C166

The tachypnoea evoked by non-peptidic delta opiate receptor agonists, but not that due to chest compression, is blocked by Naltrindole in the anaesthetised rabbit

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Non-peptidic δ-opioid receptor agonists offer the prospect of analgesia free from the respiratory depression due to μ-receptor agonists (as with oral SB 235863 in the rat, Pozzi *et al.* 1998). Given to anaesthetised rabbits I.V., SB 235863 actually evoked a tachypnoea abolished by cervical vagotomy (Sears & Banks, 2002) as now described for two other δ agonists. New Zealand white rabbits (2.0-3.0 kg) were anaesthetised with Na pentobarbitone (40-60 mg/kg I.P.) and subsequently by constant I.V. infusion (6-8 mg/kg/h). Carotid BP was measured and ear and jugular veins cannulated for giving fluids and drugs. Silver ball electrodes sealed 3.0 mm apart on a plastic strip recorded the EMG from the abdominal surface of the diaphragm; tracheal airflow and alveolar CO_2 were also measured. An oil-filled syringe on a rack and pinion was lowered so that its plunger applied a steady force to the sternum as detected by syringe pressure. Signals were acquired via a CED 1401*Plus* and Spike 2v5 software. At 0.1 mg/kg I.V., Pfizer UK321130 evoked a short latency tachypnoea of more than 5 min duration (Fig. 1A), a 0.75% fall in alveolar CO_2 and small increases in HR and mean BP (10 mmHg). Pressure on the sternum evoked an abrupt increase in instantaneous respiratory rate (20-40/min, not shown). After 30 min, the δ-opioid receptor antagonist Naltrindole (1.0 mg/kg, I.V.) was given. Five minutes later, the respiratory responses to the δ agonist were completely blocked (Fig. 1B), whereas those due to chest compression persisted (Fig. 1C); similar results were obtained with SNC80. Cervical vagotomy abolished the tachypnoea evoked by Pfizer UK321130 and the other agonist tested (SNC80). Although tachypnoea also occurred with higher doses of the agonists, slowing and eventually apnoea supervened (in some cases partially reversed by Naloxone or sternal pressure) and brief latency falls in HR and BP invariably occurred. The actions of these δ-opioid agonists given I.V. are complex; nevertheless the differential effects of the antagonist Naltrindole and vagotomy suggest that different groups of receptors mediate the tachypnoea; further studies are needed to identify their nature and location.

Pozzi O. et al. (1998). Soc Neurosci Abstr 24, P352.16

Sears TA & Banks D (2002). Eur J Physiol 443, S242.

The authors would like to thank Glaxo SmithKline for their support.

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

C167

Different mechanisms underlie respiratory rhythms in muscle and thermoregulatory vasoconstrictor sympathetic activity

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The pattern and rhythm in sympathetic activity regulating many targets indicates cardiorespiratory coupling (Numao et al. 1987; Smith & Gilbey, 2000). Smith & Gilbey (2000) concluded that central respiratory drive (CRD)-related rhythms in renal nerve activity arose directly from central respiratory networks, whereas activity supplying thermoregulatory circulations (CVC) resulted from entrainment of autonomous sympathetic rhythm generators (T-rhythm: Chang et al. 1999). Here we investigate the mechanism(s) underlying cardiorespiratory interactions in sympathetic muscle vasoconstrictor (MVC) activity.

S-D rats (male, 280-330g, n=6) were anaesthetised (sodium pentobarbitone 60 mg kg^{-1} I.P.; supplemented with 5-10 mg α-chloralose I.V., as required until humane killing with anaesthetic), vagotomised, sino-aortic denervated and pneumothorax. During positive pressure ventilation (2.0 Hz, 1.5-2.ml) CRD (index phrenic nerve (PN) activity) was adjusted by adding CO2 to the inspired gas mixture. Arterial blood gases and pH were assessed after each data collection. A neuromuscular blocker (gallamine triethiodide 16mg kg-1 h-1; Smith & Gilbey, 2000) was administered. Autospectra were computed from rectified and smoothed (τ=20ms) population recordings from a gastrocnemius nerve (GN, MVC activity), the plantar aspect of the ipsilateral tibial nerve (TNp, activity of typical CVC activity) and PN (Huang & Gilbey, 2003).

Whereas at all levels of CRD tested a dominant peak in the autospectra of MVC activity was observed at rhythmic PN discharge frequency (fPN: linear regression analysis, 21 pts, Y-intercept=0, slope=1, r^2=1), a dominant peak in powerspectra of CVC activity was observed at fPN only when CRD was at an enhanced level (PaCO2 59±3): at normal levels of CRD and in central apnoea the T-rhythm dominated (linear regression analysis, 21 pts, Y-intercept=0.75, slope=-0.24, p=0.0006, r^2=0.47). PN-triggered averages of MVC and CVC activities showed that during enhanced CRD both nerve activities reached a peak in early expiration, however peak MVC activity was sustained (duration 0.46±0.04 of phrenic cycle), whereas peak CVC activity subsided rapidly (duration 0.08±0.02; durations significantly different P<0.003, unpaired t test). Thus, two distinct mechanisms underlie the CRD-related MVC and CVC rhythms. We provide evidence that CRD entrains autonomous sympathetic rhythm (T-rhythm) generators associated with CVC activity. No such evidence was obtained concerning sympathetic networks controlling MVC activity.

Chang et al. (1999). J Neurosci 19 3183-3197

Numao et al. (1987). Neuroscience Letters 81 279-284

Smith and Gilbey (2000). J. Physiol. 523 pt 2 449-457

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

C169

Acute inflammation of the carotid body: functional manifestations

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Ventilatory responses to hypoxia are mainly mediated by carotid body (CB) chemoreceptor excitation, but no reports have been published on CB function during acute endotoxaemia. Having characterized the early steps of CB inflammation induced by I.V. infusion of lipopolysaccharide (LPS) in cats [Fernandez et al, this meeting], this preparation provided the opportunity to study its functional manifestations.

