C59

Peak cardiac power outputs in human patients with peripheral vascular disease

L.D. Hodges[1], S. Gavin[2], S. Das[3] and D.A. Brodie[1]

[1]Research Centre for Health Studies, Buckinghamshire Chilterns University College, Buckinghamshire, UK, [2]Department of Health and Human Sciences, London Metropolitan University, London, UK and [3]Faculty of Medicine, Imperial College, London, UK

Peripheral vascular disease (PVD) is a condition characterised by atherosclerotic occlusive disease of the lower extremities, low functional capacity and low exercise tolerance. Little empirical data are available concerning the cardiovascular response to maximum exercise tests in patients with PVD. The purpose of this study was to examine cardiovascular variables in patients with peripheral vascular disease.

Fifty patients (67 ± 9 years) completed an incremental exercise test (2 min stages, 3.2 km/h, with increases of 2% every 2 min) to maximum claudication pain. Peak oxygen uptake ($\dot{V}O_{2peak}$) was assessed on a breath-by-breath basis by online expiratory gas analysis (CardiO2, Medical Graphics Corp., St Paul, MN, USA). Following a 30 min rest period, patients exercised at the highest level attained during the first test and cardiac output ($\dot{Q}_T$) was measured using the non-invasive rebreathing method (Defares, 1958). Cardiac power output peak (CPO_{peak}), in Watts (W), was then computed using the equation described by Cooke et al. (1998).

Mean ± SD values were; $\dot{V}O_{2peak}$ 13.85 ± 4.14 ml kg min^{-1}; maximum walk time (MWT) 357 ± 227 s; mean arterial pressure peak 127 ± 15 mmHg; $\dot{Q}_T$ 9.8 ± 2.39 l min^{-1}; CPO 2.86 ± 0.87 W. Regression analysis showed CPO scaled for weight to be the best independent predictor of MWT (r = 0.606, Standard error of estimate, 3.31).

Patients with peripheral vascular disease demonstrate attenuated levels of cardiovascular capacity, equivalent to values reported for heart failure patients (Williams et al. 2001). Measurement of CPO may provide additional information to stratify individuals who are at a higher risk of mortality.

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

C60

Muscle vasodilatation in acute systemic hypoxia in the rat: modulation by oestrogen

C.L. Tomlinson, A.M. Coney and J.M. Marshall

Department of Physiology, The University of Birmingham, Birmingham, UK

Premenopausal women have a reduced incidence of cardiovascular disease compared to men of a similar age and postmenopausal women: the vascular effects of oestrogen (E_2) have been implicated (Farhat et al. 1996). Acute systemic hypoxia induces vasodilatation in skeletal muscle that is partly adenosine mediated acting via an increase in nitric oxide synthesis (NO; Ray & Marshall, 2002). E_2 can facilitate NO synthesis (Chen et al. 1999), thus, we hypothesised that E_2 may facilitate hypoxia-induced muscle vasodilatation.

Experiments were performed on anaesthetised (Saffan; 4-8mg kg^{-1} h^{-1} i.v.) age-matched male (n=12; 233g±2g) and female Wistar rats, the latter being divided into low E_2 (n=9; 163±4g) and high E_2 (n=5; 184±11g) groups: oestrous cycle stage was determined by vaginal smears. The response evoked by breathing 12% O_2 for 5min was tested before and 30, 60 and 90min after administration of 17β-oestradiol (10μg kg^{-1} i.v.). Arterial blood pressure (ABP) and femoral blood flow (FBF) were recorded and femoral vascular conductance (FVC=FBF/ABP) was calculated. Results are expressed as mean ± S.E.M.

In male rats, systemic hypoxia evoked a fall in ABP (30±4mmHg, $P<0.001$) and an increase in integrated FVC (of 1.03±0.1 CU, $P<0.05$). After administration of E_2 the hypoxia-induced increase in integrated FVC was depressed (0.67±0.08, 0.41±0.06, 0.44±0.05 CU at 30, 60 and 90min after E_2 respectively: $P<0.01$ ANOVA and Dunnets post hoc test). In low E_2 females, hypoxia evoked a similar increase in integrated FVC (1.03±0.3 CU) to that of males, and after E_2 administration, hypoxia-induced increases in integrated FVC were also depressed (to 0.59±0.2, 0.4±0.09, 0.4±0.12 CU, $P<0.05$). However, hypoxia-induced increase in integrated FVC in high E_2 females was only 50-60% relative to males *before* E_2 administration (0.56±0.12 CU) and no further depression after acute administration of E_2.

These results contrast with our hypothesis. They suggest that in female rats, when endogenous E_2 is high, hypoxia-induced muscle vasodilatation is already depressed. A similar depression can be induced in males and low E_2 females by administration of exogenous E_2. We therefore propose that E_2 affects the balance of vasoconstrictor and vasodilator influences that are exerted upon muscle vasculature in hypoxia. E_2 may decrease the vasodilator component, which is mediated by adenosine and NO and/or facilitate the vasoconstrictor component, which is mediated by sympathetic activity and vasoconstrictor hormones.

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C61

Vasoconstrictor responses in rat femoral artery: effects of early chronic systemic hypoxia

S. Mukerji, P. Kumar and A. Coney

Physiology, University of Birmingham, Birmingham, UK

Vascular tone is dependent on the balance of vasoconstrictor and vasodilator influences. Systemic hypoxia induces vasodi-

latation in skeletal muscle (Coney et al. 2004). This effect is observed when the hypoxia is applied acutely for 5min and also persists when the hypoxia is maintained chronically (CH) for up to 3 days. A role for adenosine and nitric oxide has been proposed for this effect (Walsh & Marshall, 2006). However, changes also occur in vasoconstrictor mechanisms and 3-4 weeks of CH results in attenuated adrenergic vasoreactivity of aortic rings (Doyle & Walker, 1991) and a reduction in sympathetically-evoked vasoconstriction *in vivo* (Coney et al. 2004). In this study we hypothesised that there might be an effect on vasoreactivity in the first few days of CH which may contribute to the tonic vasodilatation in skeletal muscle vessels.

Male Wistar rats (200-300g) were divided into 3 groups: Normoxic (N; n=13), 1 day CH (1CH; n=12) and 3 day CH (3CH; n=5). CH was induced in an hypoxic chamber maintained at 12% O_2 (Coney et al. 2004). Femoral arteries were excised under terminal anaesthesia (halothane 1-3% in O_2) rings (3-4mm length) were mounted on a Mulvany myograph bubbled with 95% O_2:5% CO_2 and normalised to 100mmHg. Responses to the α_1-agonist, phenylephrine (PE; 10, 100 & 300μM) were measured. The maximum constriction that could be totally blocked by phentolamine (1mM) was determined and all tensions expressed as a percentage of this maximum. Data are presented as mean±SEM and analysed by ANOVA and post hoc test. Significance was taken as $P<0.05$.

The maximum tension recorded was not significantly different between all groups. In rings from N rats, PE induced a dose-dependent increase in tension (34±2, 52±2 and 68±2%, respectively). Rings from 1CH also showed a dose-dependent increase which was significantly increased, compared to N rings, at 10 and 100μM (50±3 and 67±3% respectively) but was not significantly different at 100μM (71±4%). In contrast, 3CH rings showed no dose-dependent increase (29±3, 33±3 and 41±4% respectively) with all responses significantly depressed compared to N rings.

Our data demonstrates that the ability of α_1-adrenoceptors to induce vasoconstriction in rat femoral arteries can be modulated differentially after 1 and 3 days of chronic hypoxia. Specifically we propose that, after 1 day of CH, attenuated adrenergic vasoreactivity does not contribute to the tonic vasodilatation seen at this time but it may contribute after 3 days of CH.

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C62

NPY receptor contributions to sympathetic responses of tail artery in streptozotocin-diabetic rats are increased: molecular and functional evidence

E. Kerlin[1], Y. Zhao[2], D. Bell[2], N. Scholfield[1] and C. Johnson[1]

[1]Physiology, Queen's University, Belfast, Belfast, UK and [2]Phamacology & Therapeutics, Queen's university, Belfast, UK

Diabetes is associated with several cardiovascular problems, including systemic hypertension. Recent studies using the streptozotocin-induced diabetic rat model indicated increased contributions from purinergic and adrenergic sympathetic cotransmitters to responses in tail arteries (Donnelly et al. 2004). NPY is a known co-transmitter, thought previously to act exclusively via NPY-Y1 receptors in normal tail arteries (Bradley et al. 2003). In this study we used molecular and functional techniques to determine changes in expression of NPY Y1 and Y2 receptor subtypes and their contribution to vasoconstriction in diabetic rat tail arteries.

Sprague-Dawley rats (8 weeks, male) were made diabetic by injection (60mg kg^{-1}, i.p.) of streptozotocin, and maintained for 12 weeks. Animals having a blood glucose with values >10 mM were deemed diabetic (n=9, blood glucose 39±2 mM). Tail arteries were excised after animals had been killed and RNA extracted (1 preparation from 2-3 vessels). Expression of NPY receptor subtype mRNA was determined by real time PCR normalized to GAPDH mRNA.

In control tail arteries, NPY Y1 was expressed more abundantly than NPY Y2 mRNA (mean±SEM, cycle times (Ct) for tail arteries 30.4±0.3 and 33.5±0.6, n=2). NPY Y1 and Y2 receptor mRNAs were elevated (12±2-fold and 90±47-fold, respectively, n=2) in diabetic vs. control tissue. Endothelium was removed and tail artery rings cut into 3-5 mm lengths. Isometric contractions were measured in response to 5 electrical stimuli (5 impulses, 1ms duration at 20Hz) delivered every 90 seconds. Specific NPY Y2 antagonist, BIIE0246 (100 nM), reduced diabetic responses by 29±8% (from 0.31±0.04 to 0.22±0.03 g, n=11, $P<0.01$) but did not change control responses. NPY Y2 receptor agonist, PYY_{3-36} (10^{-12}–10^{-7} M) had no direct vasoconstrictor affect on either tissue. Electrically evoked diabetic tissue responses were increased by 65±11% (from 0.36±0.11 to 0.44±0.1 g, n=3, $P<0.05$, paired Student's t test) but were unaffected in controls. Specific NPY Y1 receptor antagonist BIBP3226 (1 μM) reduced responses in both diabetic and control tissue (diabetic: 39±9%; 0.36±0.06 to 0.22±0.05 g, n=6, $P< 0.05$; control: 36±18%, from 0.50±0.28 to 0.37±0.27 g, n=2, $P< 0.05$). There was no significant difference in this reduction between tissues (unpaired Student's t test).

These preliminary data indicate an increase in mRNA NPY Y1 and Y2 receptors in diabetic rat tail artery, evident functionally as an increased contribution from Y2 receptors in sympathetically evoked contraction.

