNA or by selective PI3Kd inhibitors. The reduction in HDAC2 gene expression as a result of hypoxia may be reversed by macrolide antibiotics, although the molecular mechanism for this effect has not yet been defined. Although our research has focussed on severe airway disease it is likely that similar mechanisms may apply in other inflammatory diseases.

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Funded by the Wellcome Trust and MRC

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SA04

Hypoxic Pulmonary Vasoconstriction (HPV): The Redox Hypothesis for Regulation of Potassium Channels

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HPV matches ventilation to perfusion and optimizes systemic oxygenation in the adult and, in the fetus, minimizes flow to the unexpanded lung. In HPV, small, resistance pulmonary arteries (PA) constrict in response to local alveolar hypoxia. This mechanism is conserved across species and is intrinsic to the pulmonary artery smooth muscle cells (PASMC), being strongest in distal PAs (<200μM). The "Redox Hypothesis" defines the mechanism of HPV as a rapid, reversible, redox regulation

of K^+ channel function[1]. According to this hypothesis (summarized in 2), HPV is initiated by a mitochondrial O_2 -sensor, that regulates production of diffusible redox mediators (reactive O_2 species, ROS and redox couples) by the activity of Krebs' cycle and the electron transport chain. This controls the redox state of the cytosol and ion channels. The production of ROS occurs in proportion to alveolar PO_2 (more oxygen=more ROS3). These reactive O_2 species (ROS, particularly diffusible hydrogen peroxide) in turn, control the gating of specific K^+ channels. Decreased ROS (or a reduced redox state) inhibits K^+ channels in PASMC, leading to membrane depolarization, calcium entry via the L-type calcium channel and pulmonary vasoconstriction. Whole cell patch clamp technique directly proved that hypoxia uniquely inhibits K^+ channels in PA but not systemic arterial myocytes4. Subsequently, the voltage gated potassium channels (Kv) inhibited by hypoxia (Kv1.5 and Kv2.1) were identified5. Supporting an important role for Kv1.5, HPV and O_2-sensitive potassium current are reduced Kv1.5 knockout mice6. Moreover, the preferential occurrence of HPV in resistance PAs relates to their enrichment in the expression of O_2-sensitive Kv channels (e.g. Kv1.5, Kv2.1, Kv3.1)7 (as well as the presence of mitochondria that vary ROS production in proportion to PO28). In conditions where HPV is suppressed (chronic hypoxia), Kv gene therapy can restore HPV9. Over the past 2 decades, the redox theory has met with mixed reviews. The K^+ channel mechanism is generally accepted and has been reproduced by man labs. O_2-sensitive K^+ channels are a conserved mechanism in other specialized O_2-sensing tissues, such as the carotid and neuroepithelial bodies. However, the specific K^+ channels involved are diverse. TASK and trp channels are important to HPV. Moreover, the release of cytosolic calcium and activation of rho kinase also contributes to HPV. Controversies as to the effect of physiologic hypoxia on ROS production are ongoing, although there is increasing consensus that the mitochondria are important O_2 sensors and regulate vascular tone through redox regulation of K^+ channel gating, which is the essence of the redox hypothesis. This pathway for oxygen-sensing is also relevant to Pulmonary Arterial Hypertension, a condition in which Kv1.5 channel expression is suppressed.