Experiments were performed on 11 cats anaesthetized with sodium pentobarbitone 40 mg/kg I.P. supplemented I.V. as required, breathing spontaneously through a tracheal cannula connected to a pneumotachograph, and whose body temperature was maintained at 38ºC by a regulated heating device. LPS was infused I.V. (0.75 mg/kg at 0.35 ml/min along 20 min) and its effects were observed for at least 4 h. LPS administration evoked marked increases in respiratory frequency (to 156.6 ± 12.5% of control, at 210 min; mean ± SEM), heart rate (to 110.7 ± 4.4%, at 90 min) and haematocrit (to 125.2 ± 2.9%, at 210-270 min), accompanied by systemic hypotension (mean arterial pressure reduced to 74.2 ± 5.1% at 150 min). LPS decreased ventilatory responses to 10 s 100% N_2 breathing (to 62.5% of control responses, at 210 min). Ventilatory responses to I.V. nicotine were diminished in sensitivity (ED_{50} displaced from 13.4 to 57.2 μg/kg, at 150 min) and maximal reactivity (65.3% of control, at 210 min). LPS increased basal frequency of carotid nerve chemosensory discharges in normoxia (> 2-fold from 30 min on), but decreased CB chemoreceptor sensitivity to stimulation by I.V. nicotine (LED displaced 20-fold to the right after 210 min), without significant changes in maximal chemoreceptor responses to brief hypoxic exposures. Ventilatory chemosensory drive, evaluated by breathing 100% O_2 for 60 s under control conditions (normoxia) and after 1 min hypoxia (10% O_2 in N_2), was diminished from 90 min after LPS administration. LPS-induced tachypnoea was prevented by bilateral carotid neurotomy. Cats were killed by an overdose of pentobarbitone given I.V. at the end of experiments.

Our results suggest that LPS-induced endotoxaemia rapidly increases the basal level of CB chemosensory discharges, but decreases the sensitivity of CB chemoreceptor responses and ventilatory chemosensory drive; LPS also induces tachypnoea but reduces ventilatory chemoreflexes. Results support the idea that many pathophysiological reactions associated with the presence of high levels of LPS in the bloodstream may be originated from the CBs.

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

C170

Regulation of a TASK-like potassium channel in rat arterial chemoreceptor cells by ATP

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Carotid body type I cells respond to hypoxia with membrane depolarisation, voltage-gated calcium entry and neurosecretion. The inhibition of TASK-like background potassium channels by hypoxia plays a key role in initiating this response (Buckler et al. 2000). The mechanism by which hypoxia modulates TASK-like channel activity is however unknown. We have previously reported that TASK-like K-channel activity is also markedly reduced by inhibitors of mitochondrial energy metabolism (Williams & Buckler, 2004) suggesting that cytosolic ATP may play a role in regulating channel activity. We have therefore investigated the effects of ATP upon background K-channel activity.

Type I cells were obtained as previously described (Buckler et al. 2000). Briefly, carotid bodies were excised from 19 anaesthetised (4% halothane) 11- to 15-day-old Sprague-Dawley rat pups and disassociated by collagenase-trypsin digestion. All animals were killed by decapitation whilst anaesthetised. Isolated cells were maintained in HAMs F-12 culture media under 5 % CO2 in air at 37 °C on glass coverslips coated with poly-D-lysine and used within 2-6 h.

Channel activity was recorded in excised inside out patches taken from type-1 cells at a membrane potential of -70 mV and is reported as NPo values ((N = number of channels, Po= open probability, mean ± SEM). Upon patch excision channel activity ran down abruptly. Following rundown, application of ATP (0.1-20 mM) to the cytosolic side of the membrane patch resulted in a dose dependent activation of channel activity. Responses to ATP were apparent at concentrations as low as 0.5 mM (control = 0.02 ± 0.01, 0.5 mM ATP = 0.03 ± 0.01, $p < 0.05$, paired t test, n=8) and appeared to reach a maximum at 10 mM where an 8-fold increase in NPo was observed (0.17 ± 0.05, $p < 0.01$, n = 10). The dose response was adequately described by a Michaelis-Menten function with an EC50 of 2.3 mM ($R^2 = 0.99$). The non-hydrolysable ATP analogue AMP-PCP (10 mM) also increased channel activity (control = 0.02 ± 0.004, AMP-PCP = 0.08 ± 0.02, $p < 0.01$, n = 8) suggesting that ATP sensitivity may be mediated through nucleotide binding to some form of regulatory domain rather than through phosphorylation.

In conclusion, the TASK-like oxygen sensitive K-channel of rat carotid body type-1 cells has the potential to respond to changes in cytosolic ATP levels within the physiological range. Thus direct ATP sensing by the channel, or associated regulatory protein, could account for the potent stimulatory effects of metabolic poisons on chemoreceptor activity. These data are also consistent with the metabolic theory of oxygen sensing wherein oxygen is indirectly sensed through changes in mitochondrial ATP synthesis.

Buckler KJ Williams BA & Honore E (2000) J Physiol 525, 135-142.

Williams BA & Buckler KJ (2004) Am J Physiol Lung Cell Mol Physiol 286, L221-L230.

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

C171

Na/K ATPase pump current is increased in ventricular myocytes isolated from phospholemman knockout mice

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Phospholemman (PLM) is a member of the FXYD family of small single transmembrane domain proteins that have been shown to be tissue-specific regulators of Na/K ATPase. We have previously shown that PLM forms an integral part of the cardiac Na/K ATPase complex (Fuller *et al* 2004) and may provide the link between adrenergic stimulation and pump activation (Silverman *et al* 2004). We have therefore investigated the effects of

PLM knockout on Na/K pump function in myocytes isolated from PLM knockout (-/-) (KO) and wildtype (+/+) (WT) mice. Mice were anaesthetised using pentabarbitone (60mg/kg ip), hearts excised and ventricular myocytes isolated using standard enzymatic digestion techniques. Whole-cell Na^+/K^+ ATPase pump current (I_p) was recorded using the perforated patch technique during the descending phase of a voltage ramp (+40 to -80mV) and was defined as that sensitive to extracellular K^+ ($[K^+]_o$). Pipette and extracellular solutions were designed to prevent activation of other endogenous currents.

In 5mM $[K^+]_o$, I_p at 0mV was increased from 2.90±0.19pA/pF (n=19 from 8 animals: mean±sem) to 3.91±0.14pA/pF (n=15 from 5 animals) in myocytes isolated from WT and KO animals respectively ($P\langle 0.05$ Student's t test). Normalised I-V relationships from KO and WT myocytes were identical indicating the voltage-dependence of I_p was unaffected by PLM deletion. The relationship between $[K^+]_o$ (1-20mM) and I_p at 0mV was investigated in KO and WT myocytes. In both groups, mean data were best fitted by a single exponential association. Maximally activated I_p (V_{max}), estimated from each curve fit, was increased by 28.3% in PLM KO cf WT controls. The absence of PLM had no effect on the K^+ affinity of the pump at 0mV. The respective $K_{1/2}$s for K^+ activation, measured at 0mV, were 1.31 and 1.28mM in KO and WT myocytes. However, K^+ affinity was decreased at more negative potentials in KO animals (for example, at -60mV $K_{1/2}$ = 1.43 and 1.06mM in KO and WT respectively).

