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Brain angiotensin II and superoxide modulate the acute hypertensive response to emotional stress but not feeding

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We reported previously that the angiotensin II (AII) - superoxide (O_2^-) signalling pathway modulates, in the rostral ventrolateral medulla (RVLM), the acute hypertensive response to emotional (air-jet) stress in rabbits (Mayorov & Head, 2003; Mayorov *et al.* 2004). This study determined (i) whether AII and O_2^- regulate the hypertensive response to stress in the dorsomedial hypothalamus (DMH), and (ii) whether AII and O_2^-, in the DMH or RVLM, also modulate cardiovascular arousal associated with appetitive eating behaviour.

Two weeks before the experiment, under fluothane anaesthesia, rabbits were implanted with guide cannulae for bilateral microinjections into the DMH or RVLM. At the beginning of the experiment, a catheter was placed into the central ear artery under local xylocaine anaesthesia to record blood pressure. The air-jet stress was induced by directing a fine stream of compressed air to the face of the rabbit for 8 min. Animals were monitored during stress and the experiment terminated if excessive distress was observed. Feeding (which lasted 5-8 min) was initiated by presenting a small bunch of straw (~10 g) to rabbits. Upon completion of the experiment, rabbits were humanly killed with an overdose of pentobarbitone. Data (means $\pm$ S.E.M.) were analysed using ANOVA and significance taken at $P<0.05$.

Air-jet stress and feeding elicited similar tachycardic responses ($+51\pm6$ bpm and $+47\pm4$ bpm, respectively, n=17). However, the pressor response to feeding ($+9\pm1$ mmHg) was lower than that caused by stress ($+16\pm1$ mmHg, $P<0.05$). Inhibition of the DMH with muscimol (500 pmol; n=8) attenuated pressor and tachycardic effects of stress by 56 ± 11% and 63 ± 24%, respectively ($P<0.01$), and evoked anorexia. Local injection of a glutamate receptor antagonist kynurenate (10 nmol; n=4) decreased pressor and tachycardic responses to stress by 46 ± 9% ($P<0.01$) and 82 ± 36% ($P=0.08$), respectively. The cardiovascular response to feeding was similarly reduced by kynurenate, although rabbits showed no apparent changes in eating behaviour. Microinjection of a selective AT_1 receptor antagonist candesartan (500 pmol; n=9) into the DMH attenuated pressor and tachycardic responses to stress by 31 ± 5% and 33 ± 11%, respectively ($P<0.05$). Local injection of an O_2^- scavenger tempol (20 nmol; n=7) decreased these responses by 50 ± 13% and 36 ± 8%, respectively ($P<0.05$). Neither candesartan nor tempol altered cardiovascular arousal caused by feeding. Likewise, microinjection of tempol (20 nmol; n=6) into the RVLM decreased the hypertensive response to air-jet stress (-49 ± 5%, $P<0.01$), but not to feeding (-3 ± 10%, n.s.).

These results indicate that AII and O_2^- modulate neuronal responsiveness in the DMH and RVLM in a stimulus-dependent fashion. In particular, local AII - O_2^- signalling may specifically subserve neural mechanisms that regulate cardiovascular arousal associated with the defence response, but not with appetitive feeding behaviour.

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The NHMRC (Australia) funded research.

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

C36

Increased angiotensin type 1A receptor activity in the rostral ventrolateral medulla of normotensive rats induces a transient increase in blood pressure

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Neurons located in the rostral ventrolateral medulla (RVLM) are essential for the tonic and reflex regulation of sympathetic vasomotor activity (1). These neurons are excited by angiotensin II acting through AT_1 receptors (2). In spontaneously hypertensive rats, but not in normotensive Wistar Kyoto (WKY) rats, activation of AT_1 receptors in RVLM contributes to the observed hypertension (3). The current experiments aimed to determine whether increased AT_1 receptor density or activity in the RVLM of WKY rats could chronically increase blood pressure.

All experiments were performed in accordance with the Australian NH&MRC code of conduct and were approved by the Institutional Animal Experimentation Committees. Metacam (1 mg/kg) was administered subcutaneously 1 h before each surgery as an analgesic. Under equithesin anesthesia (0.3 ml/100g), male WKY rats (240-280g) were implanted with blood pressure telemeters (DSI). Following 7-10 days recovery blood pressure was recorded daily (between 3 and 4 p.m.) for 7 days. On the 8th day bilateral microinjections of replication-deficient adenoviruses (4 x 100 nl of $1x10^7$ PFU/μl) were made into the RVLM under isoflurane anesthesia (1.8-2.4% in room air). Two viral constructs were used inducing expression, under the control of the cytomegalovirus promoter, of green fluorescent protein (GFP) and either a wild-type AT_{1a} receptor or a constitutively active AT_1 receptor mutant (N111G). Blood pressure was recorded for another 7 days. The rats were then deeply anesthetized, perfused with 4% paraformaldehyde and brains processed for localization of GFP.

The results describe experiments where adenovirus-induced expression of GFP was confined to the RVLM bilaterally. Increased AT_1 receptor activity (N111G) increased mean arterial blood pressure for 4 days before it returned to control levels (n=5, control, 99±0.3 mmHg; 3 days post injection, 113±3 mmHg). Increased expression of the wild-type AT1 receptor (n=5) did not affect blood pressure.

We demonstrate that increased AT_1 receptor activity in the RVLM induces a transient increase in blood pressure. Transgene expression, under the control of the CMV promoter, occurs in astrocytes in the RVLM (4) indicating that local changes in astrocytic function may lead to changes in the activity of RVLM premotor neurons.

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This work was supported by the Australian NH&MRC and the National Heart Foundation of Australia.

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

C37

Is there a role for protein kinase B in the rostral ventolateral medulla for maintaining hypertension?

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It is thought that the presence of a phosphatidylinositol-3-kinase (PI3K) dependent Akt signalling in noradrenergic neurones of the brainstem in the spontaneously hypertensive rat (SHR) is responsible for the maintenance of hypertension (Yang & Raizada, 1999). Our aim was to establish whether Akt activity within the rostral ventrolateral medulla (RVLM) contributed to the hypertension in the SHR.

We employed real time RT-PCR using unilateral tissue punches containing the RVLM from 16 week old humanely killed SHR and WKY rats, which were used to quantify differences in Akt mRNA expression for all three Akt isoforms (Akt 1, 2 and 3). Using a comparative CT method of calculation, RT-PCR revealed a highly significant 2.4-fold increase ($p<0.01$) in Akt3 mRNA expression in the SHR (n=5) compared to the WKY (n=6). In contrast, there was no difference in mRNA expression for either Akt1 or Akt2 isoforms between the two rat strains. Double immunofluorescence was employed to reveal co-localisation of phosphorylated Akt (pAkt), which is the active form of Akt, with tyrosine hydroxylase (TH) - a marker for noradrenergic neurones. pAkt expression was found in some TH positive RVLM neurones in both the SHR (n=3) and the WKY (n=3).

We conclude that since Akt3 is the major neuronal isoform of Akt, and Akt3 mRNA expression in the RVLM is higher in the SHR, this supports the hypothesis that increased PI3K/Akt signalling in catecholamine neurones contributes to the maintenance of hypertension in the SHR.

Yang H & Raizada M (1999). *J Neurosci* 19, 2413-2423.

Supported by Pfizer and the British Heart Foundation.

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

C38

Role of 5-HT1A receptors in the cardiovascular response elicited from the dorsomedial hypothalamic nucleus

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The dorsomedial hypothalamic nucleus (DMH) mediates the cardiovascular response to stimuli such as air jet stress or cold stress (1). The sympathoexcitatory vasomotor and cardiac components of the DMH-evoked response are dependent upon neurons in the rostral ventrolateral medulla and raphe pallidus in the medulla (1,2). Both of these regions contain 5-HT1A receptors. Intravenous administration of the selective 5-HT1A agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) centrally inhibits sympathetically mediated cutaneous vasoconstriction induced by cold stress (3). The aim of this study was to investigate the effect of systemic administration of 8OH-DPAT on the cardiovascular response evoked by DMH activation and, as a control, on the reflex responses to chemoreceptor and baroreceptor stimulation.

Sprague-Dawley rats (body weight 410-480 g) were anaesthetized with urethane (1.3 g/kg, i.v. after induction with 2% isoflurane). Bicuculline microinjections (10 pmol in 20nl) into the DMH resulted in a modest increase in mean arterial pressure (MAP; 16 ± 2 mmHg), heart rate (HR; 89 ± 13 bpm) and renal sympathetic nerve activity (RSNA; 47 ± 4% of baseline). Intravenous injection of 8-OH-DPAT (0.1 mg/kg) resulted in small but significant decreases in resting MAP (12 ± 1 mmHg, $P<0.05$) and HR (32 ± 7 bpm, $P<0.05$) and had no significant effect on resting RSNA (change 0 ± 3% baseline), but greatly reduced the increases in MAP, HR and RSNA evoked by DMH activation (to 3 ± 2 mmHg, 15 ± 4 bpm and 4 ± 2%, respectively; $P<0.001$ compared with control values, in all cases). After subsequent administration of the selective 5-HT1A receptor antagonist WAY-100635 (0.1 mg/kg) the DMH-evoked increases in MAP, HR and RSNA (14 ± 2 mmHg, 94 ± 10 bpm, and 49 ± 7%, respectively) were not significantly different ($P>0.5$ in all cases) to those observed before 8-OH-DPAT administration. Administration of 8-OH-DPAT did not alter either the increase in RSNA evoked by chemoreceptor stimulation with sodium cyanide (0.1 mg/kg, i.v.) or the cardiac-related component of the RSNA response. The results indicate that activation of 5-HT1A receptor blocks the cardiovascular response evoked from the DMH but does not affect sympathetic vasomotor tone or chemoreceptor and baroreceptor reflexes. All animals were humanely killed at the end of the experiments.

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Supported by the National Health and Medical Research Council of Australia.

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

C39

5-HT depletion reduces cardiac baroreflex sensitivity in awake and anaesthetised rats

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Blockade of central 5-HT_7 and 5-HT_{1A} receptors interferes with reflex-evoked vagal bradycardias (Kellett *et al.* 2005) implying that 5-HT is released during the reflex activation of cardiac vagal preganglionic neurones. This is supported by the

observation that 5-HT depletion inhibits reflex-evoked vagal bradycardias in anaesthetised rats (Kellett *et al.* 2004). The present experiments examine the effect of 5-HT depletion with para-chlorophenylalanine (*p*-CPA; Koe & Weissman, 1966) on the baroreflex of awake and subsequently anaesthetised (α-chloralose 80 mg kg^{-1}, i.v.) rats. Adult male Sprague-Dawley rats (300-350 g) were given daily *p*-CPA (350 mg kg^{-1} s.c.; $n = 7$) or saline ($n = 7$) for 2 days. Catheters were implanted in the femoral artery and vein under isoflurane anaesthesia (2.5% in O_2) 24 h before the experiment. During the experiment animals were given atenolol (1 mg kg^{-1}, i.v.). Baroreflexes were elicited by i.v. phenylephrine (PE, 1 μg) and sodium nitroprusside (SNP, 1 μg). Absolute changes in MAP and pulse interval (PI) were measured, and reflex gain calculated by linear regression (Su *et al.* 1992). At the end of the experiment animals were humanely killed with an overdose of pentobarbitone.

5-HT immunocytochemistry showed a marked reduction in 5-HT immunoreactive fibres within the nucleus tractus solitarius of *p*-CPA treated rats. Measurement of tissue monoamine content, using HPLC (Géranton *et al.* 2004), confirmed that *p*-CPA depleted hindbrain tissue of 5-HT (mean±S.E.M.: 0.2±0.1 *vs.* 3.1±0.6 μM; t test, $P<0.01$, $n = 3$), but not of noradrenaline or dopamine.

Baseline MAP was significantly higher in depleted vs. control rats (110±4 *vs.* 97±4 mmHg; 2-way ANOVA, $P<0.05$), but this difference was abolished by anaesthesia (71±5 *vs.* 78±6 mmHg). Baseline PI was similar in all groups. PE- and SNP-evoked baroreflex gains were attenuated by depletion in both awake and anaesthetised rats (see Fig. 1). Also, PE-evoked changes in PI (another measure of reflex sensitivity) were also attenuated by 5-HT depletion (23±5 *vs.* 50±10 ms), and in control animals they were potentiated by anaesthesia (79±6 *vs.* 50±10 ms), suggesting inhibitory mechanisms are active in the awake state.

The data indicate that 5-HT release is involved in the cardiac baroreflex in both awake and anaesthetised rats.

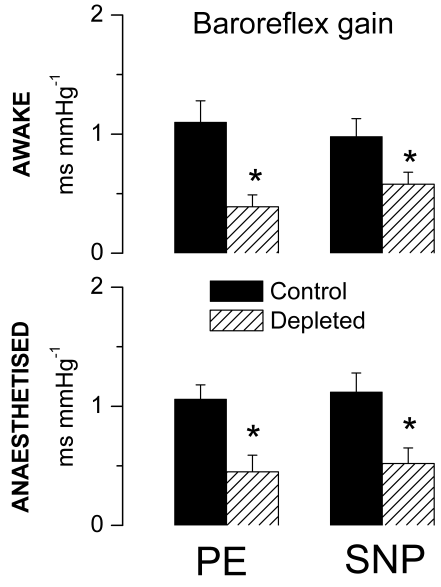

Figure 1. Mean (± s.e.m) baroreflex gain evoked by PE and SNP in control and 5-HT depleted awake and anaesthetised rats (n = 7). * P<0.05 (cf. control).

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

C40

Optical imaging of the ventral medullary respiratory network during eupnoea and gasping in situ

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Voltage-sensitive dyes can be used to monitor changes in membrane excitability from neuronal networks (Briggman et al. 2005; Onimaru & Homma, 2003; Tominaga et al. 2000). The information gleaned gives both temporal and spatial patterns of activity. In the present study we applied voltage-sensitive dyes to the mammalian respiratory network to assess the feasibility of this technique. Our initial question was to assess whether there was any spatio-temporal differences of neuronal activity within the ventral respiratory group (VRG) between eupnoea and gasping.

Rats (Wistar; male; 80-100g) were anaesthetised deeply in 4% halothane. Once there was an absence of a withdrawal response to noxious pinching of the tail or a paw, an arterially perfused in situ working heart-brainstem preparation was prepared. This has good mechanical stability of the brainstem and light scattering is minimised by using a cell-free perfusate. Using a lateral approach the ventrolateral medulla was exposed (see Dutschmann & Paton, 2003). Contained within a glass micropipette di-2-ANEPEQ (50μg/ml; Molecular Probes) was microinjected into the VRG as defined by mass respiratory related activity recorded extracellularly. Neuronal activity was recorded using an optical recording system (MiCAM02 system, Brain Vision Inc., Tsukuba, Japan) through a 510-550 nm excitation filter, a dichroic mirror and a 590 nm absorption filter. Illumination was provided by a tungsten-halogen lamp (150 W) and a CCD camera (180 × 120 pixels) with a maximum time resolution of 3.5 ms was used to detect a change in fluorescence of the voltage-sensitive dye. Changes in fluorescence (F) were measured as ΔF/F and expressed as au. Magnification was provided by a ×2 objective (Olympus Optical) that resulted an imaging area of 4.2 × 3.25 mm^2.

Phrenic nerve related patterns of neuronal activity were seen throughout the VRG (n=4); these included regions exhibiting inspiratory (0.66±0.07) and post-inspiratory (0.27±0.02) related changes in fluorescence. During gasping (induced with 7% oxygen; n=4), activity was confined to a restricted region that exhibited inspiratory related changes in fluorescence only (2.54±0.5).

We conclude that voltage-sensitive dyes can be used in situ and that temporal and spatial information can be obtained from the VRG under conditions of both eupnoea and gasping.

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This work was supported by the NIH and British Heart Foundation.

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

C78

Functional consequences of increased expression of α4, β1 and δ $GABA_A$ receptor subunits in the periaqueductal grey matter: heightened responsiveness to a panicogenic agent in female rats

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In humans and animals panic-like behaviour can be elicited by activating neuronal circuitry in the dorsal half of the periaqueductal grey matter (dPAG) (Lovick, 2000). In a recent study in female rats we have shown that late dioestrus (equivalent to the late luteal phase in women) is associated with an increase in the number of GABAergic neurones in the PAG that express α4, β1 and δ $GABA_A$ receptor subunits (Griffiths & Lovick, 2005; Lovick et al, 2005). We have investigated whether the increase in expression of α4, β1 and δ $GABA_A$ receptor subunits in late dioestrus is associated with an increase in the responsiveness of the PAG circuitry to a panicogenic compound compared with other phases of the cycle.