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C92

Gene expression profile of adhesion molecules and cytokines in the nucleus tractus solitarii of the spontaneously hypertensive rat (SHR)

H. Waki, S. Kasparov and J.F.R. Paton

Physiology, Bristol Heart Institute, University of Bristol, Bristol, UK

Human essential hypertension is a complex polygenic trait with underlying genetic components that remain unknown. Since the brainstem structure – the nucleus of the solitary tract (NTS) - is a pivotal region for regulating the set-point of arterial pressure (e.g. Doba & Reis, 1974), we proposed a role for it in the development of primary hypertension. Recently, we identified that junctional adhesion molecule-1 (JAM-1; a leukocyte/platelet adhesion molecule) was over expressed in endothelial cells in the NTS of SHR compared to normotensive Wistar-Kyoto rat (WKY; Waki et al. 2005). Moreover, over expression of JAM-1 by adenoviral gene delivery in the NTS induced hypertension in the WKY indicating that JAM-1 may be pro-hypertensive (Waki et al. 2005). In the present study, we have assessed the level of gene expression of other major leukocyte/platelet adhesion molecules and cytokines in the NTS of SHR relative to WKY.

The NTS was micro-dissected from 15-week-old male SHR (n=5) and WKY (n=5) rats. Total RNA was extracted and real-time RT-PCR was performed. Expression of target genes relative to an internal control, β-actin, was derived in each sample using the comparative ($2^{-\Delta\Delta CT}$) method. Data were expressed in fold differences against the average value of WKY.

Gene expression of monocyte chemoattractant protein -1 (MCP-1) was significantly higher in the NTS of SHR (SHR: 1.70±0.14, n=5; WKY: 1.08±0.21, n=5, $p<0.05$) while interleukin-6 (IL-6) was significantly lower in the NTS of SHR compared to the WKY (SHR: 0.41±0.12, n=5; WKY: 1.03±0.12, n=5, $p<0.01$). In contrast, expression of adhesion molecules including: intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and junctional adhesion molecule-3 were not different.

Our data suggest that the NTS of the SHR exhibits a specific inflammatory state. The high expression level of mRNA JAM-1 and MCP-1 and lowered IL-6 in the NTS of the SHR is a unique characteristic. Whether this expression profile contributes to the hypertensive state via alteration of neuronal circuitry regulating cardiovascular autonomic activity remains to be resolved.

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C93

Selective attenuation of sympathetic component of the baroreflex by angiotensin II in the nucleus tractus solitarii

J.W. Polson[1], R.A. Dampney[2], P. Boscan[3], A.E. Pickering[1] and J.F.R. Paton[1]

[1]Physiology, University of Bristol, Bristol, UK, [2]Physiology, University of Sydney, Sydney, NSW, Australia and [3]Anesthesia & Critical Patient Care,Veterinary Medical Teaching Hospital, University of California, Davis, CA, USA

It is well established that the heart rate (HR) component of the baroreflex is depressed following exogenous application of angiotensin II (ANGII) to the nucleus tractus solitarii (NTS; Casto & Phillips, 1986). However, the effect on baroreflex modulated sympathetic nerve activity (SNA) is unclear. We sought to determine if ANGII in the NTS also depresses baroreflex-evoked sympathoinhibition in the upper-middle and lower thoracic-lumbar sympathetic chain.

Experiments were performed in the rat using the in situ 'working heart-brainstem' (Paton, 1996) and 'decerebrate arterially perfused rat' preparations (Pickering & Paton, 2006). Rats were terminally anaesthetized with halothane, decerebrated precollicularly and perfused via the heart or descending aorta with modified, oxygenated Ringer solution (32°C). Perfusion pressure, HR and SNA either from the inferior cardiac nerve, upper-middle thoracic chain or lower thoracic-lumbar chain were recorded. Perfusion pressure was increased to stimulate baroreceptors and baroreflex changes in HR and SNA were measured before and after injection of ANGII (500 fmol) or the $GABA_A$ agonist isoguvacine (500 pmol) bilaterally into the NTS. Baroreflex gain was calculated as the ratio of either ΔHR or ΔSNA (expressed as % of basal)/Δperfusion pressure. Data are expressed as mean ± SE. Statistical significance was determined using a Student's *t* test.

ANGII in NTS reduced baroreflex gain in HR (54% reduction, control 1.9 ± 0.2 vs 0.9 ± 0.1 bpm/mmHg, n=22, $p<0.01$), inferior cardiac (31% reduction; control 3.5 ± 0.4 vs 2.4 ± 0.4%/mmHg; n=12, $p<0.05$) and upper-middle thoracic SNA (46% reduction; control 3.9 ± 0.6 vs 2.1 ± 0.5%/mmHg; n=5, $p<0.05$) but not in the lower thoracic-lumbar SNA (9.8% increase, control 2.9 ± 0.3 vs 3.2 ± 0.4; n=9, p=0.57). In contrast, inactivating NTS with isoguvacine inhibited both the HR (by 90%, $p<0.01$) and all sympathetic baroreflex components measured (by 65-75%, $p<0.05$).

Our data indicate that ANGII in the NTS does not attenuate the baroreflex inhibition of SNA in a uniform manner, but appears to be organised depending on the spinal level of origin or target organ being innervated.

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C94

High salt intake elevates the prolonged blood pressure and renal sympathetic nerve activity response to intracerebroventricle angiotensin II infusion

B.L. Houghton, C. Huang and E.J. Johns

Physiology, BioSciences Institute, University College Cork, Cork, Ireland

Sodium intake, central angiotensin II (ANG II), and autonomic control of the cardiovascular system may be linked. AT_1 receptor expression in the brain is upregulated by elevated sodium intake (Wang et al. 2003) and impacts on sympathetically mediated renal function (Huang & Johns, 1998). This may lead to a prolonged elevation in BP and renal sympathetic nerve activity (RSNA). We hypothesised that the prolonged BP and RSNA response to intracerebroventricle (ICV) ANGII infusion would be augmented in rats treated on a high salt (HS) diet versus a low salt (LS) diet.

Four week old male Wistar rats were treated on a HS (3.0% NaCl, n=5) or LS (0.03% NaCl, n=5) diet for 6 weeks. Each animal was anaesthetised by 16.5mg/250ml chloralose/urethane I.P. injection. Cannulae were inserted into the right femoral artery (BP measurement) and vein (saline/anaesthetic infusion). A guide cannula was placed into the right lateral ICV for ANGII infusion (25ng/µl, 1 µl/min, 2 min). The left kidney was exposed and recording electrodes were sealed onto a renal nerve. BP and RSNA were averaged over a 5 min period during baseline measurements and 30 min post ICV infusion. Five minutes post ICV infusion, BP and RSNA were averaged over a 1 min period. RSNA was calculated as percentage of baseline values. Means ± S.E.M. were compared by ANOVA. Linear regression analysis compared %ΔRSNA versus %ΔBP at 30 min post ICV infusion. $P<0.05$ indicated significance.

Baseline BP was similar between groups (HS: 89±3mmHg, LS: 84±3mmHg). Five minutes post ICV ANGII infusion, both groups showed a sharp increase in BP (HS ΔBP: 16±3 mmHg, LS ΔBP: 10±1 mmHg, $P<0.05$ for both groups) and reflex reduction in RSNA (HS RSNA: 88 ± 3%, LS RSNA: 78 ± 8%, $P<0.05$ for both groups). Thirty minutes post infusion, only the HS group showed an elevation in BP (HS ΔBP: 5.5±0.4mmHg, $P<0.05$, LS ΔBP: 4±2 mmHg) and RSNA (HS RSNA: 113 ± 2%, $P<0.05$, LS RSNA: 100 ± 3%) compared to baseline values. There was also positive linear relationship between % ΔRSNA versus % ΔBP in the HS group ($r^2=0.84$, $P<0.05$), but not in the LS group ($r^2=0.009$).

Following ICV ANGII infusion, both groups exhibited an initial baroreflex response characterised by a sharp rise in BP and fall in RSNA. The HS group further showed a prolonged secondary response characterised by a concurrent elevation in BP and RSNA. These results suggest that chronic high dietary salt intake can elicit a uniquely biphasic response to ICV ANGII infusion.

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C95

Involvement of nitric oxide in the mechanisms underlying salt and water excretion in salt-loaded hooded (Aguti) rats

F. Mojiminiyi, C.N. Anigbogu, O.A. Sofola and S.A. Adigun

Usman DanFodio University, Sokoto, Nigeria

Experimental hypertension studies are few in the hooded (Aguti) rat. However, a recent work demonstrated its usefulness for experimental hypertension studies (Mojiminiyi et al. 2005). The present study was designed to investigate some of the mechanisms underlying the development of hypertension in this rat stain during dietary salt and/or L-NAME (N-nitro-L-arginine-methyl-ester) loading.

Hypertension was induced in inbred 8 week old hooded rats (n=8 each) by administering 8% salt in the diet for 6 weeks (Sofola et al. 2003) and/or 100 mg/kg/day L-NAME in the drinking water for 4 weeks (Pollock et al. 1993). Urine was collected from the rats weekly, its volume determined and stored. At the end of 6 weeks the blood pressure of the rats was measured invasively following anaesthesia with a 0.25mg/ml urethane and 0.01mg/ml chloralose mixture given intraperitoneally at a dose of 5ml/kg. Blood samples were collected from the rats by cardiac puncture during anaesthesia and serum extracted. The cation concentrations of serum and stored urine samples were measured by flame photometry.

Results are presented as mean ± SEM. Statistical analysis was done using one-way ANOVA and a post hoc Student Newman-Keuls test. $P< 0.05$ was taken as statistically significant.

Water consumption, urine volume and Na^+ excretion increased significantly in salt-loaded and salt+L-NAME groups compared with control ($P<0.05$) but remained similar in L-NAME rats. These values were however significantly less in salt+L-NAME rats compared with salt loaded rats ($P<0.05$). Urinary K^+ excretion and serum Na^+ and K^+ concentrations remained similar in the test groups compared to control. The mean arterial pressure (mmHg) increased significantly in the test groups of rats (salt:138.3±4.0; L-NAME: 165.7±6.0; salt+L-NAME: 133.3±5.2) when compared with control (88.4±2.7; $P<0.05$).

These results confirm the earlier finding suggesting the usefulness of the hooded rat for experimental hypertension studies. Attenuation of the diuretic and natriuretic responses to salt loading in the presence of L-NAME suggests that nitric oxide is involved in the mechanisms involved in these responses. It is concluded that nitric oxide deficiency may exacerbate salt and volume retention in salt-loaded hooded rats and possibly play a role in the subtle renal defect underlying salt sensitive hypertension.