In conclusion, PLM appears to modulate cardiac Na/K ATPase by applying a tonic inhibition that, when relieved by PLM knockout, results in an increase in V_{max} without a change in K^+ sensitivity assessed at 0mV. At more negative potentials, PLM deletion slightly decreases K-sensitivity. This effect may, however, have little physiological significance as at normal $[K^+]_o$ (ie ~5mM) I_p is saturated at 93.3% and 92.9% of maximum current in WT and KO myocytes respectively.

Fuller W *et.al.* (2004) *FASEB J* **18,** 197-199.

Silverman BdZ, *et.al.* (2004) *Cardiovasc Res* (In press).

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

C172

Effects of Nitric Oxide on cardiac contractility: role of NOS isoforms and signalling pathways

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The role of NO in the cardiac function remains controversial. Several NO donors cause both positive and negative inotropic responses. Furthermore, adrenergic and cholinergic agonists induce NO production in cardiomyocytes, suggesting that NO may be important in the autonomic regulation of the heart. Cardiomyocytes express endothelial (eNOS) and neuronal (nNOS) isoforms of nitric oxide synthase, but eNOS is more abundant, while nNOS expression is restricted to the sarcoplasmic reticulum. We hypothesized that the opposite effects of NO may be related to its concentration, the NOS isoform and intracellular pathways that are activated. eNOS activation may release enough NO to trigger cGMP production to reduce contractility, whereas nNOS activation release small amounts of NO able to nitrosylate sarcoplasmic reticulum proteins.

We tested this idea in the rat heart, Langendorff preparation, using S-nitroso-N-acetylpenicillamine (SNAP) as NO donor, and Isoprenaline as inotropic agent.

Male Sprague-Dawley rats (290-300 g) were anesthetized with ketamine (90 mg kg^{-1} I.P.) and xilazine (10 mg kg^{-1} I.P.). Under deep anaesthesia, hearts were excised, and mounted on the perfusion system and paced at 360 beats min^{-1}. The contractile response was measured as change in d*P*/d*t*max (% basal).

Pulses (1-min) of SNAP 0.1, 1.0 and 10 μM increased contractility (16±5%, 25±3 and 16±1%, respectively, n=6), but 100μM SNAP decreased it (−22±3%, n=10). To assess possible nitrosylation, we used TEMPOL, a superoxide dismutase mimetic, assuming superoxide is an intermediary in this reaction. TEMPOL 100 μM abolished the positive inotropic effect of 1 μM SNAP (n=3). To analyse the cGMP route, we used ODQ, a guanylyl cyclase blocker and 8-Br-cGMP. In the presence of ODQ 10 μM, the effect of SNAP 100 μM turned to a positive inotropic effect (21±5%, n=4). Pulses of 8-Br-cGMP 1, 10 and 100 μM reduced contractility (−8±3%, −24±7% and −25± %, respectively, n=4).

The maximal response to Isoprenaline was reduced during nNOS inhibition with S-Methyl-L-thiocitrulline 300 nM (211±11% v/s 247±33% in control, p≤0.05, paired t test, n=5). On the contrary, during broad NOS inhibition with L-NAME 100 μM the maximal response to Isoprenaline increased (158±23% v/s 97±21% in control, p≤0.05, paired t test). In addition, the β3 adrenergic agonist, BRL-37344 (100 nM), reduced contractility (−24±3%, n=4), and this effect was abolished by ODQ 10 μM.

These data suggest that positive inotropism is related to nNOS activation and low NO levels, possibly due to protein nitrosylation, while the negative inotropic effects observed at higher NO concentrations involve cGMP generation.

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

C174

Cardiac power output and its relationship with bradykinin receptor gene (BK2)

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The central function of the heart is contraction and like all muscles the myocardium must make use of metabolically available energy to produce mechanical work. Cardiac power output is equivalent to the rate at which the heart imparts hydraulic energy into the arterial system to maintain the circulation of blood. All individuals have the BK2 receptor and BKBR2 gene. The expression of the absence (-9) rather than the presence (+9) of a 9 base pair repeat in the gene encoding bradykinin 2 receptor (B2BKR) is associated with greater gene expression and lower cardiac trophic responses to exercise. The purpose of this study

was to explore the relationship of the absence or presence of a 9 base pair B2BKR with cardiac power output.
Fifty seven subjects underwent maximal cardiopulmonary exercise testing. Subjects provided a mouthwash sample using a 10 ml of 0.9% sodium chloride solution. The DNA was extracted from the cheek cells contained in the sample. B2R genotype was ascertained using forward 5'TCTGGCTTCTGGGCTCCGAG-3' and reverse 5'AGCGGGCATGGGACTTCAGT-3' primers.
Genotype distributions in the cohort in which homozygotes were drawn were consistent with Hardy-Weinburg equilibrium. Analysis compared individuals who had the +9/+9 (n=12) expression vs. -9/-9 (n=14). Cardiac power output was significantly higher amongst those of +9/+9 genotype than amongst -9 homozygotes (5.19 ± 1.44 W vs. 4.25 ± 0.98 W, ($p<0.025$). However, there was a non significant difference ($p>0.05$) in between groups (33.2 ± 12.9 and 29.7 ± 6.7, respectively).
As a component of renin-angiotensin systems (RAS), angiotensin converting enzyme (ACE) degrade kinins, lessening their action upon the BK2 receptor. A polymorphic variant of the ACE gene associated with high ACE activity has been associated with improvements in skeletal muscle strength with training, and with 'power' related sports. Our data here suggest that these effects may in part be mediated through alterations in kinin activity at the BK2 receptor. They also extend to previous observations to implicate RAS in regulating cardiac (as well as skeletal) muscle function.
These novel data suggest a role for myocardial RAS in regulating myocardial energetics and contractile performance. They may have implications for the rational design of newer therapeutic targets for the treatment of myocardial contractile dysfunction and clinical heart failure.