All experiments were carried out on 200-250g urethane-anaesthetised female Wistar rats (1g kg^{-1} i.p.) in proestrus (PRO) or late dioestrus (LD), chosen to represent states of low and high expression, respectively, of α4, β1 and δ $GABA_A$ receptor subunits in the PAG (Lovick et al. 2005). Femoral arterial pressure, heart rate (HR) and respiratory rate (RR) were measured. The panicogenic cholecystokinin CCK_B receptor agonist pentagastrin (PG, 0.002-80μg kg^{-1} i.v.) evoked a dose-related increase in mean arterial pressure, heart rate and respiratory rate. The pressor response and tachycardia evoked by 80μg kg^{-1} PG in rats in LD (14.7±1.0 mmHg and 14.3±2.2 beats min^{-1}, means±S.E.M, n=8) was significantly greater than rats in proestrus (10.3±1.5 mmHg and 6.9±2.0 beats min^{-1}, n=7, $p<0.05$, ANOVA). Respiratory rate also increased in both groups but the difference between groups was not significant (increase of 17.7±3.3 and 11.9±1.4 breaths min^{-1} for LD and PRO, respectively, p=0.13, ANOVA).

In electrophysiological experiments, PG (40μg kg^{-1} i.v.) evoked excitatory responses in 5/6 presumed output neurones recorded extracellularly in the dPAG using 5-barrelled glass micropipettes. Iontophoretic application of PG (13mM, pH 8-9, 10-30nA) evoked an increase in firing rate in 14/17 cells. Ongoing activity was increased from 5.1±0.4 Hz to 9.7±1.0 Hz ($p<0.001$). In the presence of the CCK_B receptor antagonist CR2945 (10mM, pH 8-9, 60nA, Sigma) the excitatory response to PG was reduced by 64±7% ($p<0.05$, n=4).

The results suggest that the cardio-respiratory component of the response to i.v. PG may be mediated, at least in part, by activation of CCK_B receptors on neurones within the PAG. Rats in late dioestrus showed heightened responsiveness to PG, which may be linked to the increased expression of α4, β1 and δ $GABA_A$ receptor subunits that occurs on neurones in the PAG at this time.

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Supported by The Wellcome Trust.

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

C79

Carotid baroreceptor stimulation alters pulmonary arterial baroreflex control of systemic vascular resistance in anaesthetised dogs

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Recently we have established that reflex vasoconstriction in response to pulmonary arterial baroreceptor stimulation can be demonstrated at the arterial pressures normally prevailing in the pulmonary circulation (Moore et al. 2004). The aim of this study was to examine whether there is interaction between reflex responses to stimulation of pulmonary arterial receptors and those from stimulation of carotid sinus baroeceptors. In ten chloralose-anaesthetized dogs (100 mg kg^{-1} I.V.), a perfusion circuit allowed independent control of pressures distending the pulmonary, aortic arch, carotid sinus and coronary artery baroreceptors. The chest wall was sealed and a phasic intrathoracic pressure ranging from atmospheric to around – 10 mmHg (18 cycles min-1) applied. Vascular responses were determined from changes in perfusion pressure to the descending aorta (constant flow). Pulmonary baroreceptor stimulus-response curves were defined at two carotid sinus pressures (CSP) at around 65 and 125 mmHg. Sigmoid functions were fitted to the curves and indicators of baroreflex function i.e. maximal gain and set point determined. Blood samples were taken at regular intervals for determination of systemic arterial blood gases and pH. Animals were killed by exsanguination following a lethal dose of anaesthetic at the end of each experiment.

Increasing pulmonary arterial pressure from 5 to 40 mmHg resulted in an increase in systemic perfusion pressure of 38 ± 8 mmHg at 60 mmHg CSP and 33 ± 8 mmHg at 120 mmHg CSP; the percentage increases in vascular resistance were not significantly different ($P > 0.05$ paired t test). When the carotid pressure was elevated, the pulmonary baroreceptor stimulus-response curve was displaced to the right, i.e. to a significantly higher pulmonary pressure (Table 1). Under the same conditions, the peak gain was reduced in 8 out of 10 experiments; however, the difference failed to achieve statistical significance. These results confirm that the set point of the pulmonary arterial baroreflex does occur within the range of normal physiological pressures. Furthermore, this set point is modified when the pressure stimulus to the carotid sinus baroreceptors is altered. We conclude, therefore, that the pulmonary arterial baroreflex is subject to modulation by input from carotid baroreceptors, and that this may play an important role in reflex cardiovascular control.

Gain (units)		Set point (mmHg)	
CSP 60mmHg	CSP 120 mmHg	CSP 60 mmHg	CSP 120 mmHg
5.9±1.6	3.3±0.5	28.5±4.0	35.8±3.6*

Table 1. Means ± SEM for the gain (maximal slope) and set point of the baroreceptor stimulus-response relationship at two levels of carotid sinus and pulmonary baroreceptor stimulation. (* $P < 0.05$, paired t test).

Moore JP et al. (2004). J Physiol 555, 805-814.

These experiments were supported by a project grant from the British Heart Foundation.

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

C80

Mild insulin resistance and accelerated endothelial dysfunction – studies in mice heterozygous for a knockout of the insulin receptor

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Mice heterozygous for a knockout of the insulin receptor gene (IRKO) represent a non-obese model of mild insulin resistance and demonstrate a 30% reduction in stimulation of the PI3kinase pathway in response to insulin(1). We recently demonstrated loss of the vascular response to insulin in young 8-week IRKO mice but preservation of endothelial function assessed by relaxation responses to acetylcholine(2). In this study, we sought to examine the effect of a mild defect in insulin signalling on endothelial function with ageing. We studied metabolic function and blood pressure in vivo, and vascular function in aortic rings ex vivo in young (2 months) and middle aged (6 months) IRKO mice and their wild type (WT) littermates as previously described (2). All animals were humanely killed. n=6-8 per group, data presented as mean±SEM, $p<0.05$ taken as significant, Student's t test and ANOVA used where appropriate.

Systolic blood pressure (measured by tail cuff plethysmography) was significantly greater in IRKO mice than WT at 8 weeks (124±4 and 110±3 n=8, p=0.01). There was no significant change in blood pressure with ageing in either IRKO (124±4 and 119±6) or WT mice (110±3 and 106±6). At 6 months IRKO mice showed significantly augmented contraction to phenylepherine (Emax(g) 2 months 0.6±0.06; 6 months 0.96±0.02; p=0.04) and impaired acetylcholine mediated relaxation (91.9%±8 and 66.3%±7; p=0.01). IRKO acetylcholine responses were partially normalised by the SOD mimetic MnTMPyP (10μM; Emax pre 77.6±6 post 91±2; p=0.03). WT vascular responses were unchanged between 2 and 6 months.

Despite the evolution of significant endothelial dysfunction, the metabolic phenotype of IRKO mice remained mild. Fasting blood glucose was similar in IRKO (8.4±0.4 and 7.8±0.4) & WT (9.1±0.6 and 8.2±0.5) of both ages, as were blood glucose responses during insulin tolerance testing. However, old IRKO mice demonstrate significantly greater blood glucose and increment in blood glucose 30 minutes after an intraperitoneal glucose challenge (1mg/g) than WT mice (peak blood glucose: 16.9 and 13.4; p=0.001; increment from baseline: 123%±16 and 68%±15; p=0.01 respectively). They also exhibit greater visceral adiposity (mesenteric fat pad mass: IRKO 1.18mg±0.21; WT 0.74mg±0.05; p=0.04) despite similar total body weights (28.2g±0.9 and 29.2g±1.2).

We conclude that a mild defect in insulin signalling promotes significant premature endothelial dysfunction with ageing, despite minimal deterioration in metabolic phenotype. Our data supports a potential role for reactive oxygen species in this process.

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This work was supported by the British Heart Foundation.

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

C81

Guanylyl cyclase activators evoke nitric oxide release in the intact rat superior mesenteric artery

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The purpose of the present study was to investigate the role of the cyclic GMP-pathway in acetylcholine (ACh)-evoked nitric oxide (NO)-mediated relaxation in rat superior mesenteric artery.

Segments from humanely killed animals were mounted in microvascular myographs and by introduction of a NO specific microelectrode in the lumen of the artery simultaneous measurements of NO concentration and isometric force were performed. In the presence of indomethacin (3μM) and contracted with noradrenaline (0.5μM), ACh (10μM) increased NO concentration 11.3±1.9 nM and relaxed the arteries 99±1 % (n=6). The selective guanylyl cyclase activators, YC-1 (10μM) and BAY 412272 (10μM), increased NO concentration by, respectively, 11.3±4.7 nM (n=6) and 5.9±1.1 nM, and relaxed the arteries by, respectively, 94±2 % (n=6) and 95±1 % (n=6). These responses were reversed by oxyhaemoglobin (OxHb). In arteries without endothelium, BAY 412272 did not increase NO concentration and relaxation was reduced to 59±1 % (n=6). An inhibitor of soluble guanylyl cyclase, ODQ (3μM), reduced ACh-evoked increases (n=6) in NO and relaxations, and abolished S-nitroso-N-acetylpenicillamine (SNAP) relaxation without changing increases in NO concentration. An inhibitor of phosphodiesterase type 5, sildenafil (1μM) caused endothelium-dependent relaxations sensitive to OxHb, without increasing NO concentration (n=6), and leftward shifted concentration-relaxation curves for acetylcholine and SNAP. The protein kinase G (PKG) activator, 8-br-cGMP (400μM), did not increase NO concentration, but caused relaxation which was inhibited by the PKG inhibitor, 8br-PET-cGMPS (30μM). 8br-PET-cGMPS and an inhibitor of PKA, Rp-8Br-cAMPS (50μM), did not change ACh-induced increases in NO concentration. An inhibitor of heat shock protein (HSP90), geldanamycin rightward shifted acetylcholine concentration-relaxation curves.

The present study provides evidence for a guanylyl cyclase-dependent mechanism, which probably independent of protein kinase G activation enhances ACh-evoked release of NO. We propose a model, where conformation changes in guanylyl cyclase induced by NO or guanylyl cyclase activators through the scaffold protein HSP90 increases endothelial NO synthase activity and enhances NO formation. The effect on relaxation of sildenafil and ODQ confirms a role for a smooth muscle cyclic GMP-pathway in acetylcholine-evoked relaxation.

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

C82

HET0016, a selective blocker of 20-HETE production, inhibits myogenic tone induced by low level of α-adrenoceptor stimulation in rat mesenteric resistance artery

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The Cytochrome P450 (CYP450) metabolite 20-HETE plays a significant role in pressure-induced vasoconstriction (myogenic response) in resistance arteries. It is less clear whether this role is due to regulation of stretch-activated ion channels involved in the myogenic response. Transient receptor potential (TRP) channels are expressed in mammalian vascular smooth muscle cells (VSMC) and have been suggested as the stretch sensor involved in myogenic responsiveness. We investigated the possibility that specific inhibition of 20-HETE production interferes with stretch-induced activation of functionally expressed TRPC6 channels. To clarify the physiological relevance of such a mechanism we examined the effect of specific inhibition of 20-HETE production on myogenic tone in rat mesenteric arteries (RMA). Freshly excised RMA (2nd or 3rd order) obtained from humanely killed animals were mounted in a pressure myograph and superfused with HCO_3^-/CO_2-buffered PSS (pH 7.4) at 37°C. Steady-state diameter was recorded at intraluminal pressures of 0, 20, 40, 60, 80 and 100 mmHg during control and drug periods. The passive pressure-diameter relationship was obtained in Ca^{2+}-free solution (+EGTA). Whole-cell currents and Ba^{2+} fluorescence were measured on HEK293 cells transiently expressing TRPC6 channels by using the patch-clamp and fluorescence imaging techniques, and stretch-activation was induced by hypotonic solution (-90 mOsm). Specific inhibition of 20-HETE production was obtained by using HET0016 (Taisho Pharmaceutical Co., Saitama, Japan), a selective blocker of CYP450 4A enzyme catalysing 20-HETE production, and unspecific inhibition was obtained by using 17-ODYA.

RMA did not develop substantial myogenic tone under control conditions. To mimic sympathetic nerve activity, RMA were preconstricted with low phenylephrine concentration (0.05—1.0 μM) to ~85% of the resting diameter at 60 mmHg. Preconstriction resulted in decreased diameter at pressures ≥20 mmHg as compared with the passive curve ($P<0.05$, $N=4$). However, in separate RMA ($N=4$) with similar degree of preconstriction, pretreatment with 10 μM HET0016 eliminated this difference.

In TRPC6–expressing HEK293 cells, hypotonic challenge potentiated both receptor-activated (100 μM carbachol) cation current and Ba^{2+} entry about 2-fold ($P<0.01$, $n=8$), and this effect was abolished by pretreatment with HET0016 (3—10 μM) or 17-ODYA (10 μM). Hypotonic potentiation of cation current or Ba^{2+} entry was not detectable in the absence of receptor stimulation. Our data shows that 20-HETE production is important for development of myogenic tone induced by low level of α-adrenoceptor stimulation in RMA. The mechanism responsible may be 20-HETE mediated potentiation of receptor-activated TRPC6 channels in VSMC as was demonstrated in HEK293 cells.

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

PC32

The cardiopulmonary reflex recruits central 5-HT$_7$ receptors in awake rats

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In anaesthetised rats, central 5-HT$_7$ receptors play an important facilitatory role in cardiopulmonary, baro- and chemoreflex vagal bradycardia (Kellett *et al.* 2005). The present study investigated the involvement of central 5-HT$_7$ receptors in the cardiopulmonary reflex responses in freely moving awake rats, using the selective 5-HT$_7$ receptor antagonist SB-269970 (Hagan *et al.* 2000).

Adult male Wistar rats (280-330 g) were implanted with intracisternal (i.c.; Michelini & Bonagamba, 1988) 23-gauge guide cannulae under tribromoethanol anaesthesia (250 mg kg^{-1}; i.p.). After 72 h recovery, rats were re-anaesthetised and implanted with femoral arterial and venous polythene catheters. Experiments commenced 24 h later. Pulsatile arterial pressure and HR were measured from the arterial line, and cardiopulmonary afferents were stimulated with 5-HT (1 - 3 μg; i.v.) under freely moving conditions as previously described (Chianca & Machado, 1996). After at least two stable (< 10% change) control reflexes, either the 5-HT$_7$ receptor antagonist SB-269970 (100 μg kg^{-1}) or its vehicle (saline), was injected i.c. (5 μl) and reflexes stimulated every 5 min. Absolute changes in HR and MAP were analysed by 2-way ANOVA and Fisher's least significant difference test. At the end of the experiment animals were humanely killed with an overdose of thiopentone sodium.

Both reflex bradycardia and hypotension were significantly attenuated by SB-269970 within 5 min of administration (see Fig. 1), and there was rapid recovery from this effect. Neither SB-269970 nor saline significantly altered baseline MAP or HR (control baselines were 104 ± 4 mmHg and 376 ± 17 bpm, respectively). Pontamine sky blue dye given i.c. caused the densest staining in the dorsal medulla.

The data demonstrate that the important facilitatory action of brainstem 5-HT$_7$ receptors on cardiopulmonary reflex responses can also be observed in awake rats.

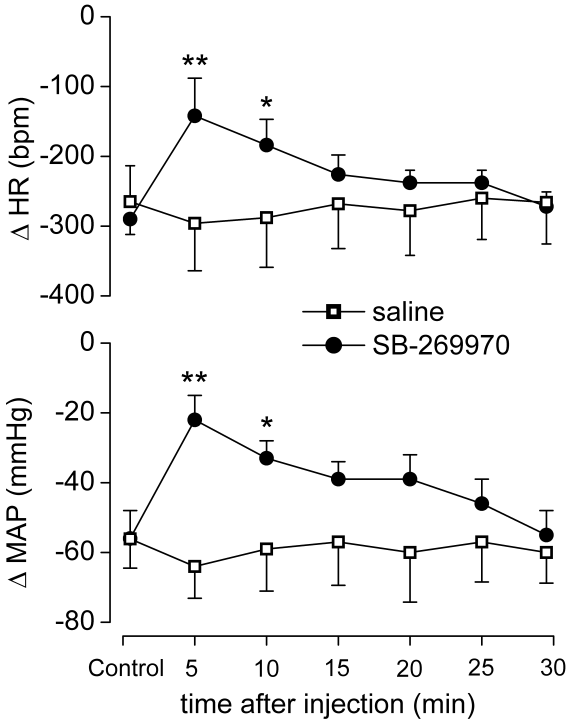

Figure 1. Mean (± s.e.m.) cardiopulmonary reflex-evoked changes (Δ) in HR and MAP in awake rats treated with i.c. saline (5 μl) or SB-269970 (100 μg kg-1; n = 5). * P < 0.05, ** P < 0.01 (cf. saline).