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SA05

Pulmonary hypoxic vasoconstriction - role of reactive oxygen species

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Hypoxic pulmonary vasoconstriction (HPV) is an adaptive mechanism that normally helps to optimise ventilation-perfusion matching in the lung by diverting blood from poorly ventilated areas. Although we know that the central mechanism (or mechanisms) of HPV resides in the smooth muscle cells of the small pulmonary arteries, there is still controversy concerning the O2 sensing mechanism and its transduction pathways. There are three competing hypotheses: the Redox hypothesis suggests that hypoxia causes a reduction in mitochondrial ROS production and a more reduced cytosolic redox state, which inhibits voltage gated K+ channels and causes depolarisation and Ca2+ entry. The ROS hypothesis however suggests that mitochondrial ROS production is increased, and that ROS activate Ca2+ release from ryanodine-sensitive stores, consequent store operated Ca2+ entry, and also importantly Rho kinase-mediated Ca2+ sensitisation. The third hypothesis does not depend on ROS, but instead invokes a change in energy state as reflected by an increase in cytosolic [AMP], which activates AMP-activated kinase. This, possibly in conjunction with elevated NADH, is thought to lead to activation of ryanodine receptors via cyclic ADP ribose. We and others have considerable evidence to support the ROS hypothesis, although as will be discussed this does not necessarily conflict with a role for AMP kinase. This includes measurements of ROS (though this is in itself highly contentious), the suppressing effect of antioxidants both exogenously applied and by over-expression of intracellular antioxidant mechanisms, and in particular the effects of exogenously applied ROS in the form of superoxide and peroxide, both of which cause constriction in pulmonary artery. Whilst the potential role of ROS in the hypoxia-induced elevation of intracellular Ca2+ will be discussed, the major focus of the this talk will concern the role of ROS in the activation of Rho kinase mediated Ca2+ sensitisation, as this is of particular importance in sustained HPV and many models of pulmonary hypertension. In summary, we believe that the balance of the evidence favours the ROS hypothesis of HPV, although as with the other hypotheses many factors remain unclear.

Supported by the Welcome Trust and British Heart Foundation.

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SA06

Cardiorespiratory Adaptations to Hypoxia: Birds at Altitude

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The bar-headed goose flies at altitudes of up to 9000m on its biannual migration over the Himalayas. Despite the severe hypoxia at these elevations, bar-headed geese must sustain high rates of oxygen transport to support the metabolic costs of flight. Based on theoretical modelling we predicted that the physiological traits with the greatest influence over oxygen transport during hypoxia would be a heightened capacity to breathe, a high affinity of the blood for oxygen, and an enhanced capacity for oxygen diffusion in the flight muscle. We subsequently found that bar-headed geese had an enhanced poikilocapnic hypoxic ventilatory response, employing a more effective breathing pattern (reduced dead space ventilation), dramatically improving arterial PO_2 compared to other birds. This enhanced HVR was not due to a greater hypoxic sensitivity but to a reduced sensitivity to hypocapnia. Barheaded geese have relatively large lungs and, as with all birds, a thin pulmonary diffusion barrier. Their blood has been shown to have a higher oxygen affinity than that of other birds, which we hypothesize can be dramatically assisted by cooling in the lungs at altitude (to assist loading) and warming in the exercising flight muscle (to assist unloading). We found that, compared to closely related geese, the flight muscles of the barheaded goose contained more type IIa fibres, a higher mitochondrial volume density, an increased capillary density, and a higher proportion of mitochondria in sub-sarcolemmal locations. At the cellular level we found no differences in oxidative capacity, no differences in metabolic enzyme activity and no differences in oxygen kinetics. Finally, while it remains possible that these birds may take advantage of updrafts and wind currents to assist them in crossing over some of the world's highest mountains, preliminary evidence suggests otherwise. Ongoing studies are designed to determine the true metabolic costs of this amazing feat and to measure physiological changes during free flight at altitude.

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SA07

Hypoxia responses in reptiles

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As ectothermic vertebrates, reptiles have low oxygen demands, yet they appear to be endowed with all of the mechanism for oxygen sensing that have been described

in mammals. Thus, when faced with hypoxia or reductions in blood oxygen carrying capacity, reptiles respond by increasing cardiac output and ventilation. In addition, reptiles can reduce the magnitude of the intracardiac shunt to elevate blood oxygen delivery, and they may lower the demand for oxygen by selecting lower body temperatures. The signals mediating these responses have been studied through independent manipulations of blood oxygen concentrations and partial pressures. These studies show that the ventilatory responses to hypoxia are solely mediated by reduction in oxygen partial pressures, while the cardiovascular responses may also be influenced by reductions in blood oxygen concentrations. The pulmonary vasculature of many species of reptiles contracts in response to hypoxia and may serve to improve the local matching of ventilation and perfusion, but the increased pulmonary vascular resistance also acts to induce a right to left shunt that may have detrimental effects on oxygen delivery.