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

C175

Inhibition of the RhoA/Rho-kinase pathway attenuates hypoxia-induced angiogenesis in the pulmonary circulation

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Chronic hypoxia leads to the development of pulmonary hypertension (PH) and right ventricular hypertrophy. The increase in pulmonary vascular resistance was previously attributed to encroachment of the remodelled arteriolar walls into the vascular lumen and loss of pulmonary blood vessels. We have recently demonstrated that chronic hypoxia leads to angiogenesis in the adult rat pulmonary circulation and increased area of the gas exchange membrane, a potentially beneficial adaptive response (Howell, K. *et al.*2003).
Recent reports suggest that inhibition of the RhoA/Rho-kinase (ROK) pathway can prevent the development of hypoxic PH. However, blockade of this pathway has also been shown to prevent migration of endothelial cells in-vitro. Hence, we hypothesised that the ROK inhibitor Y27632 would inhibit the pulmonary angiogenesis induced by sustained hypoxia.
Experiments were conducted on male Wistar Kyoto rats, anaesthetised (pentobarbital sodium 60mgkg^{-1}) and killed by exsanguination. We assessed the pulmonary artery pressure (PAP) in isolated blood perfused lung from 3 different groups of rats: a control group, a chronically hypoxic (1week, $FiO_2$0.1, CH) group and a CH group orally treated with Y27632 (30mgkg^{-1}day^{-1},CH-Y27632).
In the CH group the mean (±SEM) PAP (13.7±0.4 mmHg, n=7) was significantly higher than that in the other groups (ANOVA, $P<0.001$), whereas the PAP was similar in both CH-Y27632 (9.5±0.4, n=7) and control groups (9.3±0.2 mmHg, n=7). Acute inhibition of ROK nearly normalized the PAP in the CH group, but had no effect in control and CH-Y27632 group.
The total intra-acinar vessel length was significantly increased in CH lungs (9236±630 cmLL^{-1}, $P<0.001$) and CH-Y27632 (7864±508 cmLL^{-1}, $P<0.05$) when compared to the controls (5327±392 cmLL^{-1}). The vessel lumen diameter was similar in all groups, whereas the ratio of wall thickness to lumen diameter was significantly ($P<0.05$) increased in the CH group, indicating that the vessels had remodelled. The endothelial surface area, the capillary length and the capillary volume were significantly increased in the CH lungs when compared to the two others groups.
These data suggest: 1) that in chronic hypoxic PH the increase in pulmonary vascular resistance is not due to structural encroachment of the arteriolar wall into the lumen but predominantly due to RhoA/ROK mediated vasoconstriction and 2) that the RhoA/ROK pathway plays a key role in hypoxia-induced pulmonary angiogenesis.

HOWELL, K., PRESTON, R. J. & MCLOUGHLIN, P. (2003). Chronic hypoxia causes angiogenesis in addition to remodelling in the adult rat pulmonary circulation. *J.Physiol.* **547**, 133-145.

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

C176

Baroreceptor dysfunction induced by hypercholesteremia in the guinea pig

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We have recently demonstrated that dietary-induced hypercholesteremia causes cardiac concentric remodeling in the guinea pig. The autonomic nervous system has been implicated in mediating changes in cardiac size and geometry and is likely to play a role in the expression of this remodeling. The purpose of this study was to determine whether the baroreceptor reflex control of cardiac sympathetic and parasympathetic nerve activity was disturbed in cholesterol-fed guinea pigs.
Groups of guinea pigs were fed certified guinea pig chow with or without 1% cholesterol for 13 weeks (n = 4 per group). The guinea pigs were anesthetized with ketamine (120 mg/kg ip)-acepromazine (12 mg/kg ip) and a catheter was placed in a jugular vein to administer drugs and into a carotid artery to determine pulsatile (PAP) and mean (MAP) arterial blood pressures and heart rate. The guinea pigs were allowed at least 4 days to recover from surgery. The guinea pigs received bolus doses of the

alpha-1-adrenoceptor agonist, phenylephrine (1-40 mg/kg iv), to elicit pressor responses and bolus doses of the nitric oxide-donor, sodium nitroprusside (1-40 mg/kg iv), to elicit depressor responses. The maximal changes in heart rate in response to the changes in MAP were recorded and subjected to sigmoidal curve fitting analyses. Differences between means were analysed by repeated measures ANOVA

Cholesterol fed guinea pigs had a similar MAP to controls (77 ± 4 vs 75 ± 4 mmHg, $P \langle 0.05$). Resting heart rate of cholesterol fed guinea pigs were also similar to controls (249 ± 9 vs 269 ± 13 bpm, $P \langle 0.05$). The sensitivity (gain, Gave) of the baroreflex was markedly diminished in cholesterol-fed as compared to control guinea pigs (2.63 ± 0.14 vs 4.08 ± 0.20 bpm/mmHg, $P \langle 0.05$). The heart rate range (upper plateau-lower plateau) of the baroreceptor heart rate reflex was markedly impaired in cholesterol-fed as compared to control guinea pigs (69 ± 6 vs 178 ± 11, bpm, $P \langle 0.05$). The maximal increases in heart rate for the cholesterol-fed and control animals were 34 ± 6 and 92 ± 10 bpm, respectively ($P \langle 0.05$) whereas the maximal decreases in heart rate were 35 ± 6 and 90 ± 8 bpm, respectively ($P \langle 0.05$).

Dietary-induced hypercholesteremia causes impaired baroreceptor reflex function in the guinea pig. Further studies will address whether baroreceptor dysfunction precedes induction of cardiac concentric remodeling and plays a role in changing cardiac geometry.

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

C177

Maximum walking time in patients with peripheral artery disease: prediction of baseline and change following exercise training.

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Patients with peripheral artery disease (PAD) are characterised by suffering intermittent claudication and have greatly reduced functional capacity and limited ambulation. It is commonly believed that peripheral factors relating to limb blood flow are the major determinants of maximum walking time (MWT) (Green 2002). Data obtained from healthy subjects shows vagal cardiac modulation measured by high frequency spectral power (HF), is related to changes in exercise capacity due to training (Hautala *et al.* 2003).

The purpose of this study was to determine whether cardiopulmonary and heart rate variability measures could predict MWT at baseline and change in walk time (ΔMWT) following exercise training.

Twenty eight patients (six women, mean age 59±6) were recruited. Resting HF was derived from a five min ECG recorded in the semi-recumbent position. All patients completed a standard incremental exercise test with two minute stages. Cardiopulmonary measures were made constantly throughout the test (MedGraphics Corporation, $CardiO_2$ CP stress system. St. Paul, Minnesota, USA). Blood pressure, ratings of perceived exertion and ischaemic pain were measured at the end of each stage. Patients were randomly assigned to either a home based (HB) or supervised (SU) exercise group. The exercise group were required to complete two, 30 min sessions of treadmill walking a week. All tests were repeated after 12 weeks. Multiple stepwise regression was performed to determine the best predictors of MWT and ΔMWT.