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This work was supported by FAPESP and CNPQ (Brazil) and by the British Heart Foundation (UK).

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

PC33

Chronic treatment with mianserin prevents DOCA-salt hypertension in rats

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Central 5-HT$_2$ receptors have been implicated in the control of blood volume through the activation of a central angiotensinergic pathway to release of vasopressin (see Ramage, 2001). Vasopressin is essential for the development of DOCA-salt hypertension (Crofton et al. 1979). Experiments were carried out to determine if blockade of 5-HT$_2$ receptors with mianserin (Bonhaus et al. 1995) would prevent the development of DOCA-salt hypertension in rats.

Fifty six male Wistar rats (150-200g) under recovery anaesthesia with choral hydrate (400 mg kg^{-1}, i.p.) had i.c.v. guide cannulas inserted (see Anderson et al. 1992) and were uninephrectomized. Five days later, DOCA (15 mg kg^{-1}, s.c.) or vehicle (soybean oil, 0.25 ml) and mianserin (30, 60 or 90 µg per rat, i.c.v.) or 3µl of vehicle (10% PEG) were given twice daily for 20 days. Rats were provided with water containing 1% NaCl and 0.03% KCl. One day before and during the experimental period, daily water intake and urinary sodium content and volume were determined (Bissoli et al. 2000). On the 21st day a catheter was placed into the femoral artery, under recovery anaesthesia with ether, from which MAP and HR were measured 6 h later. At the end of the experiments, the rats were humanely killed.

The MAP and HR changes in these above groups are shown in Table 1.

Mianserin, 90 or 60 µg for 20 days prevented the development of hypertension in rats receiving DOCA-salt but did not affect blood pressure in rats on salt alone. The dose of 30 µg had no effect on the development of the hypertension nor did the vehicle. Mianserin 90 µg also prevented the increase in fluid intake, urinary flow and sodium excretion caused by DOCA-salt treatment. In DOCA-salt rats receiving vehicle, urinary flow was 14 ± 4 ml and sodium excretion was 24 ± 7 mEq kg^{-1} on day 0; however, by day 20 both values had significantly ($P < 0.05$) increased to 49 ± 17 ml and 44 ± 6 mEq kg^{-1}, whereas in the mianserin group they were not significantly different; at day 0 urinary flow was 16 ± 3 ml and sodium excretion was 18 ± 2 mEq kg^{-1}, while on day 20 they were 21 ± 5 ml and 26 ± 4 mEq kg^{-1}, respectively. In DOCA-salt rats fluid intake on day 0 was similar to that on day 20, being 75 ± 20 ml and 81 ± 10 ml, respectively. However, for the mianserin-treated rats it was reduced from 68 ± 13 ml to 47 ± 7 ml by day 20, which was significantly different from that of DOCA-salt rats.

These data indicate that this action of mianserin is not due to an intrinsic hypotensive action but an action which involves interference with the mechanism by which DOCA salt treatment causes hypertension. The data overall support the view that to induce hypertension with DOCA-salt, central 5-HT_2 receptors need to be activated to cause the release of vasopressin which has been shown to be responsible for the initiation of DOCA-salt treatment hypertension.

Experimental Groups	n	MAP (mmHg)	HR (bpm)
DOCA vehicle-salt	5	116 ± 5^{++}	376 ± 7
DOCA vehicle salt + PEG	5	114 ± 5^{++}	357 ± 18
DOCA vehicle salt + mianserin 90 µg	9	121 ±9^{++}	381 ± 13
DOCA-salt	5	150 ± 5**	358 ± 17
DOCA-salt + PEG	8	152 ± 6**	360 ± 20
DOCA-salt + mianserin 30 µg	7	151 ± 7**	374 ± 19
DOCA-salt + mianserin 60 µg	7	128 ±6^{++}	370 ± 30
DOCA-salt + mianserin 90 µg	10	126 ±3^{++}	357 ± 21

Table 1. Effects of chronic i.c.v. injections (for 20 days) of mianserin on MAP and heart rate (mean ± S.E.M.) in control and DOCA-salt hypertensive rats. **P⟨0.01 when compared to DOCA vehicle-salt + PEG. $^{++}$P⟨0.01 when compared to DOCA-salt + PEG group using repeated measures ANOVAs for the main effects, and Fisher test for paired comparisons between the means

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Ramage AG (2001). Brain Res Bull 56, 425-439.

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

PC34

Progressive baroreflex desensitisation during incremental isometric exercise in man

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In dynamic exercise of increasing intensity baroreflex sensitivity (BRS), measured by the sequence technique (Iellamo, 1998), or gain, by neck pressure change (Fadel *et al.* 2003), has been found to decrease progressively. However, baroreflex control in isometric exercise is less well understood (Carrington & White, 2001). With progressive increases in isometric force, the interaction of greater central command, mechanoreceptor stimulation and metaboreflex activation make it difficult to predict BRS. This study examined BRS during isometric exercise of increasing intensity. Ten subjects (7 male, mean±S.E.M.) aged 22.4±0.9years sat in an isokinetic dynamometer with the right knee flexed at 150 deg and the foot attached with the lower leg horizontal. Heart rate (HR) and blood pressure (BP) were measured using an ECG and Finapres. Respiratory phase was measured using a strain gauge around the chest and respiratory rate was maintained at oeupnoeic frequency by breathing to a metronome. After 290s of rest, a cuff was inflated around the right thigh to 200mmHg. Subjects then either rested (0% trial) or performed 90s of isometric plantarflexor exercise at 30, 50 or 70% of maximum voluntary contractile force (MVC). Mean resting HRs were 70±3, 68±2, 73±4 and 73±3 b min^{-1} prior to the 0, 30, 50 and 70% trials, respectively, and were 70±4, 85±4, 100±5 and 119±7 b min^{-1} by end of rest or exercise, respectively. Mean resting BPs were 86±3, 89±3, 93±3 and 95±3 mmHg prior to the 0, 30, 50 and 70% trials, respectively, and were 88±3, 109±3, 126±4 and 141±4mmHg by end of rest or exercise, respectively. Regression equations produced by sequence analysis of R-R intervals (Y axis) and systolic blood pressure (SBP) (X axis) gave mean slope and intercept values shown in Table 1. There were no significant differences between trials for slopes and intercepts at rest (repeated measures ANOVA). However, exercise decreased slope and increased intercept from rest ($P<0.05$) and these changes became progressively greater with increasing exercise intensity ($P<0.05$, *post hoc* LSD test). This study has shown that BRS decreases progressively with increasing intensity of isometric exercise, with a concomitant rightward shift of the R-R v SBP relationship. Whether this progressive change is due to increased central command, increased muscle mechanoreceptor stimulation or metaboreflex activation requires further study.

Table 1. Mean slope and intercept values from BRS sequence analysis (S.E.M.)

	Slope (ms $mmHg^{-1}$)		Intercept (ms)	
Trial	Rest	Exercise	Rest	Exercise
0%	15.5 ± 1.5	15.1 ± 1.4	–1017 ± 158	–999 ± 157
30%	15.7 ± 1.5	9.7 ± 1.2 * #	– 1160 ± 198	–694 ± 167 *
50%	14.8 ± 2.1	6.2 ± 1.5 * #	– 1160 ± 265	–345 ± 229 * #
70%	14.6 ± 2.1	3.3 ± 0.7 * #	– 1165 ± 256	–4 ± 94 * #

*Different from resting values of same trial; #different from 0% trial.

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

PC35

Oxytocin-immunoreactive axons closely appose cardiovascular neurons in the nucleus of the solitary tract and dorsal vagal motor neurons in the rat

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In addition to its involvement in reproduction, oxytocin (OXT) has roles in the regulation of feeding, water balance and blood pressure. Neurons in the dorsal vagal complex, which includes the nucleus of the solitary tract (NTS) and the dorsal vagal nucleus (DVN), are part of the neuronal circuitry regulating these latter functions. OXT-immunoreactive (IR) axons, which probably originate from the paraventricular nucleus of the hypothalamus, a centre for autonomic and neuroendocrine integration, occur in the dorsal vagal complex; but it has not yet been conclusively established which neurons in this region receive an OXT-IR innervation.

To answer this question, we identified autonomic neurons in the dorsal vagal complex in two ways. In awake rats, we lowered blood pressure by intravenous administration of hydralazine (n = 6; Stornetta et al. 2001) or raised blood pressure with a 90 min intravenous infusion of phenylephrine (n = 12; Minson et al. 1997) to activate reflexly cardiovascular neurons in the nucleus of the solitary tract (NTS) and thereby evoke Fos expression. Seven rats were anesthetized (pentobarbitone, 60 mg kg^{-1} i.p.) and neuromuscularly blocked (α-bungarotoxin, 150 $\mu g\, kg^{-1}$ i.v.) for juxtacellular labelling; blood pressure and heart rate were continously monitored and reflex responses to tail pinch were assessed regularly. In these rats, we identified and electrophysiologically characterised individual neurons in the NTS (n = 8) or DVN (n = 6) that responded to vagal stimulation and labelled them juxtacellularly with biotinamide. At the end of all experiments, rats were perfused with 4% formaldehyde under pentobarbitone anaesthesia (100 mg kg^{-1} i.p.). Fos-IR nuclei and/or OXT-IR axons were revealed with a nickel intensified diaminobenzidine reaction (black); and choline acetyltransferase (ChAT)-immunoreactivity or biotinamide, with an imidazole-intensified diaminobenzidine reaction (brown).

In all rats treated with hydralazine or phenylephrine, varicose OXT-IR axons formed a network around Fos-IR nuclei in the caudal and mid NTS and were associated with ChAT-IR neurons in the DVN. Six out of eight of the juxtacellularly labelled NTS neurons were closely apposed by at least one OXT-IR varicosity. All six labelled DVN neurons received close appositions on their proximal and distal dendrites but their cell bodies were too heavily labelled to detect appositions.

These results suggest that oxytocin may directly affect the activity of both NTS neurons and preganglionic neurons in the DVN through inputs on their dendrites.

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Supported by NH and MRC Australia, NIH USA and Wellcome Trust UK.

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

PC36

ATP mediates CO_2-chemosensitivity in the carotid body in mice

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It was shown previously that ATP acting via P2X receptors that contain the $P2X_2$ subunit, with or without $P2X_3$ subunit, contributes in a significant manner to the transmission of the sensitivity of the carotid body to changes in arterial PO_2 (Zhang *et al.* 2000; Prasad *et al.* 2001; Rong *et al.* 2003). In the present study we used mice with selective deletion of genes encoding $P2X_2$ ($P2X_2$-/-), $P2X_3$ ($P2X_3$-/-) or both subunits ($P2X_2/P2X_3$Dbl-/-) to determine whether in the carotid body ATP also mediates CO_2 chemosensory transduction.

All experiments were carried out in adult (4-6 months) knockout and wild-type mice. Animals were killed by an overdose of pentabarbitone sodium (200 mg kg^{-1}). The carotid body and the attached sinus nerve were dissected. The sinus nerve was recorded in vitro using a suction electrode. The analogue of hypercapnia was induced by perfusing the recording chamber with aCSF in which sufficient extra CO_2 had been bubbled to reduce the pH from its normal value of 7.4 to a 7.0.

It was found that (i) carotid sinus nerve resting discharge was reduced by 32% (n=9; $p<0.05$, Student's *t* test), 54% (n=9; $p<0.05$), and 83% (n=7; $p<0.05$), in $P2X_2$-/-, $P2X_3$-/- and $P2X_2/P2X_3$Dbl-/- mice, respectively; (ii) peak response to hypercapnia in $P2X_2$-/-, $P2X_3$-/- and $P2X_2/P2X_3$Dbl-/- mice was smaller than in their wild-type littermates (n=18) by 63% (n=9; $p<0.05$), 63% (n=9; $p<0.05$), and 86% (n=7; $p<0.05$), respectively; (iii) in preparations taken from the wild-type mice (n=8) P2X receptor antagonist 2′-(or-3′)-O-(trinitrophenyl)-adenosine 5′-triphosphate (TNP-ATP; 10 μM) reduced the baseline firing by 58% ($p<0.05$, Student's paired t test) and the peak response to hypercapnia by 71% ($p<0.05$); (iv) in preparations taken from the $P2X_2$-/- mice (n=5) TNP-ATP reduced the baseline firing by 76% ($p<0.05$) and the peak response to hypercapnia by 50% ($p<0.05$).

From these data we conclude that in the carotid body ATP is partially responsible for the maintenance of the baseline discharge and mediates the effect of CO_2 on the activity of the sinus nerve afferent fibres. This study provides further evidence that ATP, acting via P2X receptors that contain the $P2X_2$ and $P2X_3$ subunits, plays a pivotal role in the carotid body function.

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Rong W *et al.* (2003). *J Neuroscience* **23**, 11315-11321.

Zhang M *et al.* (2000). *J Physiol* **525**, 143-158.

The work was supported by the BBSRC.

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

PC37

Respiratory drive to sympathetic preganglionic neurones of neonatal rats

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Sympathetic outflows to particular target organs show specific patterns of respiratory modulation (Habler et al. 1994). Single unit recordings show striking differences in respiratory drive between sympathetic preganglionic neurones (SPN) (Gilbey et al. 1986). The central mechanism(s) underlying the differences in sympathetic respiratory modulation are not known. We addressed this question at the level of the SPN using whole cell recording (WCR).

This study used the working heart brainstem preparation (Paton, 1996). Neonatal Wistar rats (p8-12) were anaesthetised with halothane, decerebrated precollicularly and perfused with carbogenated Ringer solution (32°C). Phrenic nerve activity was recorded and the thoracic sympathetic chain stimulated with a bipolar electrode. After laminectomy (T5-C8), the SPN were located using extracellular recording. Patch electrodes were driven into this area to obtain WCR (solution containing Lucifer yellow). Respiratory drive could be either increased by stimulating peripheral chemoreceptors (NaCN, 10-20µg, i.a.) or arrested by topical cold saline (10°C) to the snout to evoke a diving reflex.

WCR were made from 62 spinal neurones (T2-T3, 500-900µm deep to dorsal surface). SPN were identified definitively by either antidromic activation or anatomical reconstruction. Neurones were identified as putative SPN on the basis of their distinctive electrophysiological properties (Dembowsky et al. 1986; Pickering et al. 1991).

Of the 62 spinal cells 22 were identified as SPN (3 identified definitively and 19 classed as putative). SPN were either spontaneously active (n=13) or quiescent (n=9). Many (62%) of the spontaneously active SPN had distinct patterns of respiratory-related firing (inspiratory (n=3), expiratory (n=4) or pre-inspiratory (n=1)) whereas only 22% of the quiescent cells showed respiratory modulation of excitability. The respiratory modulation was generated by phasic bursts of either excitatory or inhibitory postsynaptic potentials. Most of the spontaneously active SPN were excited by chemoreflex activation (83%), with a shift to post-inspiratory bursting, whereas quiescent SPN were mostly not excited (89%). Diving reflex activation provoked excitation in 75% and 57% of spontaneously active and quiescent SPN, respectively, with only one cell inhibited. Some quiescent SPN (n=4) showed no respiratory modulation under any condition. Spikelet firing (possibly from coupled cells) was seen in four SPN in response to either chemo-stimulation or direct current injection.

We have obtained WCR from SPN in a preparation with strong respiratory drive. In this preliminary sample, we have observed a broad range of respiratory modulation in SPN. Using WCR in this *in situ* preparation, we can examine the relative contributions of intrinsic SPN properties and synaptic inputs that determine the distinct patterns of sympathetic motor activity.

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A.E.P. is a Wellcome Advanced Fellow.

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

PC38

Modulation of sympathetic nerve discharge during eupnoea and gasping in the *in situ* arterially perfused preparation of rat

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Gasping is an important autoresuscitative mechanism that is initiated during hypoxia and ischaemia and vital for initiating breathing following a period of apnoea. For this to be effective cardiovascular autonomic motor activity must also be maintained. Recently, we have shown that gasping, but not eupnoea, depends on the integrity of the persistent sodium current, implying that respiratory pacemaker neurones drive gasping (Abdala *et al.* 2004). Here, we have investigated if sympathetic discharge persists during gasping and whether it is also dependent upon the persistent sodium current.

We have employed an *in situ* arterially perfused preparation of decerebrated rat (the working heart brainstem preparation; Paton, 1996). Decerebration was performed after killing by halothane anaesthesia. We used glass suction electrodes to record from a phrenic nerve, the central end of a vagus nerve and the left thoracic sympathetic chain. To induce gasping, we either induced tissue ischaemia by stopping the perfusion (for 50-70 s) or switched to a perfusate gassed with 5% oxygen and 8% carbon dioxide (for 90-120 s). To block persistent sodium channels, 7 µM riluzole was added to the perfusate since this dose is known to abolish gasping (Abdala *et al.* 2004).