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Real time visualization of nitric oxide release in living brainstem slices

C.R. Sunico[1], S. Wang[2], B. Moreno-Lopez[1] and S. Kasparov[2]

[1]Área de Fisiología, Universidad de Cadiz, Cadiz, Spain and [2]Physiology, University of Bristol, Bristol, UK

Nitric oxide (NO) is a freely diffusible signalling which modulates both, excitatory and inhibitory neurotransmission. Spatial and temporal characteristics of NO signalling in the brain remain unclear. At the same time, it is very important to have a realistic idea of NO spread in the brain in order to explain how it may convey any meaningful biological message.
We visualized NO production in real time using ratiometric confocal imaging of a NO-sensitive fluorescent indicator DAA (1,2-diaminoanthraquinone sulfate) and Alexa 633, used as a reference. In order to ensure that NO is produced by identifiable cellular sources, we introduced nNOS selectively into the neurones of the hypoglossal motor nucleus (HN) of the rat using retrograde transfection with adenoviral vectors.
Under deep halothane anaesthesia P3-5 rat pups were injected into the tongue with either a mixture of Ad hCMV-nNOS and/or Ad hCMV-eGFP viral vectors (~$5x10^{11}$ PFU/ml, 6 μl/pup). After 1 – 5 days acute brainstem slices containing the HN were prepared. The slices were transferred into a recording chamber and continually perfused with oxygenated artificial cerebrospinal fluid. eGFP-fluorescent neurones were identified and the mixture of DAA and Alexa was introduced into the adjacent parenchyma using a patch pipette. Dyes were imaged using Leica SP system with two separate channels (500-545 and 620-660 nm) for DAA and Alexa 633, correspondingly.
We first established that under these imaging conditions the emission spectra of the dyes do not overlap and that the DAA/Alexa ratio increases reliably when the dyes are exposed to exogenously produced NO. A robust increase of the DAA/Alexa ratio (~+30%) occurred at areas immediately adjacent (<5 μm) to the membranes of nNOS/EGFP-transfected motoneurones (n=5) after stimulation with L-glutamate (500 μM applied in the bath solution). This was not observed in the eGFP-only transfected neurones. While the increase in the ratio was the highest directly at the membrane, it dropped as a function of the distance and became insignificant at ~15-20 μm. A NOS inhibitor L-NAME(0.2-2 mM) blocked glutamate-induced NO release (n=7).
We conclude that DAA/Alexa combination can be used for ratiometric measurements of NO production in living brain slices. High levels of nNOS expression in neurones may result in activity-dependent generation of NO signals which are detectable within the surrounding brain parenchyma at distances of at least 10-15 μm.

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Glutathione depletion impairs upper airway muscle function in an animal model of sleep-disordered breathing

K.D. O'Halloran

University College Dublin, Dublin, Ireland

Sleep-disordered breathing is extremely common, affecting more than 2% of adults in the developed world and perhaps as much as 20% of middle-aged men. The condition is associated with episodic hypoxia due to recurrent apnoea. Additionally, episodic hypoxia occurs commonly in normal individuals and may even be induced as a strategy in exercise training and in the treatment of clinical disorders. We have shown that chronic episodic hypoxia impairs respiratory muscle function and CNS control of upper airway patency [1]. In this study, we tested the hypothesis that disruption of an endogenous antioxidant defence system exacerbates the effects of episodic hypoxia on upper airway muscle contractile function. Thirty two adult male Wistar rats were placed in restrainers with their heads in hoods in which the ambient oxygen concentration could be modified by controlling the gas supply to the hoods as previously described [2]. Sixteen rats were exposed to alternating periods of hypoxia (FiO_2 = 6-8%) and normoxia, twice per minute, 8 hours a day for 1 week (episodic hypoxia). The remaining sixteen animals were exposed to an air/air cycle under identical experimental conditions (control). In both groups, half the animals received daily injections of buthionine sulfoxamine (BSO; 50mg/kg i.p.), an inhibitor of the rate-limiting enzyme in glutathione synthesis [3]. The other half received daily vehicle injections. At the end of the 1-week treatment period, the sternohyoid muscles (a representative pharyngeal dilator muscle) were removed under anaesthesia (sodium pentobarbitone, 60mg/kg i.p.). *In vitro* isometric contractile properties were determined using strips of sternohyoid muscle in physiological salt solution at 30°C [4]. Fatigue properties were determined by stimulation of the muscle strips at 40Hz (train duration of 300ms), every 2 s for 5 min. Episodic hypoxia was associated with a decrease in sternohyoid muscle endurance, an effect that was exacerbated by treatment with BSO (e.g. tension at 3 min into the fatigue trial was 27±3% vs. *19±2% vs. *14±1%;
mean±SEM; % of initial tension, control (n=8) vs. episodic hypoxia (n=8) vs. episodic hypoxia+BSO (n=6), *P<0.05 ANOVA vs. control). The results suggest that episodic hypoxia-induced oxidative stress contributes to impaired upper airway muscle performance in our animal model. Our results may have particular relevance to respiratory disorders associated with episodic hypoxia such as the sleep apnoea/hypopnoea syndrome.

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PC68

Possible involvement of central prostaglandins in elevated muscle vasoconstrictor activity associated with systemic inflammatory states

M.P. Gilbey and C. Huang

Physiology, UCL, London, UK

Peripherally released cytokines enhance sympathetic drive to a number of targets (1). One probable mechanism is through the activation of PGE (EP) receptors following cytokine-induced prostaglandin release from endothelial cells of the cerebral circulation. As cytokine levels are raised in systemic inflammatory states that increase cardiovascular risk, such a mechanism may contribute to hypertension, raised sympathetic activity and other physiological disorders (1,2). Here the actions of ICV PGE1 are examined upon population of muscle vasoconstrictor (MVC) sympathetic activity recorded from a gastrocnemius nerve (GN).

Urethane-anaesthetised male rats (N=9; initial dose, 1.3 g kg^{-1}, I.P., supplemented with 5-10 mg I.V. as required) were positive pressure ventilated with oxygen-enriched air and maintained in central apnoea (3). Diaphragmatic EMG was recorded and blood gases monitored to assess central respiratory drive. Glass suction electrodes were used to record mvc and cutaneous vasoconstrictor (CVC) activities simultaneously from a GN and the ipsilateral tibial nerve plantar branch (TNp), respectively (filtered, 80-1000 Hz; rectified and smoothed (see 3)). Either PGE1 (500 ng in 5 µl ACSF) or vehicle was administered ICV, as previously described (see 4). Of three groups of animals, 2 groups received PGE1; one group was vagotomized (A: N=2) and the other additionally sinoaortic denervated (B: see 3: N=4). Animals in the time matched control group (C; N=3) received vehicle only and were vagotomized and sinoaortic denervated. MVC data were analysed only where CVC activity increased following PGE1 (positive control; see 4).

Ten minutes following the administration of PGE1 the change in MVC activity seemed greater than that seen in the group receiving vehicle (Fig. 1). The effect appeared to be sustained for 50 min following PGE1. Although the sample size in this preliminary study precluded statistical analysis on differences between treatments at each time point, the mean values from all time points (n=5) in each treatment group were compared (1-way ANOVA): MVC percentage change in each of the PGE1-treated groups (A = 51±5%; B = 63±3%) was significantly different from that in the vehicle control (14±3%, $p < 0.0001$). BP and heart rates in groups A and B were similarly significantly different from C (mean BP 114, 128 and 86 mm Hg respectively; mean heart rate 505, 528 and 460 beats/min, respectively; n=5, $p < 0.001$)

These preliminary studies are consistent with the idea that activation of CNS EP receptors can lead to an increase in MVC activity which if maintained may contribute to cardiovascular disease.

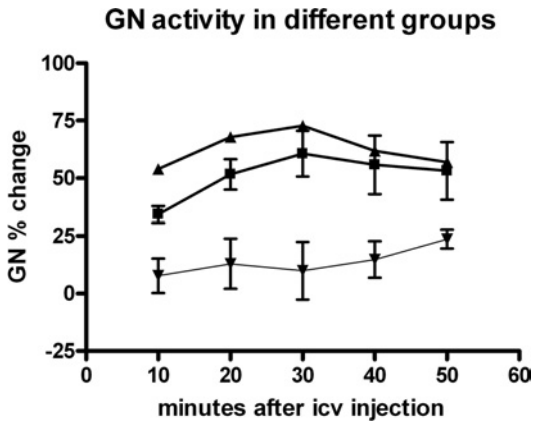

Figure 1. Triangles, Group A; squares, Group B; and inverted triangles, Group C. Means and SEMs.

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

PC69

The mammalian lung: did evolution take the wrong path?

J.B. West

Dept. of Medicine 0623A, UCSD, La Jolla, CA, USA

Some 300 million years ago the ancestors of the present reptiles emerged completely from water and committed themselves to air breathing. They were exothermic and incapable of sustained levels of high physical activity. But from them developed the two great classes of vertebrates with high levels of maximal oxygen consumption: the mammals and birds. A remarkable feature of these two groups is that although the physiology of the cardiovascular, renal, gastrointestinal, endocrine and nervous systems show many similarities, the lungs are radically different. The thesis here is that the bird lung is superior to that of the mammal, and that evolution took the wrong path for the latter.

A major difference is that the bird lung with its air sacs and gas exchanging parabronchi successfully separated the ventilatory and gas exchange functions. The combination of these two functions in the alveolar tissue of the mammalian lung results in several problems including collapse of part of the lung if an airway is blocked. This disadvantage is heightened by the fact that mammalian alveolar tissue needs to be compressible whereas the parabronchi are remarkably rigid. Another advantage of the bird lung is that ventilation through the parabronchi is unidirectional and continuous, similar in principle to that of a car radiator. By contrast reciprocating ventilation in the mammalian lung poses

the potential for uneven ventilation caused by stratification of inspired gas with that already in the lung. Furthermore this mode of ventilation relies on a combination of convection and diffusion to transport gas to the terminal air spaces which therefore must be much larger in the mammal than in the bird. As a result the pulmonary capillaries are strung out along the alveolar wall in the mammal and are poorly supported compared with the bird where they are embedded in the rigid honeycomb-like structure of the air capillaries (West et al. 2006). As a consequence the mammalian alveolar walls need a collagen cable to ensure their integrity and this interferes with gas diffusion across the blood-gas barrier because it must run through one side of each pulmonary capillary. The result is that the barrier is much thinner and more uniform in the bird. The bird lung also utilizes a cross-current gas exchange system which is superior to that of mammals as has previously been described (Piiper & Scheid 1972). The net result of all these differences is that some birds have higher mass-specific maximal oxygen consumptions, and also larger aerobic scopes than any mammals. From a structure-function standpoint, the bird lung is clearly superior.