The SU group showed significant increases in MWT (*t*-test, $P < 0.001$); there was no increase in the HB group. At baseline, peak oxygen consumption was the only predictor of MWT ($R^2 = 0.51$ and S.E.E = 167.8 s). In the SU group, peak oxygen consumption and high frequency spectral power in log units (HFln) both predicted ΔMWT ($R^2 = 0.74$, S.E.E = 90.9 s). Colinearity diagnostics confirmed the independence of these measures.

This study shows that central factors account from much of the variance in MWT in PAD patients. Higher oxygen consumption and greater cardiac vagal modulation (HFln) at baseline were indicators of a greater training response to exercise. This work indicates that those involved in rehabilitation can use baseline measures to guide the intensity of training and thus optimise their programmes.

All work conformed to the standards set by the declaration of Helsinki.

Green, S. (2002) Clinical Physiology and Functional Imaging **22**, 81-91.

Hautala, *et al.* (2003) American Journal of Physiology Heart and Circulatory Physiology **285**, H1747-1752.

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

C178

A diet rich in polyunsaturated fats lower offspring blood pressure independent of peripheral artery function.

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Western diets high in saturated fatty acids (SFA) are associated with increased risk of cardiovascular disease. Whilst diets rich in polyunsaturated fatty acids (PUFA) have been found to be cardio protective. Increasing evidence suggests that, at least in part cardiovascular disease may have its origin in utero, as a direct consequence of maternal dietary imbalance. Previously we have reported distinct effects of a SFA and PUFA maternal diets on offspring blood pressure at one year (LHS ref Br. J. Clin. Pharm). Systolic blood pressure in 1 year old offspring of PUFA fed dams were 20 mmHg lower than in the offspring of SFA fed dams

The aim of the current study was to investigate vascular function in the adult offspring of dams fed either a SFA or PUFA diet during pregnancy. The two diets are representitative of typical western and Mediterranean diets.

Sprague-Dawley rats were fed a diet supplemented with either SFA (Palm oil 20% w/w) or PUFA (Corn oil 20% w/w) for 10 days before, during pregnancy and suckling. After weaning all the offspring were fed a normal maintenance diet. Offspring were killed with an overdose of CO2 at 1 year of age. Third order branches of the mesenteric arteries were removed and mounted on a Mulvany-Halpern small vessel myograph. Constrictor func-

tion was assessed using noradrenaline (10-7 to 10-5 M), and the thromboxane analogue U46619 (10-8 to 10-6 M). Dilator function was assessed using acetylcholine (10-9 to 10-5 M) and nitric oxide (10-8 to 10-4).

There was no significant difference in the endothelium-dependent relaxation to Acetylcholine [(% relaxation: PUFA 94.8±2.12 versus SFA 87.3±3.9; n=5); (EC50: PUFA-7.56±0.02 versus SFA-7.49±0.05)], endothelium-independent relaxation to NO (% relaxation: PUFA 92.8±3.01 versus SFA 91.1±1.2; n=5) or to the constrictors noradrenaline and U46619 in third order mesenteric arteries at 1 year. Values are given as the mean ± SEM and statistical significance determined using a students t-test ($P<0.05$).

In summary, a high fat diet rich in PUFA compared to SFA during pregnancy and suckling permanently programs a blood pressure lowering effect in the adult offspring of PUFA fed dams compared to SFA fed dams. The vascular function in the same animals did not vary significantly between the groups indicating that the programming of blood pressure by maternal diet in this model is independent of small artery function.

R Jensen, P.D. Taylor, L. Poston (2004)

Developmental programming of blood pressure through high fat feeding during gestation and suckling. Poster Demonstration and Oral Presentation. Proceedings in London Hypertension Societ, British Journal of Clinical Pharmacology (in press).

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

C179

Diabetic plasma prevents the increase in platelet aggregation seen in a rat model of diabetes

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Thrombotic disease, a major complication in diabetic patients, is associated with increased platelet reactivity[1]. However, some data from experimental animal models suggest that platelets in diabetes are similarly or less reactive than control platelets[2,3]. *In vivo* we have previously reported that platelet aggregation is less in streptozotocin (STZ) diabetic rats as compared to control rats[4]. In this study, we examined aggregation responses *in vitro* in both washed platelets and platelets in the presence of plasma in this same model.

Male Wistar rats were rendered diabetic by intraperitoneal injection of STZ 50mg/kg (n=6). Control rats were injected with saline (n=6). Experiments were performed 21 days post-injection. Blood glucose concentration was 6.3 ± 0.2mM in controls and 28.3 ± 0.8mM in STZ-treated rats ($P<0.01$). Rats were anaesthetised with urethane 1.5g/kg i.p. and blood was collected from the abdominal aorta into trisodium citrate then centrifuged to obtain platelet-rich plasma (PRP). Platelets were obtained by centrifugation of PRP and resuspended in either Ca^{++}-free Tyrode solution, platelet-poor plasma (PPP) from control rats or PPP from STZ-treated rats. Responses were measured to ADP (0.3-30μM) using standard Born aggregometry. Data are mean ± SEM. Statistical analysis was by two-way ANOVA with $P<0.05$ taken as significant.

ADP-induced aggregation was increased in washed platelets from STZ-treated rats as compared to controls (Fig.1). In contrast, platelets from control or STZ-treated rats resuspended in PPP from STZ-treated rats exhibited reduced aggregation, as compared with the same platelets resuspended in PPP from control rats (Fig.2). In conclusion, platelets from STZ-treated rats are inherently more sensitive to ADP-induced aggregation than platelets from controls. However, in the presence of plasma from STZ diabetic rats, platelet responsiveness is reduced, suggesting that one or more factors are present in diabetic plasma which inhibit this aggregation. The nature of such factors remains to be elucidated.

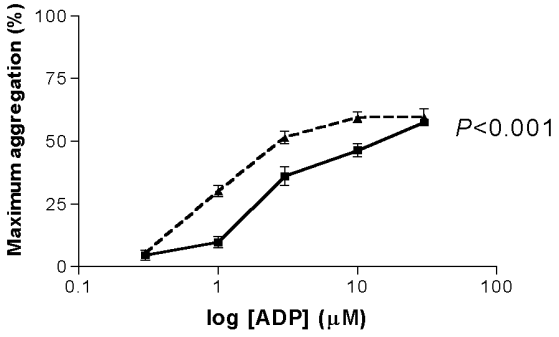

Figure 1. Aggregation of washed platelets from control (solid line) and STZ-treated (broken line) rats

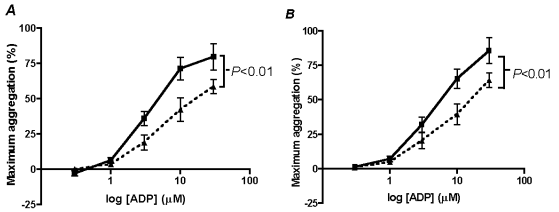

Figure 2. Graphs show aggregation of platelets from control rats (panel A) and platelets from STZ-treated rats (panel B) suspended in plasma from control rats (solid line) or plasma from STZ-treated rats (broken line)

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

C181

Are T-type calcium channels causally involved in neonatal cardiac myocyte hypertrophy?