During eupnoea, thoracic sympathetic chain activity typically exhibited discharges that coincided with pre-inspiratory and late inspiratory/early post-inspiratory phases of the respiratory cycle. During gasping (ischaemia, n = 4; hypoxia, n = 4), sympathetic activity exhibited inspiratory related discharges. The sympathetic burst was initiated 0.2-0.6 s prior to the onset of phrenic activity. In addition, baseline tonic sympathetic activity increased by 221.8 ± 43.7% in ischaemia and by 99.0 ± 48.3% in hypoxia compared with eupnoeic levels. Riluzole little affected sympathetic chain and phrenic nerve discharges during eupnoea but abolished gasping. During ischaemia or hypoxia, there was no bursting in the sympathetic chain following riluzole and the evoked

increase in tonic basal activity was unaffected compared with control (ischaemia, 189.3 ± 42.5%; hypoxia, 83.6 ± 32.9%). These results suggest that during gasping there is heightened tonic sympathetic discharge and gasp-related bursting occurs. The ischaemia/hypoxia induced increase in tonic sympathetic discharge is not dependent on the persistent sodium current but gasp-related bursting is.

Abdala APL *et al.* (2004). *Society for Neuroscience*, program no. 424.3.

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Research was funded by the British Heart Foundation, the British Council and the Japan Society for the Promotion of Science

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

PC39

The role of A2 NAergic neurones in cardiovascular regulation

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There remains uncertainty shrouding the role of noradrenaline containing (NAergic) neurones in the brainstem for regulating arterial pressure. An earlier study used a neurotoxin - 6-hydroxydopamine (6OHDA) to lesion these neurones and reported lability in mean arterial pressure (MAP) and hypertension [2]. In this study we have examined the role of A2 neurones in regulating arterial pressure in normotensive rats.

A lenti viral vector (LV-PRSx8-Kir2.1-IRES-eGFP) was designed to induce expression of an inwardly rectifying potassium channel (Kir2.1) in NAergic neurones to electrically silence them. The PRSx8 promoter in our vector (LV-PRSx8-Kir-IRES-eGFP) is a synthetic promoter of about 260 bp. It encodes the binding sites for *Phox2* transcription factor [1]. The binding has been multimerised eight times and attached to a TATA initiation site. Rats were telemetered for chronic blood pressure recordings under ketamine (60 mg kg^{-1}) and medetomidine (250 μg kg^{-1}, both I.M.) anaesthetic. Anaesthesia was reversed with a subcutaneous injection of atipamezole (1 mg kg^{-1}). The virus was injected into the caudal NTS bilaterally (108 ifu ml^{-1}; 500nl per site; anaesthesia as above). Injection sites were determined from the expression of enhanced green fluorescent protein (eGFP) and Kir2.1 expression validated with real time RT-PCR. Control animals were injected with LV-PRS-eGFP (n=4), with none of the cardiovascular changes associate with LV-PRSx8-Kir-IRES-eGFP.

Our data from the normotensive rats indicates that LV-PRSx8-Kir2.1-IRES-EGFP triggers a gradual increase in MAP (from 96.1 ± 2 to 104.1 ± 3 mmHg, mean ± S.E.M., P< 0.05 ANOVA) at 21 days post-injection without a change in spontaneous cardiac baroreflex gain (n=6). Moreover, spectral analysis of systolic blood pressure indicates an increase in the very low frequency from (3.50 ± 0.47 mmHg2 to 4.25 ± 0.45 mmHg2, P< 0.01 ANOVA) indicative of a neurohumoral mechanism. We are presently assessing the role of A2 neurones in the spontaneously hypertensive rats. To date, our findings suggest that A2 neuronal activity in the normotensive rats play a key role in the regulation of arterial pressure.

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MRC and British Heart foundation funded research.

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

PC40

Characterization of the role of metabotropic glutamate receptors in the baroreceptor reflex arc at the level of the nucleus tractus solitarii in the rat

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The nucleus tractus solitarii (NTS) is the first central site of integration for the arterial baroreceptor reflex. The baroreflex exerts a reciprocal modulation of the sympathetic and parasympathetic nervous systems. Previous work in our laboratory has found differences in the receptor pharmacology for baroreflex modulation of these two outflows within the NTS (Pickering et al. 2003). Since glutamate is likely to be the primary neurotransmitter of baroafferents in the NTS (Talman et al. 1980) we have aimed to determine the role of metabotropic glutamate receptors (mGluR) in the baroreflex arc at the level of the NTS.

Studies used the working heart brainstem preparation (Paton, 1996). Wistar rats (65-75g) were decerebrated under deep halothane anaesthesia, bisected sub-diaphragmatically. Preparations were perfused via the aorta with a carbogenated Ringer solution (+ficoll 1.25%, 32°C). Flow through the preparation was computer controlled with a peristaltic pump and used to alter baseline pressure. Baroreceptors were activated with pressure pulses/ramps. Peripheral chemoreceptors were stimulated with NaCN (i.a.). Recordings were made of arterial pressure, heart rate, phrenic nerve and thoracic sympathetic chain (T8-12) activity. Bilateral microinjections (80nl) of drug were made stereotaxically into the NTS at the site of termination of baroafferents. All values are mean±S.E.M. and paired t tests were used.

The broad-spectrum mGluR antagonist MCPG produced a dose-dependent (1-50μM), reversible inhibition of the baroreflex bradycardia by up to 81±3% (50μM, p<0.001, n=5). In contrast, MCPG did not affect the baroreflex sympathoinhibition or chemoreflex sympathoexcitation and bradycardia. The group II mGluR selective antagonists LY341495 (100μM) and EGLU (1mM) also reversibly inhibited the baroreflex cardiac gain by 75±5% (p<0.001, n=6) and 68±6.5% (p<0.001, n=5), respectively. None of the mGluR antagonists significantly affected baseline pressure, heart rate, phrenic or sympathetic nerve activity. The inhibition of the baroreflex cardiac gain by LY341495 was reversibly reduced by pre-injecting bicuculline (10μM, n=3) bilaterally into the same site of the NTS.

These results suggest that group II mGluRs play a specific role in modulating the cardiac baroreflex. Since group II mGluRs are thought to act as inhibitory autoreceptors to decrease glutamate release it is paradoxical that group II mGluR antagonists

should attenuate the cardiac baroreflex. However, the finding that bicuculline prevents this attenuation suggests that group II mGluRs may be located on a circuit involving inhibitory GABA interneurones within the NTS.

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A.E.S. is supported by the Dr WE Parkes BHF PhD Fellowship.

British Heart Foundation & Wellcome Trust funded research.

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

PC41

Contribution of medullary NO-producing neurons to hypertension in rats

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In acute experiments in spontaneously hypertensive (n=58) and normotensive (n=45) rats anaesthetized with urethane (1.7 g/kg, i.p.), we analysed the haemodynamic effects of activating and inhibiting nitric oxide (NO)-producing neurons within medullary nuclei which are known to participate in the cardiovascular control: nucleus tractus solitarius (NTS), paramedian reticular nucleus (PMn), nucleus ambiguous (AMB) and lateral reticular nucleus (LRN). We demonstrated that activation of neurons with unilateral injections of either a nitric oxide donor (sodium nitroprusside; 15.2-152 nmol), or a substrate for NO synthesis (L-arginine; 5.8-58.0 nmol) in most experiments resulted in lowering the systemic arterial pressure (SAP) level mainly due to a reduction in the peripheral vascular resistance which was more pronounced in hypertensive as compared with normotensive rats. The SAP was recorded from the femoral or carotid artery via an inserted polyethylene cannula, filled with heparinized 0.9% Ringer solution and connected with a pressure transducer and tensoamplifier. We recorded blood stroke volume (SV) using tetrapolar transthoracic impedance rheoplethysmography and calculated the cardiac output (CO) and peripheral vascular resistance (PVR). Injections of 5.8 nmol of L-arginine into the NTS of normotensive rats induced the lowering of the SAP by 18.4% ($P<0.01$) on average, those into the AMB by 23.8% ($P<0.01$), and those into the LRN by 18.2% ($P< 0.05$). Injections of 58.0 nmol of L-arginine into the NTS evoked a drop of the SAP by 29.4% ($P<0.01$), those into the AMB and LRN resulted in the SAP lowering by 25.2 and 29.8%, respectively ($P< 0.01$). In spontaneously hypertensive rats, injections of 58.0 nmol of L-arginine into the nuclei under exploration resulted in the SAP lowering by 39.8, 31.2 and 32.8% ($P<0.01$), correspondingly. Both NOS-1 and arginase are known to use L-arginine as a substrate for their metabolism and they can possibly compete for it. Therefore, we injected the NOS-1 antagonist (N^G-nitro-L-arginine; L-NNA, 4.6-23 nmol), and an antagonist for arginase (norvaline) into the medullary nuclei to see the contribution of those enzymes to the cardiovascular control in hypertensive rats.

Our results indicated that both enzymes were potentially active. This suggests that L-arginine in medullary neurons of spontaneously hypertensive rats can be utilysed not only via NO-synthase pathway, but also via arginase-mediated metabolism of L-arginine. As such, a lack of L-arginine might contribute to the high level of the SAP. Injections of either inducters of mitochondrial permeability (MP) phenylarsine oxide (PAO, 10^{-8} -10^{-12} M) or its inhibitors (cyclosporin A, melatonin, 10⁻⁸ - 10^{-12} M) into the medullary nuclei modulated the SAP level in a dose-dependent way. An increase in mitochondrial permeability induced a dose-dependent drop in the SAP level. Injections of inhibitors of MP induced dose-dependent changes in the SAP level; in normotensive rats the SAP level only slightly increased or decreased following injections of 10^{-12} M melatonin, and the responses were prolonged. In spontaneously hypertensive rats, the responses of the SAP were similar to those in normotensive rats. However, prior administration of melatonin (10^{-8} M, i.p.) enhanced the effects of injections of L-arginine. This suggests that inhibitors of MP can enhance the activity of the medullary NO-producing neurons during hypertension. Effects of NO-producing cardiovascular neurons were also modulated by injections of the superoxide dismutase into the medullary nuclei under exploration. The data obtained suggest that MP and an excess of superoxides in the medullary cardiovascular neurons may contribute to hypertension in rats.

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

PC42

Mechanisms of nitric oxide (NO)-induced elevation of intracellular calcium concentration $[Ca^{2+}]_i$ of GABAergic interneurons within the nucleus tractus solitarii (NTS)

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NO potentiates GABA-mediated IPSPs in the NTS (Wang et al. 2004). We hypothesize that NO may potentiate GABA release via elevation of $[Ca^{2+}]_i$ in NTS GABAergic interneurons. P8 rats were humanely killed and used to prepare brainstem organotypic slice cultures which were transfected with adenoviral vector containing 3.7 kb of the GAD67 promoter driving expression of enhanced green fluorescent protein (Teschemacher et al. 2005). Fluorescent GABAergic neurons were visualized by combined confocal and DIC optics and recorded in whole-cell patch clamp mode. The intracellular solution contained a red-shifted calcium indicator Rhod-2. The NO donor diethylamine NONOate (DEA/NO) concentration-dependently (1 and 10 μM) increased $[Ca^{2+}]_i$ as assessed by fluorescence intensity (FI) in different compartments of GABAergic neurons and caused a slight depolarization in the majority of neurons tested (+3.4 ± 0.9 mV; ±SEM with 10 μM, 6/9 cells). The most dramatic FI increase (+40±11%, $P<0.05$) was observed in 5 of 6 putative axons. sGC blocker-ODQ completely blocked DEA/NO actions. The IP_3 receptor inhibitor - 2-APB was unable to block DEA/NO-induced $[Ca^{2+}]_i$ rise indicating that IP_3-sensitive Ca^{2+} stores are not essential for NO action on $[Ca^{2+}]_i$. An antagonist of cyclic ADP-ribose (cADPR)

receptors, 8-Bromo-cyclic adenosine diphosphate ribose, introduced via patch pipette, completely abolished DEA/NO-induced $[Ca^{2+}]_i$ increase. 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 leads to ryanodine receptor-mediated Ca^{2+} release from stores thus promoting GABA exocytosis.

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British Heart Foundation funded research. S.Wang is an ORS award recipient.

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

PC43

Chronotropic and inotropic response to tyramine in the intact and sympathectomized albino rats

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The present study was designed to get better insight into the relationship between the developing cardiac sympathetic innervation and myocardial function. We studied the effect of tyramine on contractile properties and heart rate in control and sympathectomized albino rats from birth till adulthood. We evaluated concentrations of noradrenaline (NA) and neuropeptide Y (NPY) in the cardiac tissue. The chemical sympathectomy was performed by repeated injections of 6-hydroxydopamine (6-OHDA) immediately after birth. Experiments were carried out in 10, 20, 40 and 60-day-old animals (n=6 for control groups and n=5 for 6-OHDA groups). The contraction force (CF) of the right ventricular papillary muscle and the heart rate of the isolated right heart atrium were measured in the Tyrode solution without and with tyramine (concentrations from10^{-7} to 10^{-4} mmol/l). Atrial and ventricular concentrations of NA and NPY were measured by radioimmunoassay diagnostic kits. All procedures were performed according to current EU legislation and local guidelines. Statistical analysis was performed using one-way ANOVA ($P<0.05$) and *post hoc* Student's unpaired *t* test with Bonferroni correction.

In control rats, tyramine had positive inotropic and chronotropic effects, both effects of tyramine were inhibited by non-selective β-adrenoreceptor blocker metipranolol. Sympathectomy significantly lowered CF ($P<0.05$) in all followed groups except 60-day-old animals where no difference in CF between intact and sympathectomized rats was observed. In contrast to controls, both tyramine and metipranolol had no effect on the heart rate and CF in 6-OHDA rats of any age groups. This result is consistent with NA concentration in sympathectomized rats, which reached maximally 5% of control values ($P<0.05$) in all cardiac compartments and did not change in the whole course of experiment. No effect of tyramine and decrease in NA concentrations reflect the long-termed destructive effect of 6-OHDA on the cardiac sympathetic nerves.

NPY is known to be co-secreted with NA from sympathetic postganglionic fibers. Moreover NPY is located in the intracardiac ganglia (1). It was found that NPY contributes to the increase in L-type calcium current density (2) and rapidly enhances calcium transients and sparks (3) in the rat ventricle. In our experiments, NPY concentration in the cardiac atria and ventricles of the younger (40 days and less) denervated rats was significantly ($P<0.05$) lower than in control ones. In older 6-OHDA rats (more than 40 days), NPY concentrations increased and were comparable with control values. Our results suggested that although sympathetic postganglionic fibers are destructed by 6-OHDA, NPY from preserved intracardiac ganglia might be able to improve contractile performance of the papillary muscle probably by influence of calcium metabolism in ventricular myocytes.

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This work was supported by the Grant Agency of the Czech Republic, grant No. 305/04/0144.

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

PC44

Angiotensin II based neurogenic hypertension is dependent on renal nerves

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Angiotensin II is well established to increase blood pressure via direct vasoconstriction of blood vessels. However, the longer-term effects are thought to involve increased sympathetic nerve activity . In this study we question whether angiotensin II based hypertension may be mediated by a selective increase in sympathetic nerve activity to the kidney.

The University of Auckland Animal Ethical Committee approved all experimental procedures. New Zealand white rabbits (3-3.6 kg) were initially anaesthetised with 5% halothane in oxygen with sufficient depth of anaesthesia maintained with 3% halothane during the surgical implantation of telemetry devices and osmotic mini pumps. After recovery from the implantation surgery, the animals were returned to their home cages where they remained for the duration of the experimental protocol. During this period the animals drank 0.9% sodium chloride (NaCl) and arterial pressure (model PA-D70, Data Sciences) and renal sympathetic nerve activity (Telemetry Research Ltd) were recorded continuously using telemetry devices before and during a 23-day infusion of angiotensin II (20 ng/kg/min) via the implanted osmotic mini pump (model 2ML4, Alzet). This dose is slow-pressor and resulted in a significant increase in arterial pressure from 92± to 115±2 mmHg (mean±S.E.M., $p<0.05$) that took 8-12 days to develop. A second group of rabbits, also drinking 0.9% NaCl were renal denervated and subjected to the same angiotensin II infusion but arterial pressure did not increase over the 3 week period of infusion (83±3 mmHg control c.f. 87±8 mmHg after 14 days angiotensin II).