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PC70

The effects of 5-HT receptor activation on sympathetic rhythms in rat spinal cord slices

M.L. Pierce, J. Deuchars and S.A. Deuchars

Institute of Membrane and Systems Biology, University of Leeds, Leeds, UK

The outflow of the sympathetic nervous system has long been known to contain rhythmic activity in the intact organism (see [1] for review). Interestingly, rhythmic sympathetic nerve discharges are also observed in an *in vitro* mouse spinal cord preparation [2], suggesting that some of this activity is spinally generated. We used spinal cord slices to investigate rhythmic activity in the intermediolateral cell column (IML), the main source of sympathetic outflow from the spinal cord. The effects of 5-HT receptor activation (which is known to enhance oscillatory activity in a variety of neural networks, e.g. [3,4]) on this rhythmic activity were determined.

Wistar rats (10- to 12-days-old) were deeply anaesthetised with urethane (2g/kg I.P.) and transcardially perfused with ice cold buffer. The thoracic spinal cord was removed and transverse slices (500μm) were cut. Extracellular field recordings were made from the IML in the presence and absence of 5-HT receptor agonists, using glass micropipettes filled with a saline solution containing (in mM): NaCl (124), $NaHCO_3$ (26), glucose (10), KCl (3), NaH_2PO_4 (2.5), $MgSO_4.7H_2O$ (2), $CaCl_2$ (2). Traces were amplified, filtered and digitised before being analysed offline using Spike2 software. Oscillations were considered to be present if the trace had a sinusoidal autocorrelogram and a defined peak in the power spectrum. The degree of oscillatory activity was quantified by the area under the power spectrum peak normalised to the total power at all frequencies ("normalised power") expressed as a percentage.

12 of 27 slices (44.4%) displayed oscillatory activity (spontaneous or drug-induced) at a peak frequency of 8-17Hz (median=8.9Hz) in the region of the IML. Slices that did not oscillate under any conditions were not studied further. 5-HT (10μM) significantly (paired t test, n=6, $p<0.05$) enhanced the normalised power of the spontaneous oscillations to 41.3±16.9% compared to 27.8±13.4% in control. Furthermore, application of the specific 5-HT_2 agonist α-methyl-5-HT (10μM) mimicked this effect (n=3), increasing the power of the oscillations to 55.4±21.8% compared to 28.3±23.6% in control conditions. The oscillations could be attenuated by blocking synaptic transmission with 1μM TTX (normalised power= 18.0% in control, 4.3% in TTX, n=1) and also by the gap junction blocker carbenoxolone (100μM, normalised power=16.8% in control, 9.4% in drug, n=1).

Our results demonstrate that oscillatory activity can be recorded from the IML of rat spinal cord slices in the 8-17Hz band. Furthermore, these oscillations can be enhanced by 5-HT, probably via the 5-HT_2 receptor, although a contribution from other subtypes cannot be ruled out. Preliminary data implicate roles for both chemical and electrical synaptic transmission in generating this activity.

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PC71

Neurochemical content of GABAergic neurones in the intermedius nucleus of the medulla (InM) in the mouse

I.J. Edwards and J. Deuchars

IMSB, University of Leeds, Leeds, UK

Activation of proprioceptors in neck muscles has a sympathoexcitatory effect (Kuwagata *et al.* 1991). However, the pathways mediating these actions have not been elucidated. The intermedius nucleus of the medulla (InM) is ideally placed to participate in these autonomic responses to stimulation of neck muscle afferents – it sends monosynaptic projections to the nucleus of the solitary tract (Dallas *et al.* 2005) and receives inputs from neck muscle proprioceptors (Neuhuber & Zenker, 1989). We are investigating the anatomical organisation of the InM and here report initial findings on the neurochemistry of the GABAergic neurones in this nucleus.

Immunohistochemistry was conducted on tissue from mice expressing GFP under the control of the glutamic acid decarboxylase 65 promoter (GAD65-GFP; De Marchis *et al.* 2004) or

glutamic acid 67 GFP knock in mice (GAD67-GFP; (Tamamaki *et al.* 2003). Adult mice were anaesthetised via an I.P (60 mg/kg) injection of Sagatal and perfused transcardially with 4% paraformaldehyde (in 0.1M phosphate buffer, pH 7.4), the medulla oblongata removed, post fixed overnight and sectioned at 30μm using a vibrating microtome.

Preliminary studies revealed that the InM is rich in neurones immunoreactive for neuronal nitric oxide synthase (nNOS) as well as the calcium binding proteins parvalbumin and calretinin. The extent of co-localisation of these markers with GFP in GAD65-GFP or GAD67-GFP mice, or GAD67 immunoreactivity (GAD67-IR) is summarised in Table 1. Although investigated, the calcium binding protein calbindin was not present within the InM.

These results show that nNOS is only found to co-localise with neurones expressing the GAD65 reporter gene, parvalbumin is found in the cells expressing the GAD67 reporter gene, and calretinin is found within the GAD67 immunoreactive population. These findings suggest that the GABAergic neurones in the InM are likely to be a heterogenous population.

Antigen	nNOS	Parvalbumin	Calretinin
% Co-localised with GAD65-GFP	27.6±8.4 (n=3)	0 (n=2)	0 (n=2)
% GAD65-GFP cells double labelled	15.5±6.0 (n=3)	0 (n=2)	0 (n=2)
% Co-localised with GAD67-GFP	0 (n=2)	39.9±1.4 (n=2)	1.7 (n=1)
% GAD67-GFP cells double labelled	0 (n=2)	47.8±7.8 (n=2)	20 (n=1)
% Co-localised with GAD67-IR	n/d	n/d	33.3 (n=1)
% GAD67-IR cells double labelled	n/d	n/d	39.2 (n=1)

Table 1. Summary of immunohistochemical analysis of GABAergic neurones within the InM; n= number of animals, n/d = not done.

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PC72

A 'binary' viral system for tight tetracycline-controllable and cell-specific expression in the brain based on a transcriptional amplification strategy

B. Liu[1], S. Wang[2], J. Paton[1] and S. Kasparov[1]

[1]Bristol Heart Institute, University of Bristol, Bristol, UK and [2]Institute of Bioengineering and Nanotechnology, Singapore, Singapore

Efficient cell-specific and tightly controllable gene expression is highly desirable in studies of gene function and in gene therapy research. The tetracycline-controlled transcription system (Tet-system) was designed for inducible gene expression in mammalian systems (1). However, the original Tet-systems suffered from high levels of leak expression and poor inducibility (2, 3). More recently, a tightly regulated Tet-system has been developed and marketed by ClontechTM. TRE-Tight vectors drive highly inducible and leak proof expression in many cellular systems. However, the limitation of this system is that the induction of gene expression requires a high level of expression of the tetracycline-dependent transactivator which requires the use of strong, typically viral, promoters. Since most cell-specific mammalian promoters are relatively weak inducers of transcription, it is difficult to achieve combined transcriptional targeting and tight Tet-inducible expression. Our aim was to develop a powerful neuron-specific, tight Tet-inducible lentiviral system.

We employed a transcriptional amplification (TA) strategy. To express a tetracycline-dependent transactivator (Tet-off) we used a system in which the first copy of the synapsin-1 promoter (SYN) drives expression of a strong chimeric transcription factor Gal4/p65 and modified the second copy of SYN promoter to incorporate a unique set of Gal4 binding sites. In this system Gal4/p65 binds to multiple Gal4 binding sites upstream of the second SYN promoter leading to highly amplified expression of Tet-off. Neuronal specificity, inducibility and levels of gene expression of this system were characterized both in vitro and in vivo.

We demonstrate that the TA-amplified SYN-based Tet-regulatable system greatly increased induction of transgene expression in both PC12 cells in vitro (~8-fold compared to non-TA SYN-based system) and hypoglossal motor neurons in vivo (~12-fold) with no loss of neuronal specificity. For injections into the hypoglossal motor nucleus, male Wistar rats (~250-300 g, n = 4) were anaesthetised intramuscularly with a mixture of ketamine (60mg/kg) and medetomidine (250μg/kg). Seven days postinjection, rats were terminally anaesthetised (sodium pentobarbital, 100mg/kg, i.p.) and perfused intracardially. Importantly, this system allowed complete and reversible control of gene expression using doxycycline both in vitro and in vivo. These results will for the first time allow us to deploy TA-based vectors for powerful neuronal-specific expression, which has extremely tight control over its timing, for probing central neural circuits regulating arterial blood pressure.

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PC73

Nitric oxide (NO) potentiates GABAergic transmission within the nucleus tractus solitarii (NTS)

S. Wang[1], A.G. Teschemacher[2] and S. Kasparov[1]

[1]Physiology, University of Bristol, Bristol, UK and [2]Pharmacology, University of Bristol, Bristol, UK

Our latest data show that nitric oxide (NO) potentates GABAergic transmission via an increase in intracellular Ca^{2+} concen-

tration ($[Ca^{2+}]_i$) in the nucleus tractus solitarii (NTS) GABAergic interneurons (Wang *et al.* 2006). However, the mechanism mediating NO action remains unclear. In sea urchin eggs NO regulated calcium homeostasis via cyclic ADP ribose (cADPR)/ryanodine-sensitive stores (Galione *et al.* 1993). We hypothesised that NO-stimulated potentiation of GABAergic transmission also may be mediated by a cADPR-dependent mechanism. Organotypic rat brainstem slice cultures were transfected with adenoviral vector containing 3.7 kb of the GAD67 promoter driving expression of enhanced green fluorescent protein. Fluorescent GABAergic neurons were visualised by combined application of confocal and DIC optics and recorded in whole-cell patch clamp mode. The intracellular solution contained the red-shifted calcium indicator Rhod-2. The ratio of fluorescence intensity (F/F_0) was used to assess changes in $[Ca^{2+}]_i$. Following bath application of 10 μM DEA/NO, $[Ca^{2+}]_i$ clearly increased in somata (+20 ± 4%, n=8/9, P<0.01) and dendrites (+30 ± 4%, n=9, P< 0.001), but the most dramatic increase occurred in the putative axons (+40 ± 10%, n=5/6, P<0.05). The soluble guanylate cyclase inhibitor ODQ blocked DEA/NO action in 6 of 7 neurons tested. An antagonist of cADPR receptors, 8-Br-cADPR, introduced via patch pipette (100 μM in the patch pipette solution), almost completely abolished DEA/NO-induced $[Ca^{2+}]_i$ increases in 7 of 8 neurons. To test whether cADPR also mediates NO-induced potentiation of GABAergic postsynaptic inhibitory potentials (IPSPs) whole-cell patch clamp recordings were performed from NTS neurons in acutely prepared brainstem slices. Monosynaptic IPSPs were evoked by electrical stimulation within the medial NTS in the presence of CNQX (20 μM). 1 μM DEA/NO (~55 nM of free NO) reversibly increased the amplitude of monosynaptic IPSPs by ~32% (n=5, P<0.01). Pre-treatment with 30 μM 8-Br-cADPR essentially abolished DEA/NO-induced IPSP potentiation (n=5). Therefore, the sGC/cGMP signalling cascade may lead to activation of ADP-ribosyl cyclase via cGMP-dependent protein phosphorylation and subsequent production of cADPR. This causes sensitisation of cADPR/ryanodine sensitive stores which boost action-potential-mediated Ca2+ release and promotes GABA exocytosis, resulting in potentiation of GABA-mediated IPSPs. In summary, NO potentiates GABAergic transmission via an evolutionary conserved cGMP/cADPR/ryanodine signalling pathway.