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Cardiac myocyte hypertrophy although initially a compensative mechanism seen in response to stresses such as hypertension or myocardial infarction often becomes a major cardiovascular risk factor leading to congestive heart failure and death. T-type cal-

cium channels have been associated with cardiac hypertrophy (Nuss & Houser, 1993) but it is unclear whether they play a direct causal role. This study aims to address this question.

Neonatal rats (P1-P2) were killed by cervical dislocation and cardiac myocytes isolated by enzymatic digestion. Myocytes were cultured without electrical stimulation for 96 hours to allow 24 hours recovery, 48 hours quiescence in 0% serum then 24 hours with either 20% serum to induce hypertrophy or 0% serum control (Vara et al. 2003).

Cell size, measured as cell area under the microscope, increased significantly ($p<0.05$, t test) from 739 ± 48 μm^2 (mean ± sem, n=288 cells from 8 preparations) in control cells maintained in 0% serum to 1579 ± 51 μm^2 (mean ± sem, n=288 cells from 8 preparations) with 20% serum, a 2.2-fold increase indicating that the cells had undergone hypertrophy.

Both groups were then treated with 3 different calcium channel blockers, Mibefradil, TTL1177 (formally TH-1177, Haverstick et al. 2000) and verapamil. These compounds all caused a significant ($p<0.05$ for all groups ANOVA) dose-dependent reduction in the induced hypertrophy as measured by cell size with IC50 values of 5.8 ± 1.3 μM) for Mibefradil, 5.0 ± 2.3 μM) for TTL1177 and 14.0 ± 2.3 μM for Verapamil (all values are means ± s.e.m, n=3 preparations; 36 cells at each of 4-6 concentrations for each preparation). These values are consistent with those known to block the CaV3.1 and CaV3.2 isoforms of T-type calcium channels shown by PCR to be present in these cells. Hypertrophy was not inhibited by verapamil at concentrations sufficient to block almost all L-type calcium channels and the value given here is consistent with T-type rather than L-type calcium channel block. Similar dose-dependent reduction in the hypertrophy-induced increase in cell size was also seen with these calcium channel blockers when hypertrophy was induced by either 100 nM phenylephrine or 10 nM endothelin-1.

These data suggest that blocking T-type calcium channels is sufficient to inhibit the induction of hypertrophy seen in neonatal myocytes on application of hypertrophic stimuli and suggest that T-type calcium channels could play a direct causal role in cardiac hypertrophy.

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

PC162

Role of adenosine in hypothalamic mechanisms of body temperature regulation in conscious rats

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Adenosine is considered to be a major non-peptide neuromodulator in the brain and its receptors have been demonstrated to be present in the hypothalamus - in areas responsible for fine regulation of body temperature (Tb) and the development of fever. In this study we investigated the potential role of adenosine in the hypothalamic mechanisms of Tb regulation.
Experiments were performed in adult male Wistar rats (280-350 g) and were approved by the Institutional Animal Care and Use Committee. Rats were anaesthetised (ketamine (87.0 mg/kg) + xylazine (13.0 mg/kg)), steel guide cannula was implanted into the anterior hypothalamus (AH), and a telemetry transmitter was implanted into the abdomen for monitoring Tb. Experiments were performed after a 7-day recovery period. Fever was induced by intraperitoneal injection of *E.coli* lipopolisaccharide (LPS; 50 μg/kg). Effects of intrahypothalamic administration of the adenosine receptor antagonist 8-(p-sulfophenyl)-theophylline (8-SPT, 2.5 μg, 1 μl), ecto-5′-nucleotidase inhibitor α,β-methylene ADP (α,β-meADP, 100 μg, 1 μl), A1 receptor agonist 2-chloroadenosine (5 μg, 1 μl) or artificial cerebrospinal fluid (aCSF, 1 μl) on Tb in febrile and afebrile rats were determined. The rat was humanely killed by overdose of anaesthetic at the end of the experiment.
In conscious rats blockade of adenosine receptors in the AH following microinjection of 8-SPT resulted in a marked and long-lasting increase of Tb. Two hours after the injection of 8-SPT Tb of rats was 38.1±0.2°C (n=8) some 0.7°C higher than the Tb of rats injected with aCSF (37.4±0.1°C; n=7, $p<0.05$, Student′s t test). Four hours after treatment with 8-SPT Tb of afebrile rats was 38.6±0.3°C (n=8) some 1.2°C higher than in rats treated with aCSF (37.4±0.1°C; n=7, $p<0.05$, Student′s t test). Similar increases in Tb (38.7±0.3°C; n=6, $p<0.05$, Student's t test) were observed in rats treated into the AH with ecto-5′-nucleotidase inhibitor α,β-meADP. When the A1 receptor agonist 2-chloroadenosine was injected into the AH at the peak of LPS-induced fever a profound decrease in febrile Tb was elicited. Three hours after intrahypothalamic injection of 2-chloroadenosine Tb of febrile rats decreased to 37.6±0.1°C (n=8), 1.1°C lower than in febrile rats three hours after treatment with aCSF (38.7±0.2°C; n=6, $p<0.05$, Student′s t test).
From these data we conclude that adenosine is released tonically at the level of the AH and that this has an action in the maintenance of Tb under euthermic conditions. We also suggest that adenosine-mediated signalling may also play a role in the hypothalamic mechanisms controlling the magnitude of the febrile response.