At the completion of the angiotensin II infusion, the animals were killed humanely with sodium pentobarbitone (160 mg/kg), followed by perfusion with heparinised saline and finally with 4% paraformaldehyde. The brains from both groups were removed and underwent Fos immunocytochemistry to investigate activation of the central nervous system including areas of the hypothalamus (paraventricular nucleus and supraoptic nucleus) and medulla (ventral lateral medulla, nucleus tractus solitarius and area postrema). All areas examined showed evidence of activation (presence of Fos positive neurones) in intact and denervated rabbits exposed to salt and angiotensin II (Lohmeier et al. 2002). These data suggest that this slow-pressor dose of angiotensin II is sympatho-excitatory and that, in particular, the renal nerves are important for the development of the induced hypertension.

Lohmeier T et al. (2002). Hyp 39, 550-556.

Supported by Auckland Medical Research Foundation and Health Research Council of New Zealand.

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

PC45

Recombinant transcriptional amplification as a method for enhancing the transcriptional activity of weak cell-specific promoters

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Transcriptional targeting by specific promoters plays an important role in the development of selective vectors for many gene transfer applications. Many specific promoters have been described (Kasparov et al. 2004), but their applicability is often hindered by their relatively low levels of transcriptional activity compared with stronger but non-selective viral promoters. In this study, we used a recombinant transcriptional amplification approach as a method to enhance transcriptional activity of the weak human SYN1 promoter, which has been shown to drive neuron-specific expression. To this end, SYN1 promoter was used to drive the expression of an artificial transcriptional activator consisting of a DNA binding domain of a yeast transcriptional activator and a strong transactivation domain of a mouse transcriptional factor NF-kB p65. The resultant recombinant transcriptional activator GAL4BD-NF-kB selectively binds to synthetic GAL4 DNA binding sites (GAL4BS) inserted upstream of the second SYN promoter and potently stimulates transcription. Since GAL4BS only exist in yeast genomes and not in eukaryotic genes, there will be no interference for any other gene expressions in transduced mammalian cells. We demonstrate that this method can greatly amplify the expression levels of green fluorescent protein (GFP) and red fluorescent protein (DsRed2) both in vitro and in vivo. In vitro analysis was carried out by transfecting PC12 cells. The transcriptional activity of SYN1 promoter was increased about 10-fold by co-trasnfection with GAL4BD-NF-kB, when the molar ratio of GAL4BD-NF-kB gene expression cassette and that of GFP or DsRed2 was set to 1:1. For in vivo tests, VSV-G pseudotyped lentiviruses were produced using previously published protocol (Coleman et al. 2003). Anaesthetised intramuscularly with a mixture of ketamine (60mg/kg) and medetomidine (250 μg/kg), male Wistar rats were placed in a stereotaxic apparatus. Bilateral injections were made into hypoglossal motor nucleus at three rostro-caudal sites, giving a total of six 1 μl injections. Seven days postinjection, rats were intraperitoneally anaesthetised (sodium pentobarbital, 100mg/kg), perfused and brainstem sections were prepared. Four sections surrounding the injection tract were selected randomly and three fields from each section were used for cell counting. GAL4BD-NF-kB containing viral constructs resulted in ~5-fold higher density of fluorescent cells (expressed by the absolute number of fluorescent positive cells) as compared to conventional SYN1-containing vectors (n = 3). In conclusion, both in vitro and in vivo data indicate that the recombinant transcriptional amplification is a powerful strategy to enhance the activity of the human SYN1 promoter. This strategy can be applied broadly to strongly amplify gene expression of cell or tissue specific promoters, with potential applications for both gene therapy and functional genomic studies.

Coleman JE et al. (2003). Physiol Genomics 12, 221-228.

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British Heart Foundation funded research.

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

PC46

The effects of hypercapnia in healthy subjects and changes after the benzodiazepine agonist lorazepam

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CO_2 has been used for many years to model panic anxiety. Much of this research has used the 35% single vital capacity inhalation or 5% CO_2 inhaled for up to 20 min. We have used a 20-min inhalation of 7.5% CO_2 in volunteers to produce symptoms of anxiety and fear, and the associated physiological arousal (Bailey et al. 2005). We have also examined the effects of a benzodiazepine agonist, lorazepam (LZP) on these measures.

The local research ethics committee approved the study protocols. In the first study twenty (6 female) healthy volunteers (mean age 25.2 ± 5.02 years, mean ± S.D.) who met our inclusion criteria underwent the procedure. They inhaled medical air and 7.5% CO_2 for 20 min with a 15-min interval between gases. During each inhalation, blood pressure (BP) and heart rate (HR) were measured continuously using the Finapres. Subjective state was assessed by visual analog ratings. The second study examined the effects of 2mg LZP in 12 (4 female) healthy volunteers (mean 27.4 ± 2.5 years) in a double-blind, placebo-controlled design.

BP and HR values recorded beat-by-beat during the inhalation period were expressed as a mean for the total duration. During CO_2 inhalation, systolic BP and HR were significantly increased compared with air. Data are shown in Table 1.

At peak effects of gas 7.5% CO_2 increased subjective feelings of anxiety, fear, feel like leaving, nervous tense and worried and

decreased feelings of being relaxed and happy. In the lorazepam study the effects of CO_2 on BP and HR were similar. However, in both gas conditions LZP increased HR further (air + placebo 69 ± 3.0, mean ± S.E.M.), air + LZP 77 ± 2.8, CO_2 + placebo 74 ± 2.9, CO_2 + LZP 84 ± 3.7). The subjective effects of CO_2 were significantly attenuated by LZP (e.g. subjective anxiety at peak CO_2: placebo 31.3 ± 6.8), LZP 17.9 ± 2.7).

These experiments demonstrate measurable physiological effects of inhaling 7.5% CO_2 in healthy, non-anxious volunteers. These effects are of fairly rapid onset and are sustained for the duration of the inhalation. This cardiovascular response to 7.5% CO_2 differs to that produced by 35% CO_2 where BP is increased but a bradycardia is seen (Argyropoulos et al. 2002). LZP reduced the subjective effects of 7.5% CO_2, but did not change the cardiovascular effects. The finding of increased heart rate was unexpected and contradicts the belief that CO_2-induced anxiety can partially be explained by a cognitive misinterpretation of physical symptoms.

Table 1

Value	Air	CO2	ANOVA
SBP	154 (17.7)	172 (16.7)	df=1,19, F=19.5, p=<0.001
HR	76 (9.3)	84 (14.7)	df=1,19, F=19.3, p=<0.001

Mean HR and systolic BP values during 20 min inhalation of air or 7.5% CO2. Data are mean ± S.D, n=20.

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Bailey JE et al. (2005). Depression and Anxiety 21, 18-25.

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

PC47

Sympathetic motor rhythms are generated within the spinal cord in response to serotonin

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In vitro studies have demonstrated that neuroeffector transmission is potentiated when stimulating the innervation of the caudal ventral artery with short bursts of high frequency spike trains, rather than continuous trains of impulses with the same frequency. Furthermore, in anaesthetised CNS-intact rats, sympathetic neuronal activity regulating the tail vasculature (SNAT) tends to occur in bursts which have a robust rhythmicity in the 0.4-1.2 Hz frequency range (T-rhythm). These rhythmic discharges appear to be generated by autonomous sympathetic oscillators (Gilbey, 2001). Rostral ventromedial medullary neurons are critically involved in regulating SNAT (Korsak & Gilbey, 2004) and some contain serotonin and/or v-glut3 transporter (Smith et al. 1998; Nakamura et al. 2004). In the present study, we hypothesized that the so-called T rhythm is generated within the spinal cord, and dependent upon serotonergic and glutamatergic inputs.

Population sympathetic activity was recorded from the dorsal collector nerve (DCN) of the tail in urethane anaesthetised (1.3 g kg^{-1}, i.p., supplemented with 5-10 mg i.v. as required) rats spinalized between T10 and T11. Intrathecal (i.t.) injections (10 µl over 5 min) were delivered to L1, as sympathetic preganglionic neurons projecting to the DCN are distributed in T11-L2 (Smith & Gilbey, 1998). Nerve activity was completely abolished after spinal transection (n=15) and no spontaneous recovery was observed afterwards (n=3). The administration of 5-HT (250 nmol, i.t.) induced sympathetic rhythmic discharges (median frequency, 0.90 Hz; interquartile interval, 0.84-1.01 Hz; n=6) that were sustained for up to 90 min and were similar to those observed before spinal cord transection (median frequency, 0.77 Hz; interquartile interval, 0.72-0.83 Hz; n=6). In contrast, NMDA (1 µmol, i.t.) failed to restore the T-rhythm, but induced activity that was sustained for long periods of time (at least 4 h). This tonic activity induced by NMDA became rhythmical for periods of 30-60 min when 5-HT (250 nmol, i.t.) was additionally administered (median frequency, 0.93 Hz; interquartile interval, 0.70-0.96 Hz; n=6). In all cases, it was possible to repeatedly initiate sequences of stable rhythmic activity by performing reapplications of 5-HT. Remarkably, the level of activity was similar in all conditions. Our observations are consistent with the idea that serotonin, released from bulbospinal neurones, acts on neurones within the spinal cord to generate the T-rhythm.

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This work is supported by the Wellcome Trust grant 063954.

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

PC48

Altered cardiac parasympathetic regulation post myocardial infarction in mice: role of NOS

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Parasympathetic impairment post-myocardial infarction (MI) increases the likelihood of adrenergic induced arrhythmias. The mechanism underpinning the development of this phenotype is unknown, but may involve changes in oxidative stress post-MI. To investigate this we used a murine model 3 days post MI, sham surgery or naive single cage housing. Anaesthesia was induced with intramuscular ketamine (30mg kg^{-1})/metatomidine (0.6mg kg^{-1}) and maintained with 2% isofluorane in oxygen; this was discontinued after repair of the thoractomy. On final skin closure metatomidine was reversed with atipamezole (2mg kg^{-1}; I.M.). Post-operative care included analgesics and subcutaneous isotonic dextrose/saline. All animals were humanely killed.

The response to vagal nerve stimulation in isolated atrial preparations was increased at 3 days post-MI (naive n=10, -90 ± 12 bpm (mean ± S.E.M.) vs infarct n=11, -117 ± 14 bpm, $p<0.05$, 2-way ANOVA with Bonferroni post-hoc analysis). Non-specific NOS inhibition failed to suppress vagal response in the MI and sham groups. There were no significant differences in chronotropic response to carbamylcholine with or without NOS

inhibition suggesting a presynaptic mechanism of altered NOS function. In the MI group there was an increase in right atrial superoxide response to NADPH and decreased cytoplasmic SOD expression.
The failure of NOS inhibition to diminish vagal responsiveness in the MI and sham groups suggests that NOS is uncoupled. In the 3 day murine MI model vagal activity may be increased through a mechanism independent of the NO-cGMP pathway. Superoxide is a potential substrate that facilitates vagal neurotransmission in this model.

Research supported by the British Heart Foundation.

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

PC49

Expression of phosphoinositide-3-kinase subunits and phosphatidylinositol-3,4,5-triphosphate metabolising enzymes in the medulla-oblongata of the Wistar-Kyoto and spontaneously hypertensive rat

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Increased phosphatidylinositol-3,4,5-triphosphate (PIP_3) signalling is thought to be responsible for the augmented catecholamine release induced by angiotensin II in the brainstem of spontaneously hypertensive rats (SHR) (1). This increase in catecholaminergic signalling is thought to result in hypertension. Supporting this idea, inhibition of phoshoinositide-3-kinase (PI3K) in the rostral ventrolateral medulla (RVLM) reduced blood pressure in SHR but had no effect in the Wistar-Kyoto (WKY, normotensive control) (2). Elevated PIP_3 levels could result from an increase in the expression of PI3K enzymes or a decreased expression of PIP_3 metabolising enzymes; phoshoinositide-lipid-3-phosphotase (PTEN) and SH2-containing inositol phosphatase 1 and -2 (SHIP, 5- and 3-phosphotases, respectively). We sought to determine whether the mRNA levels of the G-protein activated PI3K subunits, p110γ and p101 as well as PTEN, SHIP1 and SHIP2 are altered in SHR medullary catecholaminergic nuclei. Total RNA was extracted from micropunches of C1/RVLM, A1/caudalVLM and A2/NTS regions of neonatal SHR and WKY (n=22; rats were humanely killed). Expression levels were assessed by real time RT-PCR based on the cycle threshold difference (ΔCt) method using GAPDH as the reference gene. SHIP2 mRNA levels were 2.3-fold lower in SHR than in WKY ($p=0.009$, unpaired t test, $\Delta Ct = 8.16 \pm 0.24$; $n = 11$ vs 6.95 ± 0.39; $n = 10$, mean ± S.E.M.) in the C1/RVLM region but no difference was observed in the A1 or A2 areas. In all the regions tested, no significant differences in p110γ, p101, PTEN or SHIP1 mRNA levels were found between WKY and SHR. This data implies that differences in PIP_3 signalling between SHR and WKY might be at least in part, related to decreased expression of SHIP2 in the C1/RVLM region, an area previously implicated in the generation of PIP_3-dependent hypertension (2). Presumably, decreased SHIP2 activity would increase PIP_3 levels resulting in abnormal catecholaminergic signalling and hypertension. As SHIP2 converts PIP_3 to $PI(3,4)P_2$ the possibility that $PI(3,4)P_2$ levels may be important in maintaining normal catecholaminergic signalling must also be considered.

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Wellcome Trust funded research.

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

PC50

Estimating respiratory sinus arrhythmia at low heart rates

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Baroreceptor responses are gated during inspiration and efferent vagal supply to the heart is modulated by respiratory activity. Such variations in vagal tonus underlies cardio-respiratory coupling in mammals, but it has been suggested that such central control is absent in non-mammalian vertebrates (Porges, 1995, 2003). We examined Antarctic (environmental temperature -1.8 to 0°C), sub-Antarctic and temperate fishes (8 to 12°C) of similar genotype and ecotype, but which face vastly different selection pressures related to oxygen supply due to their different habitat temperatures. If cardio-respiratory coupling were present to optimise oxygen uptake, then it may be expected that a difference would be observed between these thermal groups.
Fish were anaesthetised (MS222, Sigma; 0.5 g l^{-1}) and e.c.g. recording electrodes inserted close to the pericardium (0.02mm diameter, multi-strand Teflon coated stainless steel wire). Fish were allowed to recover until resting vagal tonus was re-established (determined by atropine-sensitivity in heart rate (fH) and development of significant heart rate variability (HRV). All fish were humanely killed at the end of the experiment.
The Fourier (time-to-frequency) transform was applied to the e.c.g. tachogram, where sampling frequency for an individual fish was determined by its own fH. The intrinsic fH for all the fish species studied was ~ 25% greater than ventilation rate (fV), but vagal activity produced a resting fH that was synchronised with fV in a progressive manner. Coefficient of variation in the R-R tachogram was similar between groups. Power spectral statistics showed that episodes of relative bradycardia occurred in a cyclical manner, occurring every 2-4 heart beats in temperate species but >4 heart beats in Antarctic species. The ratio of LF/HF components provides an index of the dominant oscillations responsible for overall HRV. This ratio was always <1 in temperate fish, indicating the significance of beat-to-beat components in determining overall HRV, and always >1 in Antarctic fish. These oscillatory components were controlled centrally by vagal tonus, as bilateral cardiac vagotomy abolished all peaks. Although the modulating effects of ventilation were acting in a similar manner for all fish to coordinate blood and water flow at the gills, the high oxygen content and/or the low metabolic rate as consequence of living in sub-zero water, results in a relaxed selection pressure for cardio-respiratory coupling in Antarctic species. We conclude that vagally mediated control of fH is present in fish and operates around the less variable ventilatory cycle, similar to that controlling respiratory sinus arrhythmia in mammals.

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This work was supported by N.E.R.C.

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

PC51

An *in situ* preparation for studying hypothalamic control of sympathetic activity

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This laboratory has utilised a number of arterially perfused *in situ* preparations for studying brainstem-spinal cord regulation of both the cardiovascular and respiratory systems (Chizh *et al.* 1998; Pickering *et al.* 2003; Potts *et al.* 2005). In this study, we have tested the integrity of hypothalamic structures in regulating sympathetic activity in a decorticate arterially perfused rat preparation.

Male Wistar rats (70-90 g) were anaesthetised deeply with halothane and decorticated; the thalamus was destroyed also. Animals were arterially perfused with a Ringer solution containing Ficoll (1.25%) at 31°C. Recordings of phrenic (PNA) and lumbar sympathetic nerve activities (LSNA) as well as heart rate and perfusion pressure were monitored. In order to test the integrity of hypothalamic structures, changes in perfusate osmolality were made. Following a switch from an isotonic Ringer solution (e.g. 290 mOsmol/kg/water) to a hyperosmotic perfusate (320 mOsmol/kg/water) for 40 s, we observed an increase in LSNA (78±36%, n=8), which persisted until the isotonic perfusate was reintroduced. To demonstrate a potential role for hypothalamic structures in mediating this sympathoexcitation to increased osmolaltiy, we transected the neuraxis at the precollicular level. After the transection the sympathoexcitation evoked with the hyperosmotic perfusate was reduced in both magnitude and duration (10±5%, n=3).