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PC74

Oestrogen receptor subtype expression in the nucleus of the solitary tract of male and female rats

E. Spary, A. Maqbool, J.K. Sinfield and T.F. Batten

Cardiovascular Medicine, University of Leeds, Leeds, UK

Oestrogen is known to have protective effects at the level of the heart and blood vessels via its actions on the α and β subtypes of the oestrogen receptor (ER). It has also been reported to have pronounced effects on the activity of the autonomic nervous system thereby influencing cardiovascular function (Saleh & Connell, 1999, 2000). To date, only one isoform of the ERα subtype has been cloned, whilst 5 splice-variant isoforms of the ERβ subtype have been identified: β1, β2, β1δ3, β2δ3 and β1δ4 (Maruyama et al. 1998; Peterson et al. 1998).

This study aims to investigate the presence of ER subtypes in the brainstem and the nucleus of the solitary tract (NTS), one of several medullary nuclei known to be involved in cardiovascular regulation, of both male and female Wistar rats. Adult Wistar rats (150-200g) were killed and tissue samples removed. RNA was extracted and reverse transcribed to cDNA. The presence of the subunits was examined using PCR with subtype specific primers. Levels of subtype expression were determined using real-time PCR with Taqman gene expression assays (ABI 7500). The veracity of the PCR products was verified by DNA sequencing.

The data suggest that the ERα, β1 and β2 subtypes are strongly expressed in the brainstem of both male and female rats (n=6). In addition, the ERβ splice variants β1δ3, β2δ3 and β1δ4 were also detected, although the expression of β1δ4 was much weaker than the other subtypes. Results from the male and female NTS samples suggest the presence of both ERα and ERβ.

PCR analysis of ER subtype expression in the brainstem was also investigated at various stages of the oestrus cycle of the female rat. Oestrus stages were determined histologically and the cycle split into metoestrus, dioestrus, proestrus and oestrus. Expression of all the subtypes was present in each stage; however, real-time PCR data using ERα and ERβ taqman probes suggest that the levels of these two subtypes change throughout the cycle peaking in the metoestrus and dioestrus stages.

The results of this study suggest that gonadal steroid hormones may modulate cardiovascular function via a variety of ER subtypes expressed on brainstem autonomic neurons, in both male and female rats.

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PC75

Zolpidem differentially enhances GABAergic transmission in two distinct pathways onto sympathetic preganglionic neurones (SPNs)

L. Wang, J. Deuchars and S.A. Deuchars

Biological Sciences, ISMB, Leeds, UK

SPNs receive synaptic inputs from both spinal and supraspinal regions. We focused on two monosynaptic GABAergic pathways from local interneurons in the central autonomic area (CAA) (Deuchars et al. 2005) and fibres descending in the lateral funiculus (Lf) from higher autonomic centres. Zolpidem, a $GABA_A$

receptor modulator with subtype selectivity, was used to compare the pharmacological profiles of these two pathways. It displayed high affinity to benzodiazepine (BZ) type I receptors ($\alpha1\gamma2$), low affinity to BZ type II receptors ($\alpha2/3\gamma2$) and had no effect on $\alpha5$ subunits (Ali et al. 1998).

Wistar rats of either sex (10-15 day) were anaesthetised with urethane (2g/kg i.p.) followed by a cardiac perfusion of 215 mM sucrose aCSF (Deuchars et al. 2005). Transverse thoracic spinal cord slices (300μm) were prepared for whole-cell patch clamp recording. Inhibitory postsynaptic potentials (IPSPs) were evoked by stimulating Lf (Lf-IPSPs) and CAA (CAA-IPSPs) sequentially and isolated in kynurenic acid (2mM). Zolpidem was initially dissolved in ethanol and was bath applied (final concentration of ethanol <1/1000). Amplitudes of IPSPs were measured by averaging 10 consecutive sweeps.

At a relatively high concentration of 500nM, zolpidem caused an initial increase in Lf-IPSP amplitude (115.64±10.63%, n=8). A secondary increase in amplitude was also observed in Lf-IPSPs in 6/8 SPNs (130.81±23.24%, mean±SD) after 15 min of drug application. The initial peak response of CAA-IPSPs (142.42±19.86%, mean±SD, n=6) was significantly larger than Lf–IPSPs ($P<0.01$), whereas a secondary response was less prominent. At a low concentration (100nM), Zolpidem induced a rapid increase in Lf-IPSP amplitude (within 3 min, 119.18% to 166.22%, n=2) whilst CAA-IPSPs either showed no effect or were enhanced but with a time delay (after 15 min). At an even higher concentration (1μM), both IPSPs showed a two phase increase in amplitude with similar time courses (n=2).

These data show that different mechanisms of GABAergic modulation are involved in controlling the activities of SPNs. In the descending Lf pathway, it is likely that the receptors are more sensitive to low concentrations of zolpidem than in the CAA pathway. At a concentration which may affect one or both types of receptors, IPSPs elicited by both pathways are enhanced. The secondary enhancements in Lf-IPSP amplitudes may reflect different levels of receptor phosphorylation (Thomson et al. 2000).

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PC76

Rat tail artery: constriction to dilatation in less than 0.3°C

M.P. Gilbey and K. Mahendran

Physiology, UCL, London, UK

An increase in body temperature of < 0.5°C caused a cessation of single unit sympathetic activity recorded focally from the rat caudal ventral tail artery (1). In contrast, population activity supplying the whole of the tail circulation ceased over a temperature range of ~2°C (2). These observations are consistent with the idea that on-off control of blood flow to different regions of the tail circulation (e.g. base and tip) has different thresholds (3) and therefore the quantal on-off (4) and the graded control hypotheses (5) are not mutually exclusive. Here we test the hypothesis that in response to an increase in body temperature the pressure within the ventral caudal artery at the base of the tail is regulated in an 'on-off' fashion.

Experiments were conducted upon four urethane-anaesthetised male Sprague-Dawley rats (initial dose 1.3 g kg^{-1}, I.P., supplemented with 5-10 mg I.V. as required) breathing spontaneously on oxygen-enriched air. Blood gases were monitored before and after a period of data collection. Oesophageal temperature (T) was monitored and regulated using a calibrated digital thermometer and a homeothermic blanket, respectively. Room temperature was 21-23°C. A jugular vein (administration of drugs and fluids) and carotid artery (to monitor 'central' arterial blood pressure) were cannulated. The caudal ventral artery was cannulated with the catheter tip positioned in the upper third. Oesophageal temperature was 35.0–35.5°C at the start of the protocol during which animals were heated over ~60 min to 37.5-38.0°C. Central (c) and tail (t) pressures and oesophageal temperature were monitored continuously and recorded using Spike 2 software (sampling frequency 100 Hz). Mean blood pressures were displayed (1 s moving average) and down sampled to 0.5 Hz before further processing. Pressure ratio (t/c) was plotted against oesophageal temperature. All values are mean ± S.E.M.

An example of a typical plot is shown in Fig 1. On warming two stable t pressures were observed; one at ≤ 36.2 ± 0.1°C (T1) and the other at ≥ 36.4 ± 0.1°C (T2). t/c at T1 was 0.51 ± 0.01 (n=4, t = 55 ± 3mmHg, c = 108 ± 4mmHg) and t/c at T2 was 0.77 ± 0.01 (n=4, t = 75 ± 6mmHg, c = 97 ± 7mmHg). Between T1 and T2, t/c rose rapidly (gradient = 1.1 ± 0.2°C^{-1}; linear regression analysis).

These data indicate that the narrow range in temperature over which the caudal ventral artery shifts from constriction to dilatation is mediated by 'on-off' nervous control. We speculate that this fine control is achieved through a reduction in mean firing frequency whilst the T-rhythm is maintained (1): the latter acts to maintain fidelity of neuroeffector transmission.

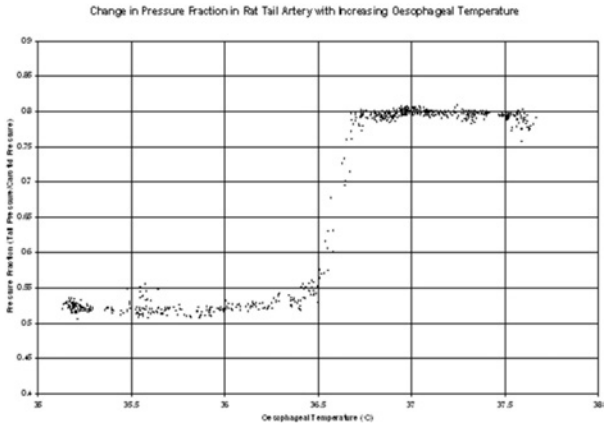

Figure 1. t/c against temperature

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PC77

Serotonin immunoreactive terminals closely appose GABAergic neurones in autonomic regions of the spinal cord

D. Conte[1], S.A. Deuchars[1], F. Erdelyi[2], G. Szabo[2] and J. Deuchars[1]

[1]*Institute of Membrane and Systems Biology, University of Leeds, Leeds, UK and* [2]*Institute of Experimental Medicine, Budapest, Hungary*

Sympathetic nervous outflow arises from sympathetic preganglionic neurones (SPNs) in the central autonomic area (CAA) and intermediolateral cell column (IML) of the thoracic and upper lumbar spinal cord. Descending serotoninergic axons heavily innervate the CAA and also the IML, where they form synapses with SPNs (Bacon & Smith, 1988). Functionally, serotonin directly excites SPNs and also induces indirect IPSPs in SPNs in spinal cord slices (Lewis et al. 1993), suggesting excitation of local inhibitory interneurones. We have identified interneurones within the vicinity of the IML (Deuchars et al. 2001) and within the CAA (Deuchars et al. 2005) that are likely to innervate SPNs. Interneurones in the CAA inhibit SPNs via a GABAergic connection. In this study we are investigating if serotonin containing terminals innervate GABAergic neurones in the IML and CAA.