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SA08

Cardio-respiratory adaptations in the master of anoxic survival - the crucian carp

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In Northern Europe, small shallow lakes and ponds often becomes anoxic for several months every winter due ice blocking both photosynthesis and oxygen diffusion from air. The only fish that survives in these waters is the crucian carp (Carassius carassius) — the wild cousin of the goldfish (C. auratus). The crucian carp is arguably the most anoxia-tolerant fish species. Among vertebrates, its anoxia tolerance is only matched by that of some North American freshwater turtles (genera Trachemys and Chrysemys). However, unlike other anoxia-tolerant vertebrates, the crucian carp remains active during anoxia, although at a reduced level. The key adaptation allowing continued activity is probably its exotic ability to convert lactate to ethanol. However, producing ethanol and releasing it to the water is a wasteful strategy as an energy-rich hydrocarbon is forever lost. Indeed, when faced with falling oxygen levels, the crucian carp strives to take up the little oxygen there is in water, thereby avoiding to turn on ethanol production as long as possible when faced with falling oxygen levels. Thus, the crucian carp has a record high hemoglobin oxygen affinity, and when exposed to hypoxia, it has the remarkable ability to remodel it gills, resulting in a 7-8 fold increase in the respiratory surface area. In sharp con-

trast to anoxia tolerant turtles, which strongly suppresses cardiac work in anoxia, the crucian carp maintain cardiac output and ventilation at normal levels even after several days in anoxia. This suggests that being active in anoxia demands an active circulatory system for shuttling glycolytic substrates and removing ethanol. To maintain activity, also the brain has to continue to function in anoxia. Brain ATP levels and ion homeostasis are protected, and there is little evidence for reduced neuronal ion permeability in crucian carp, possibly with the exception for reduced expression of glutamate receptors of the NMDA type. Elevated levels of the inhibitory neurotransmitter GABA are likely to play a major role in reducing the energy use by both the brain and body. Molecular mechanisms involved in the anoxic defense involves AMP activated protein kinase (AMPK), while there is an apparent suppression of HIF induced pathways. Experiments followed current national guidelines for animal experimentation.

The work is supported by The Research Council of Norway

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SA09

Intermittent Hypoxia- Functional Consequences

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Sleep disordered breathing manifested as recurrent apneas are the most frequently encountered respiratory disorders in adult humans and infants born preterm. Patients with recurrent apneas exhibit autonomic co-morbidities including hypertension, persistent sympathetic activation and elevated plasma catecholamines. Intermittent hypoxia (IH) resulting from apneas is the primary stimulus for evoking autonomic changes. Carotid bodies are the front-line defense during apneas and adrenal medullary chromaffin cells (AMC) are a major source of circulating catecholamines. In this presentation I review recent studies on the effects of IH on carotid body and AMC, their functional consequences, and the underlying cellular as well as molecular mechanisms. Studies on rodents demonstrate that IH sensitizes carotid body response to hypoxia and induces sensory long-term facilitation in response to repetitive hypoxia resulting in exaggerated chemo-reflex function that contributes to heightened sympathetic tone. IH markedly increases hypoxia-induced catecholamine secretion from AMC, which contributes to elevated plasma catecholamines. Reactive oxygen species (ROS) signaling is critical for mediating the effects of IH on carotid body and AMC. Cellular sources of ROS during include the activation of NADPH oxidases (Nox) and inhibition of mitochondrial complex I. In addition, IH up-regulates pro-oxidant and down-regulates anti-oxidant genes, and imbalance between transcriptional activators HIF-1 and HIF-2 contribute to this response.

Supported by grants from the National Institutes of Health, Heart, Lung and Blood Institute HL- HL-90554, HL-76537, HL-86493.

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SA10

Short and longer term consequences of systemic hypoxia for the cardiovascular system

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Systemic hypoxia is sensed by carotid chemoreceptors, which evoke a complex pattern of reflex responses that includes hyperventilation, tachycardia and generalised increase in sympathetic vasoconstrictor activity. The hyperventilation and tachycardia wane due to local influences of hypoxia that include the actions of adenosine. Further, in skeletal muscle, the sympathetic vasoconstriction is overcome by vasodilatation, which arises in large part because endothelial cells "sense" falls in O_2 concentration. Thus, NO that is generated tonically by shear stress competes with O_2 for the same binding site on endothelial cytochrome oxidase raising its K_m, such that even moderate falls in O_2 decrease ATP synthesis. Our evidence indicates this leads either directly to adenosine release from endothelial cells, or to adenosine being generated extracellularly from lower adenine nucleotides. Adenosine then acts on endothelial A_1 receptors to release NO and cause muscle vasodilatation via a pathway that involves synthesis of PGI_2 as an intermediate. Acute hypoxia also leads to generation of reactive oxygen species (ROS) in skeletal muscle. Since xanthine oxidase inhibition attenuates hypoxia-induced dilatation, while exogenous superoxide dismutase (SOD) potentiates it, we have proposed that H_2O_2 generated from O_{2^-}, partly as a consequence of adenosine metabolism, contributes to the vasodilatation.