In conclusion, we have demonstrated that the arterially perfused decorticate preparation demonstrates appropriate responses to salt loading as seen *in vivo* (Scrogin *et al.* 1999) and that these responses are likely dependent on hypothalamic structures. We suggest that this preparation is a promising model for future studies for understanding hypothalamic and brainstem mechanisms of homeostatic regulation of blood pressure and volume.

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British Heart Foundation funded research.

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

PC83

Moderate sleep apnoea increases diastolic rather than systolic blood pressure parameters obtained by ambulatory blood pressure monitoring

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Secondary arterial hypertension is one of the major under-diagnosed and under-treated cardiovascular complications of sleep apnoea syndrome (SAS), in spite of its 40-70% prevalence in adults [1,2]. Ambulatory blood pressure monitoring (ABPM) can contribute very effectively to screening and treatment control of hypertension, by detecting about 1/3 of hypertonics not observed by standard BP meassurement [3,4].
BP parameters obtained by ABPM were correlated with indices measured during whole-night polysomnographic examination in 57 adults. The patients were divided into 4 groups: simple snoring (apnoea/hypopnoea index (AHI) <5/h, n=12), mild SAS (AHI 5-25/h, n=16), moderate SAS (AHI >25/h, n=17) and a group of severe SAS treated with nasal continuous positive airway pressure (CPAP, n=12).
The time of sleep with snoring correlated with nocturnal, morning, diurnal, average and minimum diastolic BPs ($p<0.05$, n=45, linear correlation) but not with SBP. Ventilatory parameters such as AHI, obstructive apnoea with bradycardia or oxygen desaturation, hypopnoea and oxygen desaturation index, correlated significantly (at least $p<0.05$) with an increase in diastolic BP values (nocturnal, diurnal and morning 2 h after awakening, which is the most likely time for strokes). The main parameters of oxyhaemoglobin saturation (such as minimal $SatO_2$, $avgSatO_2$ and O_2 saturation <85%), correlated negatively with practically all diastolic BP data, but only with some parameters of systolic BP. Particularly the morning values measured 2 h after awakening and the diurnal, nocturnal and minimal DBP data changed. CPAP therapy improved both the saturation and BP values by decreasing the number of apnoea/hypopnoea episodes, and eliminating the intermittent hypoxaemia and increased arousal reaction and sympathoadrenergic hyperactivity.
ABPM allows the selection of the most predictive BP parameters for large-scale screening of both hypertension and SAS, to reveal diastolic hypertension appearing in early phase of the disease and to manage an effective therapy also for drug-resistant hypertension by long-term use of CPAP treatment.

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

PC84

Left ventricular contractility and cardiac troponin T plasma concentrations in experimental anthracycline-induced cardiomyopathy in rabbits

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Cardiac toxicity associated with chronic administration of anthracycline antibiotics (e.g. daunorubicin, doxorubicin) represents a serious complication of their use in anticancer chemotherapy, but can also serve as a useful experimental model of cardiomyopathy and congestive heart failure. In this study, the relation between left ventricular (LV) contractility and cardiac troponin T (cTnT) plasma concentrations was studied, using a model of repeated daunorubicin administration to chinchilla male rabbits.
Two groups of animals were used: control group (n = 10) received i.v. saline, experimental group (n=11) received daunorubicin (3 mg/kg, i.v.). Substances were administered once weekly for 10 weeks. 5 - 7 days after the last administration, dP/dtmax (i.e. the maximal rate of the pressure rise in the isovolumic phase of the systole) was invasively measured as the index of the left ventricular contractility under pentobarbital anaesthesia (30 mg/kg i.v.). Immediately after the LV contractility determination, arterial blood had been sampled for the cTnT determination and all the animals were humanely killed. The cTnT concentrations in heparinized plasma samples were measured using an Elecsys Troponin T STAT Immunoassay on the Elecsys 2010 immunoassay analyser (Roche, Switzerland).
Four animals (i.e. 36%) from the experimental group died or were moribund and had to be killed prematurely. In the control group no premature deaths occurred. The cardiac contractility (dP/dtmax) was in 7 surviving daunorubicin-receiving animals significantly lower than in control group (745.7±69.3 vs. 1245.1±86.2 kPa/s; $P\langle 0.001$), while the cTnT plasma concentrations were significantly increased (0.262±0.034 vs. 0.007±0.003 nmol/mL; $P\langle 0.001$). When the dP/dTmax values were plotted against the cTnT plasma concentrations, a linear correlation ($R=0.91$; $P\langle 0.005$; Regression equation: dP/dtmax= -1861*cTnT+1234) was found in daunorubicin-receiving group of animals.
This study shows that pathologically increased plasma cTnT concentrations closely correlate with the decreased LV contractility as measured with the use of invasive haemodynamic measurements. The cTnT plasma concentration determination, which is simple and inexpensive, can thus be used for LV systolic function assessment and contractility estimation. However, further studies are necessary to evaluate this finding in different experimental and clinical conditions.

Study supported by the Czech Science Foundation (Grants GACR 305/05/P156 and 305/03/1511).

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

PC85

Assessment of carotid plaque vulnerability in vivo using magnetic resonance imaging and finite element analysis

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Up to 50% of strokes may be caused by rupture of atheromatous plaque in the carotid arteries in the neck. Such strokes are potentially preventable by carotid endarterectomy, an operation during which the plaque is surgically excised [1]. Appropriate selection of patients for surgery is a crucial clinical issue and is currently based on the severity of carotid luminal stenosis. There is, however, growing evidence to suggest that luminal stenosis per se may not be the best predictor of subsequent strokes. Consequently, other factors such as intrinsic plaque morphology and biomechanical stress are increasingly thought to be more important risk factors for plaque rupture [2, 3]. This study explores the role of stress as a marker of plaque vulnerability using a combination of magnetic resonance imaging (MRI) and finite element analysis (FEA).

Ten patients (5 symptomatic, 5 asymptomatic) with carotid stenosis underwent high-resolution in vivo pre-operative multisequence MRI. Intact carotid plaques were retrieved after surgery for histological analysis. Segmentation was based primarily on MRI for fibrous cap and lipid core, when compared with histology. Each patient axial MR slices were segmented and all contours were traced to generate the boundaries of lipid core, fibrous cap, vessel lumen and wall based on net intensity characteristics. The mesh was generated for each slice and finite element analysis was conducted in each case to determine the stresses within every plaque. The plaque components were modelled as elastic materials with different Young's moduli and Poisson's ratios. The internal luminal pressure was assigned at 15kPa, and large deformation analysis was performed for plaque stress simulation. Statistical analysis using a non-paired t test was carried out for the comparison of symptomatic and asymptomatic patients.

High stress concentration is found at the shoulder region of the symptomatic patient, while in asymptomatic patient the stress distribution is much more averaged. The mean principal stresses in the plaques of symptomatic patients are significantly higher than those of asymptomatic patients (533.4±185.3 versus 203.5±121.3 kPa, $p<0.05$).

This study illustrates the potential application of state-of-the-art computational modelling techniques to solving important clinical problems in medicine. This FEA suggests that the maximum stresses within the plaques of symptomatic patients are higher than those of asymptomatic patients. Mechanical stress in the carotid plaque, therefore, ought to be regarded as a complementary indicator of plaque rupture risk alongside the traditional measure of stenosis. A combination of high resolution MR and FEA could potentially act as a useful tool for assessment of risk in patients with carotid disease.

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The work was supported by GlaxoSmithKline (UK) and Stroke Association.

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

PC86

The activity of NADH and NADPH mitochondrial oxidase in chronic venous insufficiency

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Chronic venous insufficiency is a frequent health problem, especially in females, with a chronic evolution and important social costs. The study aims to identify the sources of reactive oxygen species in chronic venous insufficiency, especially the NADPH and NADH mitochondrial oxidases, by way of a histochemical technique employing nitro blue tetrazolium (NBT). This method allows for the evaluation of the oxidative enzyme activity in the tissue due to NBT's property of being eliminated by the tissue dehydrogenases, with the formation of a blue non-crystalline pigment, called formazan.

For this purpose the fragments of varicose veins were taken from 31 patients diagnosed with CVI stage II/III (class CEAP 2-5) with an average age of 46.2 during a surgical procedure (venous stripping). The results were compared to those of a control group of the same sex and age distribution, taken from normal saphenous vein during the surgical procedures of coronary and peripheral by-pass. The CVI diagnosis was clinic (the presence of varicose veins, shank oedema, lipodermatosclerosis, varicose eczema and post venous ulceration scars). The arterial involvement was excluded through peripheral Doppler probe.

In chronic venous insufficiency there is an intense mitochondrial oxidant enzyme activity, which intervenes in the production of superoxide anion and indirectly of oxygenated water. These enzymes are active in the whole wall of varicose vein, but mostly in the hypertrophycal muscular layer and conjunctive layer where rich fibroblasts can be seen. The activity of NADPH oxidase is high in the luminal thrombus possibly in neutrophils and fibroblasts infiltrate. The histochemical quantification of the oxidative state of the tissue by means of using NBT proved to be an efficient method of demonstrating the excess production of reactive oxygen species and of analysing the antioxidant state of the tissue.

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

PC87

Effect of PGE_2 on isolated segments of mouse aorta

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Prostaglandin E_2 (PGE_2) mediates its effects on the blood vessels by binding to four E-prostanoid receptors, designated EP_1, EP_2, EP_3 and EP_4. EP_1 and EP_3 receptors are coupled to Ca^{2+}

mobilisation and cAMP inhibition via Gq/Gi, respectively, and mediate vasoconstriction. EP_2 and EP_4 are coupled to stimulation of adenylyl cyclase via Gs proteins and mediate vasodilatation (1). Recent studies show that the EP_4 receptor also couples to phosphatidylinositol-3-kinase (PI-3-K) that in turn activates Akt-kinase (PKB) (2). PKB can activate endothelial NO synthase. In the present study we wish to evaluate mouse aorta for isometric force measurements and determine if PGE_2 causes vasodilatation by binding to the EP_4 receptor, activating the PI-3K/Akt-dependent pathway and stimulating eNOS.

Mice were humanely killed and aortic rings were prepared and mounted in an isometric force transducer. Phenylephrine (10^{-5}M) followed by acetylcholine (10^{-6}M) were added at the start of each experiment in order to test the viability of the media and the endothelium, respectively. All experiments were conducted in aerated (5% CO_2 in air) Krebs-bicarbonate solution in the presence of the COX-inhibitor indomethacin ($5x10^{-6}$M).

Our results show that PGE_2 has divergent effect on blood vessels because it is able to cause both dilatation and constriction. PGE_2 constricted the mouse aortic rings in a concentration-dependent manner (EC_{50} (-log M) = 4.89 ± 0.06, n=8) and further constricted ($p < 0.05$) PE-preconstricted ($6x10^{-8}$ M) aortic rings (EC_{50} (-log M) = 5.89 ± 0.06, n=4).

In the presence of S18886, a TP-receptor antagonist (10^{-7} M), PGE_2 (10^{-8} M to 10^{-6} M, n=6-7) relaxed PE-constricted ($3x10^{-7}$ M) mouse aortic rings. L-NAME (10^{-4} M), a NO-syntase blocker, abolished the vasodilatation to PGE_2 in PE-constricted segments (n=9).

$PGF_{2\alpha}$ also constricted the mouse aortic rings in a concentration-dependent manner (EC_{50} (-log M) = 5.03 ± 0.13, n=8). PGE_2 caused no further constriction of $PGF_{2\alpha}$-constricted aortic rings indicating that PGE_2 and $PGF_{2\alpha}$ activate the same vasoconstrictor receptor (n=3). The constrictor effect of $PGF_{2\alpha}$ was also abolished by S18886 (10^{-7} M, n=6).

The current data indicate that PGE_2 and $PGF_{2\alpha}$ cause vasoconstriction in mouse aortic rings by activation of the TP-receptor. In the presence of a TP-receptor antagonist (S18886) PGE_2 causes vasodilatation by stimulating NO synthesis.

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

PC88

Cardiovascular autonomic dysfunction in a rat model of coarctation of the aorta (CoA)

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We have shown recently that human infants with CoA show signs of derangement in their autonomic cardiovascular function in the early neonatal period (1), and this may play a role in the high incidence of hypertension in this patient group. To investigate cardiovascular autonomic dysfunction in more detail we have developed a rat model of CoA.

In a preliminary operation in 1-3 day old rat pups (anaesthetized with ketamine, 60 mg kg^{-1}, and medetomidine, 250 μg kg^{-1}), the abdominal aorta was exposed via a retroperitoneal approach and isolated from the surrounding connective tissue. A ligature was placed around the aorta, either superior (SR; n=5) or inferior (IR; n=3) to the renal arteries, and tightened around a 25g needle which was then pulled free. A control group (n=7) consisted of exposing the aorta without placement of a ligature. Three to six weeks later rats were re-anaesthetized (2% halothane, reduced to 0.75% after completion of surgery) and carotid and femoral artery blood pressures (BP) were measured, as well as baroreceptor reflex gain following bolus injections of phenylephrine (0.5-2.5 μg). Data are expressed as means ± S.E.M. Statistical significance was determined using Student's unpaired t test. Differences were considered significant at $p < 0.05$.

Carotid BP was increased in SR CoA but not in IR CoA (129.7 ± 6.9 and 103.6 ± 3.0 v 106 ± 5.1 mmHg control, respectively). The pressure gradient across the constriction was greater in both CoA groups (21.4 ± 1.3 and 9.8 ± 0.9 v 5.2 ± 0.7 mmHg control, respectively). Baroreflex gain was reduced significantly in the SR CoA group but not the IR CoA group (0.64 ± 0.26 and 1.45 ± 0.32 v 1.51 ± 0.23 control). Post mortem examination revealed that the heart weight of the SR CoA, but not the IR CoA group was significantly heavier than control (1.3 ± 0.1 and 1.0 ± 0.1 v 1.2 ± 0.03 g control).

These data show that in the rat SR CoA, but not IR CoA, causes increased BP and reduced baroreflex sensitivity, and is similar to the autonomic cardiovascular dysfunction observed in the human CoA infant. The SR CoA rat, therefore, is a suitable model for studying autonomic changes associated with CoA.

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This work was supported by the British Heart Foundation.

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

PC89

Heterogenous changes in noradrenaline and neuropeptide Y concentrations in the hearts of diabetic rats

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Neuropeptide Y (NPY) is co-localized with noradrenaline (NA) in the sympathetic postganglionic nerve fibers supplying the heart and it has been also identified in the intrinsic cardiac neurons that exert relatively autonomous local control over the myocardial function.

In the heart, NPY mediates particularly direct vasoconstriction of coronary arteries and modulates NA release. In addition, the peptide seems to be an important trophic factor in the cardiovascular system as evidenced by NPY-mediated increase in the ventricular L-type calcium current density, cardiac hypertrophy, and angiogenesis.

Diabetic autonomic neuropathy accompanies the later stages of diabetes mellitus and contributes significantly to the increased morbidity and mortality of diabetic patients. Although numer-

ous studies dealing with cardiac NA concentrations in humans and various animal models of type 1 diabetes have been reported, evidence about putative changes in NPY levels in relation to the shifts in NA concentrations in the diabetic heart is still lacking. In the present study, the changes in concentrations of NPY and NA (determined by radioimmunoassay diagnostic kits) were investigated in the separated rat heart compartments 1, 2, 4, 6, 9 and 12 months after administration of streptozotocin (STZ; 65 mg/kg i.v.) and in the age-matched controls (n=15 per group). Hearts were removed from humanely killed rats. Statistical analysis was performed using one-way ANOVA ($P<0.05$) and *post hoc* Student's unpaired *t* test with Bonferroni correction.
About 30% of diabetic animals displayed symptoms of partial spontaneous recovery, i.e. decreasing blood glucose levels and increasing insulin concentrations in the plasma and pancreas. NPY concentrations in the diabetic atria did not differ from those in age-matched control rats 1, 2, 4, 6 months in the right atria and even 9 months in the left atria. However, uncompensated diabetes led to a significant decrease in NPY levels 9 and 12 months after STZ administration in the right and left atria, respectively ($P<0.05$). In the ventricles, NPY concentrations were significantly decreased 6 months after the onset of diabetes ($P<0.05$). Interestingly, partial spontaneous recovery of diabetes was associated with increased NPY levels in the atria ($P<0.05$). Myocardial NA concentrations increased 1 month after STZ and then declined reaching ~60% of the respective control values 12 months after the onset of the disease ($P<0.05$). Partial spontaneous recovery of diabetes had no effect on NA myocardial concentrations. Regarding preferential localization of NA in the sympathetic postganglionic fibers and that of NPY also in intrinsic ganglion neurons, intrinsic neuronal circuits seem to be less susceptible to STZ-induced damage than extrinsic nerves and they might be able to recover after amelioration of diabetes.