Targets of serotonin in thoracic spinal cord were investigated using immunohistochemistry on transgenic reporter mice expressing GFP in cells containing glutamic acid decarboxylase (GAD)65 (De Marchis et al. 2004). Adult mice (n=5) were anaesthetised with an intraperitoneal injection of Sagatal (60mg/kg) and perfused transcardially with 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4. Thoracic spinal cord was sectioned at 50μm on a vibratome (Leica). Serotoninergic terminals were localised using rabbit anti-5-HT (1:500, Neuromics Inc, USA) and visualised with donkey anti-rabbit Alexa^{555} (1:1000, Invitrogen, UK). Stained sections were analysed for close appositions between serotoninergic terminals and GFP expressing cells using an epifluorescence microscope.

The distribution of GFP expressing cells was similar to that observed in our previous studies of cells expressing GAD detected by *in situ* hybridisation (Deuchars et al. 2005). Numerous GFP containing cells were visible in the CAA (dorsal and lateral to the central canal). Although labelled cells were sparse within the IML, they were more common around the edges, similar to the location of pre-sympathetic interneurones. Serotonin containing terminals were highly concentrated in the IML and CAA as previously described by Bacon & Smith (1988) and were visible at low magnification in the vicinity of GFP expressing cells. High power examination revealed serotoninergic terminals were indeed closely apposed to both GFP cell bodies and dendrites.

These results suggest that 5-HT containing terminals can innervate GABAergic neurones in the vicinity of the IML and in the CAA. This provides an anatomical substrate for the IPSPs induced in SPNs in spinal cord slices by 5-HT. Descending pathways may therefore exert control of sympathetic outflow indirectly via local interneurones as well as through direct actions on SPNs.

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

PC78

Targeted neuronal nitric oxide synthase gene transfer into cardiac noradrenergic neurons reduces the amplitude of the potassium-induced increase in $[Ca^{2+}]_i$

L. Wang[1], C.J. Mee[2], J.S. Taylor[1], D.B. Sattelle[2] and D.J. Paterson[1]

[1]*Department of Physiology, Anatomy and Genetics, Oxford University, Oxford, UK and* [2]*MRC Functional Genetics Unit, Department of Physiology, Anatomy and Genetics, Oxford University, Oxford, UK*

Noradrenergic cell specific gene transfer with neuronal nitric oxide synthase (nNOS) can inhibit cardiac sympathetic neurotransmission (unpublished observations). Since neurotransmitter release strictly depends on transmembrane Ca^{2+} influx, we investigated whether selective nNOS gene transfer can modulate calcium handling in isolated cardiac sympathetic neurons. Stellate sympathetic ganglia were dissected from neonatal Sprague Dawley rats and digested using a combination of collagenase and trypsin. Dissociated neurons were purified by panning on a collagen coated dish and plated onto poly-L-lysine/laminin substrate before transduction with an adenoviral vector encoding nNOS driven by a noradrenergic promoter (1). An empty adenoviral vector was used as control for comparing the effect of viral transduction on the neurons. Fura-2 based, ratiometric measurements of intracellular free calcium $[Ca^{2+}]_i$ were obtained from cells bathed in HEPES buffered Tyrode solution using a Perkin Elmer UltraView imaging system housed on an inverted Olympus IX70 microscope equipped with a 40×, 1.3 N.A. oil-immersion objective. Ca^{2+} imaging was performed 48 hours post-transduction. Cell depolarization was induced by using 30mM KCl in HEPES Tyrode buffer for 30 seconds. The results showed nNOS gene transferred sympathetic neurons had an 80% lower potassium induced increase in $[Ca^{2+}]_i$ compared to neurons transfected with an empty vector. These results demonstrate that noradrenergic neuron-specific gene transfer with nNOS can reduce depolarization-induced increase in $[Ca^{2+}]_i$. The results suggest that nitric oxide (NO) modulation of intracellular Ca^{2+} may be a key step in NO decreasing cardiac sympathetic neurotransmission.

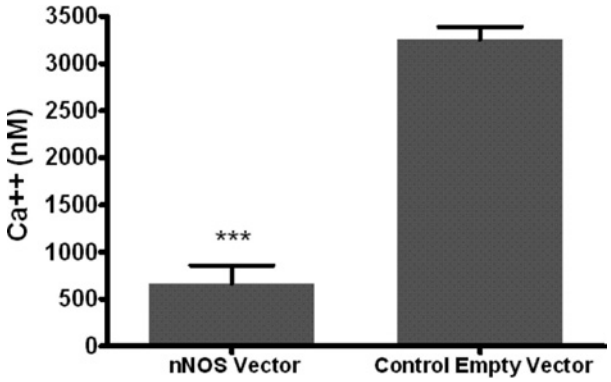

Figure 1. Average increase in $[Ca^{2+}]_i$ in sympathetic neurons treated with nNOS vector and empty vector control. n=10 p<0.01.

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

PC79

The temporal role of adenosine in the muscle vasodilatation of early chronic hypoxia *in vivo*

K.E. Glen and J.M. Marshall

Physiology, University of Birmingham, Birmingham, UK

In rats, acute systemic hypoxia (5 min) evokes skeletal muscle vasodilatation (increase in femoral vascular conductance, FVC) that is partly mediated by adenosine acting at A_1-, but not A_{2A}-receptors (Bryan & Marshall, 1999). Further, a tonic increase in FVC is present in rats exposed to chronic systemic hypoxia (CH) for 1 and 3 days, which is partially reversed by the A_1-receptor antagonist, DPCPX (Walsh & Marshall, 2000). A_1-receptors have been shown to redistribute from cell surface to cytoplasm during continued exposure to adenosine (Saura *et al.* 1998). Thus, we have tested whether the vasodilator role of adenosine changes over the first 6 h of CH.

In male Wistar rats anaesthetized with Saffan (4-8 mg kg^{-1} h^{-1} I.V.), cardiovascular variables were recorded during 6 h of either normoxia (N; n=7) after vehicle or CH (breathing 12% O_2), either after vehicle (n=7), DPCPX (0.1 mg kg^{-1} I.V.; n=7) or 8-PT (10 mg kg^{-1} I.V., an adenosine receptor antagonist which is non-selective between sub-types; n=7). At the end of the experiment animals were killed. Variables were analysed at 0, 5 and 10 min and at 10 min intervals for the first hour and then hourly. All variables remained steady during N. CH induced a maintained fall in P_aO_2 (from 87±2 to 44±2 mmHg, mean ± S.E.M). At 5 min of CH, mean arterial pressure (MAP) had fallen from 123±2 to 72±5 mmHg (P<0.001, ANOVA and Fishers post hoc), but returned to 103±6 mmHg at 1 h of CH and was maintained until 6 h. Concomitantly, FVC was increased at 5 min CH (0.013±0.0016 to 0.024±0.003 ml min^{-1} $mmHg^{-1}$; P<0.01), gradually waned back to baseline levels at 2-3 h, but increased again to 0.018±0.002 ml min^{-1} $mmHg^{-1}$ by 4 h where it remained until 6 h of CH (P<0.05). Neither DPCPX nor 8-PT affected baselines. However, both antagonists attenuated the initial fall in MAP and increase in FVC (to ~90-100 mmHg and ~0.012-0.020 ml min^{-1} $mmHg^{-1}$, respectively, P<0.05) at 5 min of CH, but had no significant effect from 1-6 h of CH.

Thus, 6 h of CH induces muscle vasodilatation that wanes by 2-3 h and partially recovers by 4-6 h. We suggest that the muscle vasodilatation of the first hour is dependent on adenosine acting on A_1-receptors but not on the lower affinity A_{2A}-receptors that are blocked by 8-PT (see Bryan & Marshall, 1999). Other dilator mechanisms become important after 2-3 h of CH, while A_1-receptors may be recycling to the cell surface to become operational by 1-3 days CH (see Walsh & Marshall, 2000).

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PC80

Changes in protein expression and localization in rat skeletal muscle following chronic systemic hypoxia *in vivo*

K.E. Glen[1], A. Gonzalez[2] and J.M. Marshall[1]

[1]Physiology, University of Birmingham, Birmingham, UK and [2]Molecular Neuroscience Group, University of Birmingham, Birmingham, UK

We showed that early chronic hypoxia (CH) lasting 1-3 days induces vasodilatation and increased vascular permeability in skeletal muscle of rats (Walsh & Marshall, 2000). By 14 days there is increased arteriolar branching (Smith & Marshall, 1999), and by 3 weeks capillary angiogenesis (Deveci *et al.* 2001). We have also shown increases in mRNA for inducible nitric oxide synthase (iNOS) and vascular endothelial growth factor (VEGF) in Tibialis Anterior (TA), Soleus (SOL) and Spinotrapezius (SP) muscles in rats exposed to CH from 2 h to 14 days, but no change in endothelial (e)NOS mRNA (Glen *et al.* 2004). We have now investigated the changes in protein expression and localization of iNOS, VEGF and eNOS.

Experiments were performed on normoxic (N) rats and on rats exposed to 12% O_2 (CH) for between 2 h and 14 days (n=6 in each case). Under anaesthesia (Sagatal 60 mg kg^{-1}) muscles were removed and frozen in liquid N_2-cooled 2-methylbutane. All animals were killed by anaesthetic overdose. Western blots were performed to measure protein expression of iNOS, VEGF and eNOS in TA, SOL and SP muscles. Immunohistochemistry with fluorescent tags was performed on serial sections of SOL and TA from N rats, and rats exposed to 6 h and 1 day CH, to localize these proteins and to detect HIF-1α, which is known to increase the expression of VEGF and iNOS. Alternate sections were stained for muscle fibre types using myosin ATPase and succinate dehydrogenase techniques and with lectin to identify capillaries.

No iNOS protein was detected in any muscles, by Western blot or immunohistochemistry. VEGF and eNOS protein was detected in all muscles. However, densitometry analysis showed increases in VEGF protein from 1 day CH and were maintained to 14 day CH, whereas eNOS protein did not change with CH. Immunohistochemistry indicated eNOS in capillary endothelium of muscles in all rats. VEGF was also present in capillaries and is associated with the sarcolemma of glycolytic fibres at 1 day CH. HIF-1α was diffusely present in muscles of N rats, but appeared to be concentrated in the nuclei of muscle fibres at 1 day CH.
We suggest that the increase in VEGF protein seen in muscles in the first few days of CH is consistent with the increase in vascular permeability and onset of angiogenesis (see above) and may be triggered by HIF-1α.