The effects of chronic systemic hypoxia depend critically on stage of development. In adult rats exposed to chronic hypoxia (CH), adenosine exerts a tonic dilator influence on skeletal muscle via A_1 receptors and NO, which is resolved by day 7 when haematocrit is increasing. Moreover, on days 1-7 and at 3-5 weeks of chronic hypoxia, the adenosine-mediated dilator influence of acute hypoxia is accentuated, suggesting the endothelial "NO-adenosine sensing" mechanism is up-regulated in CH rats.

By contrast, rats exposed to chronic hypoxia from birth for 6-7 weeks (CHB), show more pronounced secondary waning of ventilation and heart rate than controls in acute hypoxia, such that arterial pressure falls more profoundly and cerebral blood flow falls. Moreover, whilst their secondary hypoventilation and bradycardia are attributable to adenosine, the muscle vasodilatation is not mediated by adenosine. On the other hand, rats that are exposed to chronic hypoxia in utero, but reared in air (CHU), show no exacerbation of the secondary hypoventilation and bradycardia in acute hypoxia, but again their substantial muscle vasodilator response is not medi-

ated by adenosine even though it is NO-dependent. Thus, the endothelial "NO-adenosine sensing" mechanism is apparently disturbed in CHB and CHU rats.
Our recent findings suggest that differences in oxidant status may play a critical role in determining the long-term cardiovascular consequences of chronic hypoxia at different stages of development.

I would like to gratefully acknowledge BHF who have generously supported this work

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SA11

Integrated responses to hypoxia signalling

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A drop in cellular oxygenation triggers tissue specific compensatory reactions aimed at preserving energy charge with the reduction in aerobic ATP production. The integrative nature of this response is pointed out by the resetting of respiratory and cardiovascular control and the promotion of anaerobic substrate flux and catabolism in skeletal muscle with chronic exposure of human subjects to extreme altitudes above 4000m sea level (Flueck 2009). In seeming contrast, intense physical activity in moderate hypoxia, i.e. altitudes of 2500 to 4000m above sea level, modifies this response in the untrained by elevating local aerobic capacity and possibly preventing muscle fibre atrophy (). The frequent lowering of muscle oxygen with physical work indicates that local hypoxia is an important signal for muscle plasticity.
We have addressed this contention by studying adjustments of the muscle trait of aerobic power output with a multi-level approach. Transcript profiling in heterozygous deficient mice for hypoxia inducible factor 1 alpha (HIF-1A) identified broadly positive transcript control of gene ontologies involved in glycolysis, mitochondria respiration and capillary neo-formation by HIF-1A in severe hypoxia (10.5% O_2); Däpp et al 2006). By contrast, expression control of lipid metabolism was inhibited in a HIF-1A dependent manner. Measures of selected gene messages in exercised human supported the implication of HIF-1A in hypoxia signalling towards the genome response in the first 24 hours of recovery and exposed that the outcome of hypoxia signalling is importantly modified by muscle differentiation. This was indicated by a delay in exercise induced up-regulation of HIF-1A dependent transcripts in untrained subjects by exercise in a hypoxic environment (12% O_2; Schmutz et al 2010). By contrast, a specific promotion of mitochondrial, angiogenic and glycolytic transcripts was evident after endurance training under the co-stimulus of hypoxia compared to normoxic exercise. The reciprocal control of muscle gene expression in hypoxia trained and untrained state corresponded to changes in arterial oxygen saturation during exercise and related to a selectively elevated subsarcolemmal mitochondr-

ial density and capillary-to-fibre ratio in locomoter muscle and increased aerobic performance in a hypoxic environment.
The results support the view that hypoxia signalling in muscle towards gene regulation is aimed at preserving energy supply and is a function of the severity of hypoxic stress and modified by the makeup in the pathway of respiration.

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The work was supported by a grant from the Swiss National Science Foundation a start up grant from Manchester Metropolitan University to MF.

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