Supported by Grant Agency of the Czech Republic, grant No. 305/04/0144.

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

PC90

Temporal changes in the IGF-I/IGFBP-1 axis in a murine model of diet-induced obesity

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Insulin and insulin-like growth factor I have complementary roles in the regulation of glucose homeostasis. Observational studies suggest that patients with type II diabetes have lower levels of circulating IGF-I. IGF-I is also believed to have a direct effect on insulin sensitivity. IGF bioavailability is modulated by a family of binding proteins, most acutely by IGFBP-1 which is also regulated by insulin and nutritional status. We assessed the longitudinal changes in IGF-I, IGF-I sensitivity and IGFBP-1 expression in dietary-induced obesity in mice.
Male C57 Bl/6 mice received an obesogenic diet and were compared with chow-fed controls (6-16 per group). Body weight and fat pad mass were recorded. Insulin sensitivity was assessed by insulin tolerance tests. Fasting IGF-I levels were measured by ELISA and hepatic IGFBP-1 expression was measured by real-time PCR. Subcutaneous IGF-I tolerance tests (0.2g/μg) were also performed. All animals were humanely killed.
Results are shown in Table 1, with $p<0.05$ considered significant. Mice receiving a high-fat diet had a higher body weight and fat pad mass. Insulin resistance as assessed by hypoglycaemic response to exogenous insulin was increased at 4 and 8 weeks. The hypoglycaemic effect of IGF-I was significantly diminished and IGF-I levels were found to be significantly higher. There was no difference in hepatic IGFBP-1 expression.
These data, contrary to previously reported observations suggest that IGF-I levels increase as insulin sensitivity decreases and there is an accompanying decrease in IGF-I sensitivity.
Table 1

Weeks feeding	4-6	8-10
Body Mass (g)		
Chow	22.2(0.4)*	24.9(0.6)*
High fat	27.1(0.4)	35.7(1.0)
Epididymal fat pad mass (mg)		
Chow	8.3(1.13)*	9.9(1.32)*
High fat	28.0(3.16)	35.6(2.70)
Fasting insulin (μU/ml)		
Chow	12.4(5.3)	21.1(4.8)*
High fat	27.3(8.1)	45.9 8.5)
Fasting IGF-1 (ng/ml)		
Chow	238(16)	298(27)*
High fat	287(28)	404(26)
BM % change (30 min) insulin challenge		
Chow	46.1(4.0)*	45.2(2.5)*
High fat	46.1(4.0)*	28.7(4.1)
BM % change (30 min) IGF-I challenge		
Chow	27.8(3.7)	38.3(3.4)*
High fat	18.3(3.7)	12.6(4.7)
IGFBP-1 expression (U)		
Chow	1(0.26)	0.78(0.25)
High fat	1.52(0.77)	0.77(0.25)

Data are expressed as mean and S.E.M.; n=6-16 per group. *$P< 0.05$ chow against high fat fed.

This work was supported by the British Heart Foundation.

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

PC91

Effects of low ethanol ingestion in the rat

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Ethanol (ETOH) is commonly used as a drug solvent in experimental treatments of research animals, frequently without adequate controls to dissect out the effects of ETOH. We have

explored the effects of ETOH administered to adult male Wistar rats in the drinking water (1% ETOH; 8 days). Controls rats were identically housed and given regular tap water.
We have explored: a) the general status of the animals (red and white blood cells and platelets, blood glucose and protein levels); b) the catecholamine levels in renal artery, superior cervical ganglion and carotid body; in the last tissue we also studied catecholamine synthesis and release), and; c) the general redox status of the animals (glutathione potential and lipid peroxides in lung, liver and diaphragm and antioxidant power of plasma (PAP). We also monitored angiotensin-converting enzyme (ACE). Animals were humanely killed at the end of the experiment. Statistical significance was evaluated by unpaired t test.
There were not differences between groups in body weight gain or in the amount of water drunk. ETOH ingested by experimental animals amounted to 0.27 ml/rat/day. Red cell count, haemoglobin, hematocrit leucocytes and platelets were not statistically different in both groups. Proteins levels were identical in both groups, but glucose (142.6 ± 8.43 mg/100ml) increased in the ETOH group to 251.0 ± 28.45; n =10). Norepinephrine levels in renal artery and superior cervical ganglion of control animals were, respectively, 11.64 ± 1.10 and 175.60 ± 11.26 pmol/mg tissue and decreased in the ETOH group to 7.20 ± 0.58 and 114.10 ± 9.47 pmol/mg tissue (n = 11-16; $p < 0.001$ in both cases). In the carotid body norepinephrine and dopamine levels were not different between groups, but the rate of synthesis and release of dopamine increased significantly in the ETOH group. The glutathione redox potential in diaphragm (-184.0 ± 2.05 mV) and liver (-223.3 ± 0.82 mV), but not in lung, rose in ETOH group to -190.9 ± 1.07 and -232.4 ± 2.75 mV, respectively, and lipid peroxides levels decreased in the same tissues. The PAP in control animals was equivalent to 1,349 ± 163.6 mM FeSO4 and in the ETOH rose to 2,036 ± 196.7 mM (n 13-16; p=0.011). ACE (332.8 ± 20.43 U/l) was significantly lower in ETOH group 187.3 ± 9.63 U/l; n = 10; $p < 0.001$).
This wide range of effects of low doses of ETOH advise the use of adequate controls in studies that use this agent as solvent. Additionally, the data indicate that moderate doses of ETOH might protect against oxidant damage at the cost of producing marked hyperglycaemia.

Supported by Spanish MEC grant (06394/BFI) and by RED RESPIRA (ICiii).

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

PC92

Autoresuscitation by gasping and arousal and resuscitation effects of gasp-, sniff- or hiccough-like aspiration reflex

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Gasping developed spontaneously in 23 of 24 moribund infants with cardio-respiratory monitoring and saved 15% of them [1]. Gasping developed also during experimental ventricular fibrillation and autoresuscitated 10% of pigs and rats [2]. However, various methods of stimulation of the nasopharynx or adjacent regions in cats can regularly induce powerful spasmodic inspiratory efforts called gasp-, sniff- or hiccough-like aspiration reflex having similar resuscitation properties as gasping [3,4].
Whole-night polysomnographic examination with detection of >30 parameters was performed in 101 adults with sleep disordered breathing (SDB) and divided into five groups of severity according to their apnoea/hypopnoea index (AHI). AHI was <5/h in simple snoring (SS; n=11), 5-10/h in upper airway resistance syndrome (UARS; n=33), 10-20/h in mild sleep apnoea syndrome (SAS I; n=14), 20-40/h in moderate (SAS II; n=18) and >40/h in severe (SAS III; n=25).
Arousal from sleep was increased in all groups, but in SAS III it was caused by respiratory arousal: 75.8±15.3/h due to severe hypoxaemia- average O_2 saturation 86.6±1.2% (M±SE) and minimum O_2 saturation 59.7±2.6%, $p<0.05$ for all compared with other groups (t test). Typical features for separate groups are: sigh (augmented breath) + micro-arousal) for SS, as well as hyperventilation+ autonomic arousal and hypertension for UARS. In obstructive SAS there are apnoeic episodes followed by choking + arousal. In severe asphyxia with depressed cough reflex, the persisting aspiration reflex supports development of aspiration pneumonia or sino-bronchial syndrome. In very severe asphyxia autoresuscitation by gasping induces gradual normalisation of blood pressure and gases, EEG activity, brainstem evoked potentials and breathing. However, the autoresuscitation may fail in sudden infant death syndrome or sudden cardiac death. Using the immunohistochemical c-Fos method in anaesthetized cats (40 mg/kg i.p.; and humanely killed at the end of the experiments), a significant 1.6- to 12-fold increase in the number of many brainstem neurones subserving respiratory and cardiovascular control mechanisms and in both ascending and descending neurones of reticular formation activated by aspiration reflex ($p<0.05$-0.001, Mann-Witney test), was observed in 14 of 35 nuclei. Strong recruitment of neurones in brainstem cardiovascular centres suggests vigorous sympathoadrenal activation, which may stop paroxysmal tachyarrhythmias, including ventricular fibrillation in animals and humans. The aspiration reflex proved to interrupt also laryngospasm, bronchospasm and even severe hypoxic coma accompanied by apnoea and arrhythmias in cats and resuscitate them from nearly a clinical death [5]. The aspiration reflex provokes activity in many brainstem neurones in the reticular formation and the respiratory and cardiovascular control mechanisms, imitating various typical features of SDB and could provide an effevtive tool for both research and treatment. We suppose that the aspiration reflex could interrupt various paroxysmal episodes of functional character, such as hiccough, spastic events, etc.

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

PC93

Brainstem mechanism of gasp-sniff and hiccough-like aspiration reflex in cats: C-fos study

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Mechanical stimulation of the nasopharynx or adjacent regions, particularly in cats, pigs and dogs, induces forceful spasmodic inspiratory efforts, called aspiration reflex (AR) [1,2], closely resembling sniffing or hiccough [3].
Under pentobarbitone anaesthesia (40 mg/kg i.p.) airflow and blood pressure were recorded in 11 cats (6 with induction of 300 AR-es in 30 min and 5 quietly breathing controls). After humane killing, parallel histo-chemical preparation and computer-aided counting, the Fos-like immunoreactive (FLI) dots were compared by Mann-Whitney test, n= 6 and 5.
A significant increase in number of FLI dots, indicating strong neuronal activation was observed mostly in the following 12 of 35 selected brainstem nuclei, localised according to Berman's stereotaxic atlas [4]. Respiratory group neurones indicated a large increase in FLI as follows: commissural nuclei of NTS (10 ×, $p<0.01$), ambigual, paraambigual and facial nuclei (12 ×, $p<0.02$). This strong recruitment of active neurones reflects the powerful spasmodic inspiratory activity of the AR. Also the cardiovascular group neurones showed increased FLI: particularly in Nucl. retrofacialis (10 ×, $p<0.01$) indicating strong cardiovascular effects of the AR with possible implication also in cardio-pulmonary-cerebral resuscitation. The neurones of the Reticular activating system showed also an increased number of FLI dots. Such strong recruitment explains the powerful arousal and revitalisation effects of the AR. Recruitment was observed particularly in the medullary lateral and magnocellular tegmental fields (11 ×, $p<0.02$), in pontine lateral and magnocellular fields (3 ×, $p< 0.02$) and in area mesencephalis ventralis -tegmentum tsai (1.6 ×, $p< 0.05$). The descending reticular tract neurones were strongly activated too, explaining the strong influences on various tonic activities [5]. Particularly the Raphe nuclei (5 ×, $p<0.02$), and the paragigantocelular lateral nucleus (2.5 ×, $p<0.02$), were involved.
Immunohistochemical c-Fos study indicated very strong neuronal activation of the brainstem respiratory and cardiovascular control mechanisms and both the ascending and descending parts of Reticular formation by AR in cats. The results suggest that the AR can be a very useful tool for studies of various influences, including drugs on 3 main vital functions and particularly their mutual interaction

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

PC94

Role of nitric oxide in cardiogenic depressor reflexes formation in different animal species

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The aim of this study is to compare the role of nitric oxide (NO) system in realization of cardiogenic depressor reflexes in different animal species. Acute experiments were performed on anesthetized dogs and rats. Cardiogenic depressor reflex evoked by stimulation of cardiac receptors with veratrine (reproduction of the Bezold-Jarish reflex). Veratrine injections (5 μg, intracoronary and i.v.) resulted in a reflex decrease of mean arterial pressure and relaxation of coronary and peripheral vessels (1). Systemic inhibition of NOS by L-NNA (30 mg/kg, i.v.) significantly decreased the depressor reflex in the dogs but not in the rats. On the other hand inhibition of neuronal NOS by 7-nitroindazole (25 mg/kg, i.p.) decreased cardiogenic depressor reflex in the rats. We carried out the separate series experiment with NADPH-d staining to evaluate the distribution of NOS containing neurons in the medulla of dogs and rats. The main results of these experiments are: (i) the average number of NOS-containing neurons in the dorsomedial and ventrolateral medulla per section in dogs was larger than that in rats, while the density of the positive cells in the both regions in dogs was less than that in rats. (ii) Within the dorsal motor nucleus of vagus a lot of NOS-containing cells (preganglionic vagal neurons) were observed only in dog. Differences in the distribution of NO-generating neurons in the medullary cardiovascular centers and heterogeneity in the basal level of NO release may contribute to the peculiarities of the hemodynamic responses under formation cardiogenic reflexes in various species of animals. Thus NO-dependent mechanisms play an important role in realization of cardiogenic reflexes, predominantly related to n.vagus. These NO-dependent reflex reactions decrease cardiac afterload, therefore they could be admitted as compensatory reactions under cardiac pathology.

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PC95

Effect of tyramine on noradrenaline release from the diabetic heart atria

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Diabetes mellitus is frequently associated with cardiovascular autonomic neuropathy that represents a serious complication of diabetes and seems to be an important factor related to the development of diabetic cardiomyopathy.

The purpose of this study was to evaluate how diabetes affects the content of noradrenaline (NA) in the heart atria in early and later stages of streptozotocin (STZ; 65 mg/kg, i.v.)-induced diabetes in female rats, i.e. 1, 4 and 20 months after the onset of the disease (STZ1, STZ4 and STZ20, respectively). In addition, carrier-mediated release of NA from the sliced atria superfused with oxygenated Krebs-Henseleit solution was studied *in vitro* in the presence or absence of tyramine. Chronotropic effect of tyramine was evaluated on the isolated spontaneously beating right atria (n=6 per group). NA concentrations in the superfusates and tissue extracts were measured by radioimmunoassay. All animals were humanely killed at the end of the experiments and all procedures were performed according to current EU legislation. Statistical analysis was performed using one-way ANOVA ($P<0.05$) and *post hoc* Student's unpaired *t* test with Bonferroni correction. Data are presented as mean±S.E.M.

The diabetic state significantly affected NA concentrations in the heart atria. An initial significant increase in STZ1 atria (n=7; $P<0.05$ vs controls) was followed by a sustained decline in tissue NA concentrations that became significantly different in STZ20 atria (n=6; $P<0.05$ vs controls).

In the control right atria, the spontaneous beating rate was 223±6 min^{-1} and it did not change significantly with age. Tyramine increased the heart rate in a concentration-dependent manner (30% over basal values in the concentration 0.1 mmol/l) and its effect was comparable in all age groups. In contrast, diabetic STZ1 and STZ4 atria had significantly lower spontaneous beating rate (140±9 min^{-1}; $P<0.05$ vs controls). Tyramine increased the beating rate by 30% in STZ1 and STZ4 samples and by 110% in STZ20 atria, i.e. significantly more than in the age-matched controls ($P<0.01$).

Basal release of NA from the control atria of all age groups was 0.44±0.2 ng/g/min (n=6 per group) and tyramine (1µmol/l) increased the NA output by 300%. In the diabetic preparations (n=6 per group), tyramine effect varied in relation to the disease duration: In STZ1 atria, the effect of tyramine was similar as in the age-matched controls despite increased atrial NA concentration in the diabetic group. In STZ4 atria, tyramine enhanced NA outflow to a lesser extent than in controls and in STZ20 diabetic samples, tyramine-induced release was by 270% greater than in the control preparations ($P<0.01$).

In conclusion, STZ-induced chronic diabetic state seemed to lead to the cardiac sympathetic denervation as evidenced by decreasing atrial concentrations of NA in the course of the disease. However, this quantitative defect might be at least partly compensated by the increased release of NA from the remaining sympathetic fibres.

Supported by Grant Agency of the Czech Republic, grant No. 305/04/0144.

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

PC96

Physiological and psychological responses to bithermal caloric stimulation

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Rapid head reorientations that activate the vestibular system may present a challenge to physiological homeostasis. Thus, the vestibular system offers a short latency mechanism by which to instigate compensatory autonomic and ventilatory responses.

Hence, we sought to test whether unilateral vestibular activation by bithermal (30°C & 44°C) external auditory canal irrigations provokes a modification of autonomic and ventilatory regulation, and whether they relate to subjective experiences.