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PC81

Heart rate variability during combined head-up tilting and isometric handgrip exercise in humans

M.S. Meah, M. Casu and L.J. Sabanathan

School of Health & Bioscience, University of East London, London, UK

Heart rate variability (HRV) measures variations between consecutive heart beats (RR intervals) and is assessed by time and frequency domain indices (1). Time indices include the standard deviation (SD) of RR (SDNN), SD of differences between consecutive RR (SDSD) and the percentage of consecutive RR intervals > 50 ms (pNN50). Frequency indices include high frequency (HF) and low frequency (LF) representing parasympathetic (PNS) and sympathetic (SNS) activity, respectively.
Postural stress (lying to sitting) increases HRV (2). However, little is known about HRV during a combination of two autonomic stressors. The aim is to see how combined stressors (tilting at 60 and 80 deg and isometric handgrip) affect HRV.
Twenty-four healthy subjects, 21 Asians and 3 Afro-Caribbean (15 men, 9 women) with mean weight, height and age ± SD; 64.83 ± 10.9kg, 166.10 ± 7.8cm, 25.08 ± 4.4years respectively, volunteered for this study.
Subject's maximum voluntary contraction (MVC) were assessed on a hand grip transducer (MIE Medical Research Ltd). Subjects lay in the supine position for 5 min, during which HRV was measured using a chest belt and watch (Polar S810i), followed by isometric handgrip exercise for 1 min whilst maintaining 30% of their MVC and recording HRV. This procedure was repeated after tilting to 60 and 80 deg using a motorized tilt table. HRV was analysed using Polar and Prism 4.0 software. Repeated measures ANOVA with Bonferroni post-test was used to analyse differences in HRV indices.
From control to the combined stressors there is an increase in HR (decreased RR interval), LF and LF/HF ratio, whilst SDNN, SDSD, pNN50 and HF decreased (Table 1). Differences between control and stressors were significant ($P<0.05$) for all variables except LF which was only significant at 60 deg head up tilting and handgrip.
Our results show increased HRV with a higher dominance of SNS over PNS during combined stressors compared to single stressors or control, but the larger decrease in HF during combined stressors suggest a larger decrease of the PNS effect.
Table 1

Variable	TT Control	IHG Control	TT60	IHG + TT60	TT80	IHG + TT80
Heart rate (bpm)	72.8±2.4	75.4±2.3	80.5±1.9	83.8±2.0	83.5±1.9	85.0±2.0
SDNN (ms)	133.3±27.2	130.6±26.6	96.0±19.6	83.9±17.1	90.4±18.4	91.54±18.6
SDSD (ms)	107.6±13.5	80.7±13.4	75.7±9.4	46.7±6.2	73.8±9.5	42.5±6.1
pNN50 %	16.6±2.1	13.4±2.1	8.5±1.1	6.0±1.3	6.8±0.9	6.0±1.0
LF %	11.5±2.0	12.9±2.5	14.9±3.01	16.1±3.3	15.3±2.7	13.6±2.5
HF %	19.8±4.2	15.2±3.5	14.2±3.3	11.0±3.0	13.1±3.2	6.0±1.9
LF/HF ratio %	10.5±1.7	25.2±7.4	21.1±4.5	34.5±7.6	28.8±6.2	54.0±14.1

Mean (N=24±SEM) HRV during head up tilting (TT) and isometric hand grip exercise (IHG): TT60 and TT80, tilting at 60 and 80 deg; IHG+TT60 & IHG+TT80, tilting and handgrip at 60 and 80 deg.

Berntson GG et al. (1997). Psychophysiol 34, 623-648.

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

PC82

Functional and structural cardiac alterations associated with β-adrenergic chronic stimulation. Role of aldosterone

B. Martín-Fernández[1], A. Couto-Davel[2], M. Miana[1], N. de las Heras[1], D. Sanz-Rosa[1], L. Rossoni[2], V. Lahera[1] and V. Cachofeiro[1]

[1]Physiology, School of Medicine, Universidad Complutense, Madrid, Spain and [2]Pharmacology, Institute of Biomedical Science, University of Sao Paulo, Sao Paulo, Brazil

Several studies have reported that chronic β-adrenergic stimulation is associated with functional cardiac alterations as well as ventricular hypertrophy. It is well known that aldosterone is a profibrotic factor which participates in the development of ventricular hypertrophy and heart failure. However, the participation of aldosterone in cardiac alterations produced by chronic stimulation of β-adrenergic receptors is not established. Therefore, the aim of this study was to evaluate the cardiac effects of an antagonist of mineralocorticoid receptors in rats with chronic stimulation of β-adrenergic receptors. To this end, male Wistar

rats (250g), N=6 in each group, were treated with either isoproterenol (ISO; 3mg/kg/day s.c.) or vehicle for 15 days. Half of the animals from each group were treated with spironolactone (SPIRO; 200mg/Kg/day s.c). At the end of the treatment, heart rate (HR) was measured. In addition, systolic arterial pressure (SAP), diastolic (DAP) and mean (MAP), left ventricle final diastolic pressure (pDf), + dP/dt and - dP/dt were measured through a catheter inserted into the right carotid artery. Animals were anesthesiced with ketamine (0.07ml/100mg body weight) and Xilacyne (0.03ml/100mg corporal weight) by intraperitoneal injection. Also, cardiomyocytes diameter and collagen content present in the miocardium was evaluated with optical microscopy techniques. As index of cardiac hypertrophy, the ratio heart/body weight was calculated. Neither ISO nor SPIRO were able to modify SAP, DAP, MAP, HR, + dP/dt y - dp/dt. However, pDf was higher ($p<0.001$) in rats treated with ISO as compared with controls. Treatment with SPIRO normalized these values ($p<0.001$). Relative heart weight was greater in the rats treated with ISO ($p<0.001$) than in controls. Treatment with SPIRO reduced heart weight ($p<0.01$) without becoming normalized. No differences in cardiomyocyte diameter were observed in any group. Collagen content in the myocardium of rats treated with ISO was greater ($p<0.001$) than in control rats. Treatment with SPIRO reduced collagen content ($p<0.05$) without reaching levels observed in controls. In conclusion, these data suggest that chronic stimulation of β-adrenergic receptors in rats induces diastolic dysfunction and cardiac hypertrophy associated with an increase in extracellular matrix. Aldosterone participates in these alterations.

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

PC85

A hypothalamic vasopressinergic pathway mediates the sympathoexcitation induced by hyperosmolality

V.R. Antunes[1], S.T. Yao[1], A.E. Pickering[2], D. Murphy[1] and J.F.R. Paton[2]

[1]Henry Wellcome LINE, University of Bristol, Bristol, UK and [2]Physiology, Bristol Heart Institute, University of Bristol, Bristol, UK

An osmotic challenge elicits a sympathoexcitatory response that involves hypothalamic projections to the intermediolateral cell column (IML) of the spinal cord [1]. Moreover, fibres immunoreactive for vasopressin make up an abundant terminal network in the IML [2], which has been associated with sympathoexcitation [3,4]. Using a novel *in situ* rat preparation, we sought to investigate whether hypothalamic vasopressinergic spinally projecting neurones are activated during acute increases in plasma osmolality to elicit sympathoexcitation.

Male Wistar rats (70-90 g) were anaesthetised deeply with halothane (5%) and decorticated, which included removal of the thalamus but preserved hypothalamic structures. Animals were arterially perfused with Ringer solution containing Ficoll (1.25%) at 31°C. Via a suction electrode lumbar sympathetic nerve activity (LSNA) was monitored while switching the perfusate from being isosmotic (291±1 mosmol (kg water)$^{-1}$) to hyperosmotic (321±1 mosmol (kg water)$^{-1}$) over 40s. For intrathecal injections, a calibrated glass micropipette was positioned into the subarachnoid space and a volume of up to 0.5 µl was delivered.

Initially, we evaluated the response of intrathecal injections of a V_1 receptor agonist (Phe^2,Ile^3,Orn^8-Vasopressin; 1 µg ml^{-1}) on LSNA before and after intrathecal injections of a V_{1a} receptor antagonist (β-mercapto-β-β-cyclopentamethylenepropionyl^1,O-me-Tyr^2,-Arg^8-Vasopressin; 1 µg ml^{-1}). The V_1 receptor agonist induced sympathoexcitation (18±1%; n=3). This increase in LSNA was attenuated significantly by prior intrathecal delivery of a V_{1a} receptor antagonist to 6±2% (P<0.05, n=3). This dose of antagonist was then used to assess the hyperosmotic perfusate induced increase in LSNA. In control, hyperosmotic perfusate increased LSNA by 29±6% (n=5) but this was attenuated reversibly to 4±1% (P<0.001, n=5) at 2 min after intrathecal injection of the V_{1a} receptor antagonist (1 µg ml^{-1}). Thirty minutes later the hyperosmotic-induced increase in LSNA had recovered to control levels (25±3%).

Our findings demonstrate the functional importance of the spinally projecting vasopressinergic pathway from the hypothalamus. This acts to stimulate spinal V_{1a} receptors and mediate the sympathoexcitation following acute salt loading.

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PC86

Delayed failure of baroreflex function caused by chronic infusion of angiotensin II in a normotensive rat

M.A. Toward, S. Kasparov and J.F.R. Paton

Physiology, University of Bristol, Bristol, UK

In conscious rabbits renal sympathetic nerve activity is reduced during systemic infusion of a pressor dose of angiotensin II (AngII) for 7 days (Barrett *et al.* 2003). This was attributed to long term activation of the baroreflex. Here, we tested whether the baroreflex can be modulated in a similar manner by AngII in the Wistar-Kyoto rat, the standard normotensive control for the popular spontaneously hypertensive rat.

Rats (male, 12-14 weeks; n=6) were anaesthetised with a mixture of ketamine (60 mg kg^{-1}) and medetomidine (250 µg kg^{-1}, both i.m.) and radio-transmitters installed for chronic measurement of arterial pressure using radio-telemetry. Continuous recordings of arterial pressure (AP) and heart rate (HR) were made for 3 days prior to, and 10 days during, osmotic minipump driven infusion of Ang II (800ng/kg/min; s.c.). Hey Presto software (Waki *et al.* 2006) was used to evaluate AP and heart rate as well as their power spectra so that changes in autonomic cardiovascular activity could be assessed. The spontaneous cardiac baroreceptor reflex (sBRG) was also computed.