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PC163

Disparate conduction slowing in guinea pig left and right atrium during gap junction uncoupling

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Gap-junction (GJ) channels, composed of connexin (Cx) proteins, are important determinants of action potential (AP) propagation in the heart. Chamber-specific patterns of Cx expression contribute to regional differences in conduction velocity (CV). The effects of Cx reduction on CV are poorly understood; Cx-knockout studies produce conflicting results and attempts at pharmacological manipulation have been hindered by effects on active membrane properties, which also determine CV. Carbenoxolone, a Cx43-uncoupler, has recently been shown to reduce rabbit ventricular and atrial CVs without altering membrane ionic currents (de Groot et al, 2003). CV reductions were of a similar magnitude despite distinct patterns of Cx40 and 43 expression and different control CVs. Our study aimed to explore how CV slowed during GJ uncoupling of right and left guinea pig atrium.
Male guinea pigs were killed by cervical dislocation. Trabeculae from left and right atrial appendages were superfused with Tyrode's solution (5%CO2, 24mM NaHCO3, pH 7.4, 37ºC). Preparations were stimulated with an extracellular bipolar electrode to initiate conducted APs at 1Hz. CV, AP upstroke velocity (Vmax) and the time constant of the AP foot (τap) were measured before, during and after superfusion with 20μM carbenoxolone. Intracellular resistivity (Ri) was calculated from the 1-D cable equation. Data are mean ± S.D. Differences between means ($p<0.05$) were examined by Student's t-test.
Control CVs were similar in the left and right atrium (78.8±4.29 vs.77.6±6.42 $cm.s^{-1}$, respectively n=5), However τap was smaller and Vmax greater in left compared to right atrium (0.20±0.03 vs. 0.33±0.12ms, n=5 p=0.04 and 107.7±5 vs. 75.4±13.5 $V.s^{-1}$, n=5 p=0.003). Calculated Ri, assuming a cell radius of 4.6μm and 4.2μm for left and right atrium respectively (Wang et al, 1999), was significantly greater in the left atrium (192±46 vs. 116±35Ω.cm, n=5 p=0.02). Carbenoxolone slowed CV more in the left atrium (21.3±7.9 vs. 11.8±5.0 % reduction respectively, n=5 p=0.05). In left atrium, Vmax increased from 101.3±5.6 to 138.4±6.3 $V.s^{-1}$ (n=4, p=0.01), AP amplitude (APA) increased from 105.2±1.6 to 118.6±3.3 mV (n=4 p=0.004) and Ri increased from 192±46 to 342±103 Ω.cm (n=5, p=0.04). Values of right atrial Vmax, APA and Ri were not statistically altered by carbenoxolone. The effects on CV were fully reversible on washout, but Vmax remained elevated in left atrial preparations. AP duration (75% repolarisation), τap,and membrane potential were unaffected by carbenoxolone.
Thus carbenoxolone slowed atrial conduction by increasing Ri with a greater effect on the left compared to right atrial appendage.

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

PC164

Cat chemosensory and ventilatory responses to hypoxia are potentiated by exposure to intermittent hypoxia

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It has been proposed that carotid body (CB) chemoreceptors play a main role on the enhanced hypoxic ventilatory response induced by chronic intermittent hypoxia (CIH). Therefore, we studied the effects of cyclic hypoxic episodes of short duration on cat cardiorespiratory reflexes, heart rate variability, and CB chemosensory activity. Cats were exposed to cyclic hypoxic episodes (PO_2 ∽ 75 Torr) repeated during 8 hours for 4 days. Acute experiments were performed the morning of the day after the end of hypoxic exposures. Cats were anaesthetized with sodium pentobarbitone (40 mg Kg^{-1} I.P., followed by 8-12 mg I.V.), and ventilatory and cardiovascular responses to several isocapnic levels of oxygen (PO_2 ∽ 20 to 740 Torr) were studied. After studying of the reflex responses, we recorded the CB chemosensory responses to hypoxia. The experimental protocol was approved by the Ethical Committee of the Facultad de Ciencias Biologicas of the Universidad Catolica de Chile and met the guidelines of the Chilean National Fund for Scientific and Technological Development (FONDECYT). All results expressed as mean ± S.E.M. Results showed that exposure of cats to CIH enhanced the ventilatory responses (VT and VI) to acute hypoxia. The curves for the relationships between PO_2 and VI and VT were significantly different in 4 CIH cats as compared with 6 control cats ($P < 0.001$, two-way ANOVA) and the Bonferroni test showed that VI and VT were statistically higher ($P > 0.05$) at a PO_2 of about 20 Torr. Similarly, exposure to CIH increased basal CB discharges (63.2 ± 7.3 vs. 36.3 ±5.1 Hz, $P < 0.05$, Student T test, n = 6 CIH and control cats, respectively) and the responses to acute hypoxia ($PO_2 < 100$ Torr, $P < 0.05$ two-way ANOVA followed by Bonferroni test, n = 6). Exposure to CIH did not increase basal arterial pressure, heart rate, or their changes induced by acute hypoxia. However, we found that the low/high frequency ratio of heart rate variability was significantly higher ($P < 0.001$, Student T Test) in 6 CIH cats (2.4 ± 0.1) as compared to 6 control cats (0.6 ± 0.1). Thus, the enhanced CB reactivity to hypoxia may contribute to the augmented ventilatory response to hypoxia, as well as to modified heart rate variability due to early changes in autonomic activity.

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PC165

Baroreflex regulation of renal sympathetic nerve activity in anaesthetised rats with heart damage.

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In man and in animal models of heart failure, there is activation of the sympathetic nervous system (SNS) to increase cardiac output to meet the metabolic demands of the body. There is evidence from animal models using coronary artery ligation induced heart failure, that there is a derangement in the arterial baroreceptor regulation of the SNS. A less well investigated model is the high output model, using ateriovenous fistulae or catecholamine administration, to produce an increase in cardiac output leading to ventricular damage (Leenen et al, 2001). This study aimed to determine whether the baroreflex regulation of renal sympathetic nerve activity (RSNA) was deficient in a rat model of catecholamine mediated cardiac damage.

All experiments accorded with current European legislation. Male Wistar rats, 180-240g, were kept for 2 weeks on a regular diet and given either tap water to drink or water containing caffeine (at a concentration to deliver 80mg/kg/day) plus sc isoprenaline (1mg/100g) every 72h. The animals were anaesthetised, 1ml chloralose/urethane (16.5/250mg/ml) ip, and prepared for recording activity in the sympathetic nerves to the left kidney. Phenylephrine and nitroprusside, each of 10 μg, were given iv over 40s to increase and decrease blood pressure, respectively and the changes in RSNA and heart rate were used to generate baroreflex curves (Kent et al, 1972). The animals were killed using an anaesthetic overdose. Means ± SEM were subjected to the Students 't' test and significance taken when $P<0.05$.