AngII produced a persistent elevation of mean AP (from 94±4 to 151±6 mmHg by day 8, mean ± S.E.M; Fig. 1a) associated

with increased drinking. There was an immediate decrease in HR from 366±5 to 262±7 bpm on day 2 ($P<0.01$; one-way ANOVA). However, from day 3, HR increased gradually so that by day 9 it was higher than before the infusion (406±18 bpm; $P<0.05$) and continued to increase until the end of the experiment on day 10 (425±10 bpm, $P<0.01$). The high frequency power of pulse interval (HF of PI;an index of cardiac vagal activity) followed a similar temporal pattern to that of the HR: initially it increased from 15.9±1.0 pre-infusion to 40.5±1.9 ms^2 ($P<0.01$) on day 2 and then decreased for the remainder of the recording period. The sBRG remained unchanged until day 5 and by day 10 it was significantly depressed (Fig 1b; -2.1±0.1 pre-infusion vs -1.2±0.4 beats min^{-1} $mmHg^{-1}$; $P<0.05$). Finally, the low frequency (LF) component of the spectra of systolic blood pressure (an index of the sympathetic vasomotor activity) was depressed from day 3 to 6 of the AngII infusion (3.0±0.1 pre-infusion vs 2.1±0.1 $mmHg^2$ on day 3; $P<0.01$) but then returned to control levels by day 7 where it remained (Fig 1c).

These data suggest that in the rat chronic infusion of AngII causes a sustained increase in blood pressure that is accompanied by a relatively transient baroreflex mediated bradycardia and sympathoinhibition. However, by day 7 the parasympathetic component of the baroreflex fails and sympathetic vasomotor activity increases to control levels. These data suggest that chronically increased peripheral levels of AngII may cause hypertension not only via the classical actions of peripheral vasoconstriction and water retention but also via central modulation of the baroreflex, which is consistent with our earlier data (Paton & Kasparov 1999).

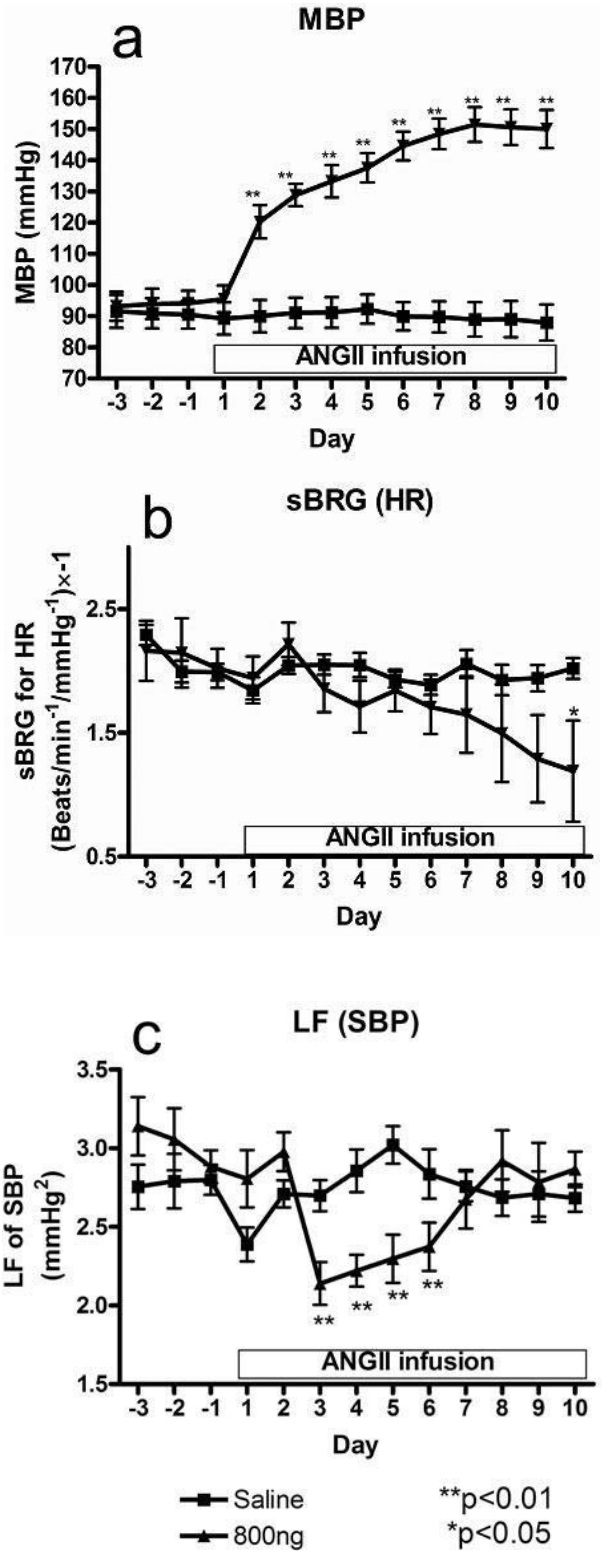

Figure 1. Cardiovascular effects of chronic AngII infusion.

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Paton JF & Kasparov S (1999). *J Physiol* **521**, 213-225.

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PC87

Changes in the pattern of spinal and cranial respiratory motor outflows after micro, transverse sectioning of the pons *in situ*

A.P. Abdala[1], J.C. Smith[2], I.A. Rybak[3] and J.F.R. Paton[1]

[1]Department of Physiology, Bristol Heart Institute, School of Medical Sciences, University of Bristol, Bristol, Avon, UK, [2]Cellular & Systems Neurobiology Section, Laboratory of Neural Control, National Institute of Neurological Disorders & Stroke, National Institute of Health, Bethesda, MD, USA and [3]School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA, USA

Following transection of rostral pontile regions in anaesthetised cats *in vivo*, Lumsden (1923) described apneusis, whereas a complete removal of the pons resulted in gasp-like discharges. Due to confounding problems relating to anaesthesia and haemorrhage, as well as not knowing where exactly the lesions were made, we refined these experiments to avoid effects of anaesthesia/poor brainstem perfusion and reconstructed brainstem transections precisely.

Wistar rats (male; 65-85g) were anaesthetised deeply with halothane until they became unresponsive to noxious pinching of the tail. They were decerebrated at the pre-collicular level and perfused arterially (Paton, 1996). Recordings of the phrenic (PN), central vagal (X) and abdominal (AB) motor nerves were made during transverse sectioning of the brainstem.

In the *in situ* preparation, eupnoea was characterized by 'ramp-like' inspiratory activity of the PN, presence of post-inspiratory activity (PI) in recordings of X and little or no activity in the AB. Three distinct pontine levels were transected as confirmed histologically in sagittal sections in separate rats: (1) rostral pons (5.1-5.7 mm rostral to calamus scriptorius, CS, n=5); (2) mid-pons (4.4-4.8 mm rostral to CS; n=3); (3) caudal pons or immediately rostral to the facial nucleus (i.e. pontomedullary junction; 4.0 mm rostral to CS; n=8). Transection at all 3 levels resulted in apneusis, increased expiratory time and loss of PI (Table 1). Transection of the rostral pons resulted in apnoea in 2 of 5 preparations but the rhythm could be reinstated by either a subsequent transection or increasing respiratory drive (7.5% CO_2). Changes in PN parameters did not differ between rostral and mid-pontile transections ($P>0.05$, t test) compared to control. However, caudal pons transection produced a smaller depression of amplitude and rate of PN compared to the more rostral cuts (Table 1). In addition, tonic expiratory activity in the AB nerve occurred after transection of the caudal pons.

Consistent with Lumsden's (1923) observations we conclude that the pons is essential for the generation of the normal eupnoeic motor discharge *in situ*. The apnoea and depressed respiratory motor outputs evoked after disconnection of either rostral or mid-pons is caused, in part, by descending inhibitory drives generated in the caudal pons, perhaps by the A5 cell group. Finally, the pons seems to depress AB expiratory activity, as it is not present until its removal.

Table 1. PN parameters after pontile transections

	TI	TE	Rate	Amplitude
Rostral pons	+424±160%*	+301±105%*	-69±6%*	-51±5%*
Mid-pons	+394±58%*	+630±196%*	-84±3%*	-43±5%*
Caudal pons	+229±75%*	+132±36%*†	-54±6%*†	-30±6%*†

Values are means±SEM. *Different from control, $P< 0.05$, paired t test. Different from rostral/mid-pontile transections, $P<0.05$, ANOVA.

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PC88

Re-visiting myocardial infarction in the guinea pig: a new approach and model

T.A. Dawson, Z. Barber, C. Carr, D. Stuckey, M.N. Lokale, C. Lee and D.J. Paterson

Physiology, Anatomy and Genetics, University of Oxford, Oxford, Oxfordshire, UK

The guinea pig has a similar electrophysiological and cardiac autonomic profile to human physiology and therefore could be a good model to investigate the effects of acute myocardial infarction. However, a previous report suggests that myocardial infarction cannot be induced in the guinea pig by ligation of either one or two coronary arteries due to high collateral vascularisation1. We have re-visited this model to determine whether it is possible to induce a significant myocardial infarction that results in an impaired cardiac neural phenotype. Guinea pigs (452±99g SD) (58 male and female) were anaesthetised using a protocol which involved induction with a mixture of oxygen and 4% isofluorane followed by intramuscular (IM) ketamine (30μg/g) and medetomidine (0.6μg/g). Anaesthesia was maintained using a mixture of oxygen and 2% isofluorane. A thoracotomy and pericardectomy was performed in the sham group (n=22) with continuation of surgery in another group to coronary artery ligation of the left anterior descending artery and its branches/collateral feeders using four discrete sutures (n=36). Once the thoracotomy was repaired buprenorphine was administered IM (1.2μg/gram) for post operative pain relief during intensive care management. All animals were killed 3 days post surgery.

Post mortem histological analysis of the left ventricle and septum using Sirius Red staining confirmed 9.3±1.1% collagen deposition, n = 6 in the infarct group compared with 1.1±0.7%, n = 6 in the sham group ($p<0.001$, unpaired t test). Myocardial infarction caused 28% mortality in the infarct group (n=10) without evidence of cardiac rupture post mortem. Of the sham animals 9% (n=2) also died suddenly. Prior to histological pro-

filing; seven infarct, sham and naïve guinea pigs were anaesthetised with isofluorane and in vivo vagal nerve stimulations were performed. The infarct group had a significant reduction ($p<0.05$, 1-way Anova, Tukey post-hoc analysis) in their chronotropic response to a 15 V, 3 Hz, 1 ms pulse, 30 s duration right vagal stimulation (-51±15 bpm) postbilateral vagotomy compared with both the sham (-91±41 bpm) and the naïve groups (-95±34 bpm). A further group of guinea pigs had right atrial 3H ACh measured in vitro. The infarct group had less 3H ACh release (1.8±0.7% increase from baseline, $n = 8$, $p<0.01$) compared to naïve animals (3.6±1.1%, n=11) but there was no difference compared to the shams (2.4±8%, n=6) suggesting the changes were secondary to surgical stress. In conclusion, we have successfully produced a guinea pig model of myocardial infarction that resulted in a sudden cardiac death phenotype associated with vagal dysfunction.

Maxwell MP, Hearse DJ & Yellon DM (1987). Cardiovasc Res 21, 737-746.

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