The rats given water to drink increased body weight from 180±10 to 231±8 g which was greater ($P<0.05$) than in rats given caffeine and isoprenaline (207±5 to 240±9g). In the acute study, basal blood pressure was 103±2 mmHg in the control rats but was slightly lower, at 94±4 mmHg in the caffeine/isoprenaline treated rats. The range of the baroreflex curve (A1) for RSNA was similar in both control and experimental groups of rats, 93±2 versus 97±1μV/s but the slope (A2) was lower in the experimental group (0.14±0.01 versus 0.20±0.05 μV/s/mmHg, $P<0.05$) as was the mid point blood pressure (115±2 versus 100±3 mmHg, $P<0.05$). By contrast, A4, the range of RSNA, was greater in the experimental than control groups (54±8 versus 33±2, μV/s, $P<0.05$). These data show that in this model of catecholamine induced heart damage, the animals grow more slowly, and there is a depression in the sensitivity of the baroreflex regulation of RSNA. Whether this defect in baroreflex control resides in the afferent, central or efferent limbs of the reflex remains to be resolved.

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PC167

Hypoxia induces changes in gene expression pattern in carotid body cells and sensory neurons

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The carotid body (CB) is composed of chemoreceptor cells that are innervated by sensory neurons. Exposure to chronic hypoxia enlarges the CB and increases its chemosensory response to acute hypoxia. Considering the time required by

hypoxia to enhance CB chemoreception, changes in the pattern of gene expression in CB cells and/or sensory neurons are to be expected. Therefore, we studied the gene expression profile induced by chronic hypoxia in CB cells and sensory neurons. Cells from the CB and the petrosal-jugular-nodose (PJN) complex ganglion were obtained from 64 Sprague Dawley rats (100 g) anesthetized with sodium pentobarbitone (40 mg/kg i.p.). Each experiment was repeated 3 times. 8 CB and 8 PJN were cultured in normoxic (95% air - 5% CO2) and hypoxic conditions (95% N2 - 5% CO2) at 37°C for 1 week. The total RNA was extracted and amplified using a RiboAmp Kit (Arcturus). The amplified mRNA was used as a template to synthesized cDNA-32P, which was hybridized on macroarray membranes containing 1176 genes (Clontech), and revealed in a phosphoimager. The images obtained were analyzed with the Atlas-Image Software (Clontech). We considered a threshold ratio (RT) (hypoxia/normoxia) of 2.0 and a threshold difference (DT) of 15000 pixels of intensity to be a significant change in gene expression. The experimental protocol was approved by the Ethical Committee of the Universidad Catolica de Chile.The CB cells expressed 203 genes (17.3%) in normoxia, 252 genes (21.4%) under hypoxia, and 132 genes in both normoxic and hypoxic conditions (11.2%). In the PJN cells, 104 genes (8.8%) were expressed in normoxia, 149 genes (12.7%) in hypoxia, and 81 genes in both conditions (6.9%). After one week of hypoxia, 20 genes increased their RT or DT expression, and 11 genes decreased their expression in CB cultured cells, whereas 5 genes increased and 12 genes decreased their RT or their DT expression in the PJN cultured cells. For CB cultured cells, only 4 genes showed RT and DT above the threshold (c-jun prontooncogen, G1/S cyclin c, intrinsic factor precursor, rab GDI-β species), while 6 genes showed RT and DT below the threshold (medium chain acyl CoA dehydrogenase, cytocrome oxidase, liver arginase, puctative v-fos transformation effector protein, proteasome subunit RC7-1, gastric inhibitory polypeptide). In the PJN cultured cells, only 2 genes showed RT and DT above the threshold (G2/M cyclin specific G, serine proteinase-rPC7 precursor), while 4 genes showed RT and DT below the threshold (dopamine transporter, alcohol dehydrogenase A subunit, putative v-fos transformation effector protein). Our results suggest that CB cells and PJN neurons change their gene expression during one week of exposure to hypoxia.

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PC168

Intracarotid hypertonic saline activates PVN and sympathetic neurones by a PVN spinal pathway in anesthetised rats

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Previous studies have shown that orthograde injection of hypertonic saline into one carotid artery can activate neurones in the organum vasculosum lamina terminalis (OVLT) which in turn excites parvocellular neurones in the paraventricular nucleus of the hypothalamus (PVN) and thus may effect neurones influencing arterial blood pressure (Chen & Toney, 2001). In the present study we sought to show that the osmotic stimulus also activates sympathetic neurones to the kidney and to determine whether this is via a PVN spinal pathway.

In 10 Wistar rats anesthetised with urethane, chloralose mixture (650 mg kg-1, 50 mg kg-1) i.v., a catheter was placed orthogradely in the right carotid artery for injection of hypertonic saline (1.7 M, 0.1 ml NaCl) and normal saline (0.9% NaCl) as a control. Arterial blood pressure (BP) and heart rate (HR) were monitored and renal sympathetic nerve activity (RSNA) was recorded from a renal nerve exposed retroperitoneally on the left side. Change in the efficacy of spinal pathways was tested by intrathecal (i.t.) application of drugs via a catheter inserted via the foramen magnum so that its tip lay at T10. The animal was placed in a stereotaxic frame and a glass micropipette was positioned in the PVN for injection of drugs. Statistical analysis was performed using a two-tailed, paired Student's t test. Rats were killed by overdose of urethane anaesthetic at the end of the experiment. Intracarotid injection of NaCl significantly increased RSNA by 48.9 ±6.1% ($p<0.05$). HR was significantly decreased by -36.1±1.4 b.p.m. ($p<0.05$). BP was little changed (3.3±0.8 mm Hg). This action of NaCl was virtually abolished by prior microinjection of the glutamate receptor antagonist kynurenic acid (4mM, 100 nl) into RSNA exciting sites in the PVN (RSNA 5.9±2%,$p<0.01$; BP no change; HR decreased by -5.9±2.1 b.p.m., $p<0.05$). Spinal kynurenic acid (4mM, 10µl, i.t.) also reduced the RSNA response to intracarotid NaCl to 10.9±61.% ($p<0.01$), BP 0.8±0.9% (ns) and HR -16.5±4.5 b.p.m., $p\leq0.05$, (n=7). Similarly i.t. application of V1a antagonist ((β-mercapto-β,β-cyclopentamethyleneprop ionyl[1],-O-Et-Tyr[2], Val[4], Arg[8]) vasopressin, Sigma, 0.05mM, 10µl) before NaCl virtually abolished all the changes (RSNA 2.6±5.7%, BP 1.5±0.6 mm Hg, HR -7.8±2.2 b.p.m., $p<0.01$, n=9).

From these data we suggest that osmoreceptor stimulation in the forebrain mediates cardiovascular changes via a glutamate dependent synapse in the PVN which then activates glutamate and vasopressin neurones projecting to spinal sympathetic neurones.

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