14 healthy subjects (8 male; mean age 32.4 ± 4.2 years) were tested, all of whom had given informed consent to participant in the study, which had received local ethics committee approval. Continuous recording of breath-by-breath respiratory parameters (pneumotachography), heart rate (H_R; standard 3-lead ECG) and systolic blood pressure (Portapres; SBP; mmHg) were performed. The 40s surrounding (20s prior and post) the peak velocity of the evoked slow phase nystagmus (PEAK) to an irrigation was compared to two 5 minute control periods prior (PRE) and post (POST) irrigations using paired students *t*-tests. Upon completion questionnaires were administered where participants experience of sickness, faintness, and dizziness in response to the irrigations were rated, in addition to state anxiety (Spielberg Short Form).

Minute ventilation (V_I: L. BTPS min^{-1}) significantly ($p<0.05$) increased during PEAK vs. PRE (7.7 ± 0.5 vs. 7.1 ± 0.3), predominantly furnished by an increase ($p<0.05$) of respiratory rate (F_R; min^{-1}) (16.1 ± 0.8 vs. 14.1 ± 0.6). Significant ($p<0.05$) SBP augmentation (157.6 ± 4.4 vs. 150.6 ± 4.3) was also observed in the absence of significant H_R changes. All parameters returned to PRE values during POST. Few psychometric effects beyond dizziness were noted in response to repeated caloric irrigations.

In conclusion, unilateral caloric irrigations activated both autonomic and respiratory control mechanisms. Rapid increments in SBP may serve to counteract a fall in cerebral blood flow during rapid head reorientations. The provocation of tachypnoea is unlikely to be simply due to anxiety/panic as these symptoms were not reported by the subjects. The mechanisms and physiological importance of vestibular evoked autonomic and ventilatory modulation requires further study.

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

PC98

ECG arrhythmias after voluntary apnoea during head-out cold water immersion

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Suited subjects submersed under cold-water experience ECG arrhythmias during and after volitional apnoea (1). We studied 32 healthy men immersed in cold (11°C) water, wearing trunks (mean(s.d.) age 21(3.5) years, height 1.78(0.09) m, mass 76.6(10.6) kg). Two hours before study, subjects rested, refrained from smoking, eating and drinking caffeinated drinks. Seated subjects completed two breath-holds in air (23.5(0.23)°C) wear-

ing nose clip and mouthpiece; inspiratory frequency (fR) and volume (VI ; turbine), endtidal PCO_2 was recorded. Three-lead electrocardiogram recorded lead II. Breath-hold duration (BH_{air}:time between onset of last breath prior to breath holding, and onset of expiration) averaged 45.8(15)s. Arrhythmias were not seen during or following BH_{air}. Subjects were winched above water for 2 min; they then again breath-held, and over 28s were lowered into water at 8m min-1 to clavicular level (total immersion duration 2.5 min). BH_{water} averaged 22.7(12.3)s, significantly less than BH_{air} ($p<0.001$; paired t test, df=31). During BH_{water}, minor bradycardia was seen. Following BH_{water}, hyperventilation (fR 41.7(18.3)breaths/min, nadir PCO_2 20.04(6.3)mmHg) and tachycardia occurred in all subjects (HR 115(3)1/ min); supraventricular and ventricular arrhythmias occurred in 20/32(63%) and 4/32(13%) subjects respectively, lasting a few seconds and usually in the first 10s after breathing recommenced. Two subjects had both supraventricular and ventricular arrhythmias transiently. Sinus tachycardia lasting about 30s was seen in 8/32 subjects, uninterpretable traces contaminated by shivering EMG and movement artefact occurred in 2 subjects. Arrhythmias were noted on occasion to be linked to respiration. A second group of 4 men (19-27 years), each studied thrice, breathed freely during cold immersion. Both groups showed tachycardia during cold immersion. However, only 1 free breathing subject on only 1 trial showed arrhythmias(coupled supraventricular ectopic beats). Since both groups are subject to the same hydrostatic and cutaneous cold stimulation during immersion, neither of these mechanisms per se are the origin of increased arrythmias seen following breath holding. They may be due to release of breath hold and consequent vagal stimulation, in a cold milieu. Under natural conditions, the nature and timing of cold immersion arrhythmias depend on which of 3 factors pertain: 1. Facial stimulation, producing bradycardia; 2. Cold, producing tachycardia; 3. Breath holding, producing supraventricular arrhythmias on release in cold water. Significant hypocapnia and ECG arrhythmias occur following breath holding during cold-water immersion, in healthy subjects wearing normal swimming attire.

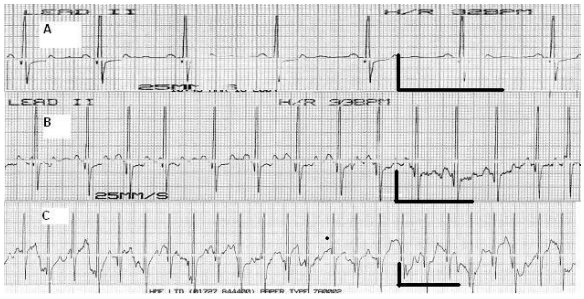

Figure 1. ECG of single subject showing sinus tachycardia and ventricular arrhythmias following break of breath hold during cold immersion. Breath hold time is diminished by cold shock to only 22 s

with subsequent hyperventilation and hypocapnia.

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

PC99

Chronic exercise improves rat aortic functions under stress

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Chronic exercise exerts beneficial effects on cardiovascular functions with multiple mechanisms. This study examined whether chronic exercise improves aortic endothelial calcium (EC $[Ca^{2+}]_i$) signalling or heat-shock protein 72 (HSP72) expression in rats under stress.

Five-week-old male Wistar rats were randomly divided into control and exercise groups. Rats in the exercise group underwent 8 week exercise training either by the access to a running wheel or by running on a treadmill. At the end of exercise periods, rats were exposed to restraint stress for 30 min, rested for another 30 min, and killed by CO_2 inhalation. The vascular functions in dissected aortas were examined by monitoring the acetylcholine-evoked EC $[Ca^{2+}]_i$ using a tissue flow chamber mounted on an epifluorescence microscope with digital ratio imaging capability, and by monitoring the vascular HSP72 protein expression using Western blotting. Stress in rats induced elevated heart rate (567 ± 54 vs. 388 ± 15 beats/min, stress vs. control, $p<0.05$, n=4), blood pressure (124 ± 22 vs. 97 ± 10 mmHg, stress vs. control, $p<0.05$, n=4), and plasma corticosterone level (455 ± 40 vs. 90 ± 25 pg/ml, 30 min post-stress vs. control, $p<0.05$, n=6). It also suppressed EC $[Ca^{2+}]_i$ signalling (250 ± 20 vs. 730 ± 140 nM at 10^{-6}M acetylcholine, for stressed and unstressed control, respectively, $p<0.05$, n=6) and might induce aortic HSP72 protein expression (0.51 ± 0.09 vs. 0.41 ± 0.01, for stressed and unstressed control respectively, $p>0.05$, n=2). Eight week exercise training ameliorated stress-induced reduction of EC $[Ca^{2+}]_i$ signalling (405 ± 60 vs. 250 ± 20 nM at 10^{-6}M acetylcholine, for exercised/stressed and unexercised/stressed, respectively, $p<0.05$, n=4) and enhanced stress-induced aortic HSP72 protein expression (0.92 ± 0.14 vs. 0.67 ± 0.19 for exercised/stressed and unexercised/stressed, respectively, $p<0.05$, n=8). Taken together, stress induced vascular endothelial dysfunction and evoked defense mechanisms, while chronic exercise ameliorated stress-induced endothelial dysfunction and enhanced stress-evoked defense mechanisms.

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Supported by grants from National Science Council, Taiwan ROC; NSC 93-2320-B-006-005, NSC 93-2321-B-006-006.

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

PC100

Estimation of the cardiovascular risk factors in a youth cardiorisk project

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The aim of this study was the identification and treatment of the cardiovascular risk factors in youths in order to reduce the incidence of coronary heart disease (CHD), ischaemic stroke and peripheral artery disease.
We studied 518 subjects (375 girls and 143 boys), aged 20±2 years. We used a simple set of questions about family history of CHD, alimentation, stress, smoking, basic behaviours and we determined systolic blood pressure, weight, height, body mass index and abdominal circumference.
A family history of CHD was present in 12.9% of cases (22% in boys and 14.2% in girls); 29.7% in smokers (38.6% boys and 26.2% girls); 18.67% with high blood pressure (36.4% boys and 11.79% girls); obesity was present in 10% of cases (22% boys and 5.36% girls); 65.2% had an unhealthy diet (77.9% boys and 60.3% girls); stress was present in 66.4% cases (51% boys and 72.3% girls); 24.7% had a sedentary lifestyle (15.8% boys and 28.1% girls). 40.7% had multiple risk factors (≥3; 54.55% boys and 35.47% girls).
In conclusion, to reduce the incidence of cardiovascular diseases it may be necessary to change risk behaviours (unhealthy diet, smoking, sedentary lifestyle) by stopping smoking tobacco, make healthy food choices, increase physical activity, and reduction of salt consumption levels at youth.

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

PC101

Prophylactic and therapeutic effects of potassium administration in hypertensive rats

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Induction of hypertension using DOCA-salt resembles in many aspects essential hypertension in human. Most of the pathophysiological aspects of this type may be contributed to the high level of serum Na^+ and the presence of Na^+ sensitivity. In the present study we demonstrated the prophylactic and therapeutic effects of oral KCl administration in hypertensive rats. Rats were divided into 4 main groups: group I, normotensive control group (8 rats); group II, hypertensive control group (8 rats); group III: concomitant K^+-treated hypertensive group (8 rats); and group IV, hypertensive-treated group (24 rats). Group IV was further subdivided into 3 subgroups: IVa, K^+-treated (8 rats); IVb, angiotensin converting enzyme inhibitor (ACE-I)-treated; and IVc, combined K^+ and ACE-I-treated. Hypertension was induced using DOCA (deoxycorticosterone acetate)-salt rat model (50mg/kg s.c. once weekly and 1% NaCl in drinking water for 4 weeks). Treatment with oral KCl (1.1 mEq/100g bw), ACE-I (7.5 mg/kg bw/day) or both continued for 4 more weeks. In group III (prophylactic group), treatment with KCl started with induction of hypertension and continued also through the 4 week treatment period. At the end of the treatment period, animals were fasted and we measured: systolic (SBP), mean arterial (MABP) and diastolic blood pressure (DBP). We then calculated: fasting serum lipids; serum Na^+ and K^+ levels (taking retro-orbital blood samples); 24 h urine and urinary Na^+ and K^+ levels. Animals were humanely killed at the end of the experiments.
In this study, mean values of SBP, DBP and MABP were significantly higher ($P<0.001$) in the hypertensive group when compared with the normotensive control group. Treatment with K^+ resulted in significant reduction of ABP, DBP and MABP levels in the concomitant K^+-treated group ($P<0.05$) and K^+-treated hypertensive group ($P<0.05$) when compared with the hypertensive control group. The combined use of K^+ with ACE-I resulted in a significant reduction in SBP, DBP and MABP levels in the hypertensive rats ($P<0.001$, $P<0.001$ and $P<0.05$) when compared with the control, K^+-treated and ACE-I-treated hypertensive groups, respectively. Supplementation and treatment with K^+ resulted in a significant decrease ($P<0.05$) in serum Na^+ level while significant increase ($P<0.05$) in serum K^+ level was observed coinciding with a significant increase in urinary Na^+ levels. Treatment with K^+ resulted in significant decrease ($P<0.05$) in serum cholesterol and LDL levels and significant increase ($P<0.05$) in serum HDL level.
Induction of hypertension using DOCA-salt resembles in many aspects essential hypertension in human. Most of the pathophysiological aspects of this type may be contributed to the high level of serum Na^+ and the presence of Na^+ sensitivity. From our data, we can conclude that oral K^+ administration causes improvement of the pathophysiological effects of DOCA-salt-induced hypertension in rats through natriuresis, similar to that of ACE-I. The prophylactic antihypertensive effect of KCl was more obvious than its therapeutic effect in this study.

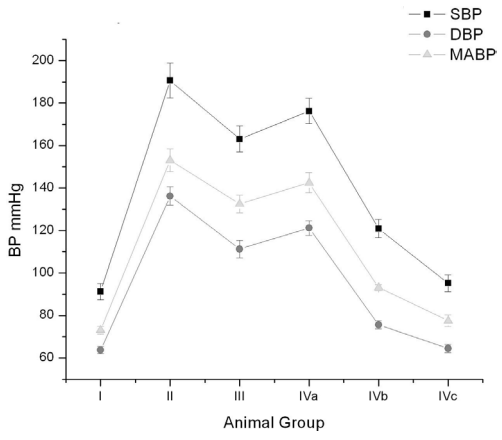

Figure 1. Effect of K^+ on ABP. All data are expressed as mean ± S.E.M. Statistics were made using Student's t test.

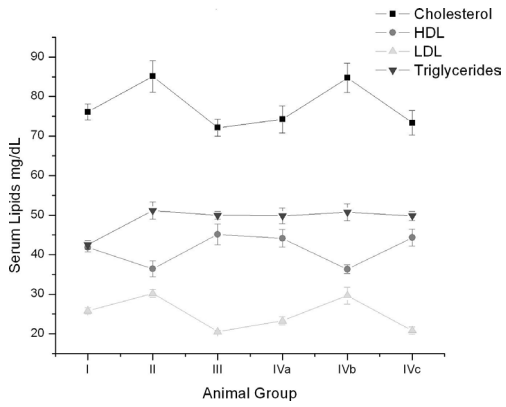

Figure 2. Effect of K^+ on serum lipids.
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We thank Prof. S. Saleh for her unlimited scientific supervision; Dr M. Hanafy and Dr H. Abdelrazek for their continous help; members of Physiology Dept-Faculty of Medicine- Minufiya University for generous assistance.

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

PC103

Chronic hypoxia enhances calcium handling in human pulmonary artery smooth muscle cells

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Hypoxic pulmonary vasoconstriction (HPV) is a widely conserved, homeostatic, vasomotor response of resistance pulmonary arteries to alveolar hypoxia. HPV mediates ventilation-perfusion matching and, by reducing shunt fraction, optimizes systemic PO_2. HPV is intrinsic to the lung, and, although modulated by the endothelium, the core mechanism resides within the smooth muscle cell (SMC). Acute hypoxia induces pulmonary vasoconstriction mediated by O_2-sensitive Kv channels (Pozeg et al. 2003) and voltage gated calcium channels (VGCCs) (Moudgil et al. 2005), whilst chronic hypoxia causes structural changes of the pulmonary vasculature including arterial hypertrophy, but the mechanism remains unclear. It is widely accepted that intracellular Ca^{2+} serves as an important signal in regulating contraction and proliferation of pulmonary artery smooth muscle cells (Ward et al. 2004). In this study, we investigated the plasticity of calcium handling ion channels and pumps in human pulmonary artery smooth muscle cells (Promocell) under chronic hypoxia stress using real time, quantitative PCR (ABI 7900) with calcium imaging and patch clamping methods.
Chronic hypoxia was induced in a hypoxia incubator with 1% O_2, 5% CO_2, balance N_2. We determined the expression levels of a number of ion channels including: TRPC3, TRPC4, TRPC5, TRPC6, TRPC7, P2X1, P2X4, P2X7, P2Y1, P2Y4, P2Y6, NCX (Na-Ca exchanger), NCKX (Na-K-Ca exchanger), VGCC α1C, VGCCα1G, VGCCα1H subunits and TASK2 channels compared with GADPH. We found mRNA levels for most of the channels examined were upregulated after 60 h exposure to 1% O_2 compared with cells growing in room air O_2 tension (with 5% CO_2). Noticeably, there were 127-fold increases in P2X1 receptor expression, 32-fold increases in TASK2 channel expression and 27-fold increases in TRPC5 channels expression. In a functional, follow up study, we measured intracellular calcium with conventional imaging methods, using Oregon Green (Molecular Probes). Our data showed that 100 μM ATP induced a dramatic cytoplasmic calcium increase, which was partially quenched by 100 μM suramin. 10 μM $\alpha\beta$methylene ATP also elicited an intracellular calcium rise. Nifedipine, 10 μM and 20μM, were used in our experiments and they exhibited a similar blockage effect on intracellular calcium elevation, suggesting that VGCCs were not the only resource contributing to the calcium rise. Whilst further studies to measure the protein levels of ion channels regulated during chronic hypoxia stress are required, these results suggest that P2 receptors might play an important role in HPV under chronic hypoxia.

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We acknowledge the support of the Dean's Initiative Fund, University of Birmingham.

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

PC104

Winter brain and body temperature in free-living pronghorn antelope

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North American pronghorn antelope (Antilocapra americana) endure hot summers and cold winters, and have a high aerobic exercise capacity. How they thermoregulate under these conditions is unknown. Our study was aimed at measuring their brain and blood temperatures, investigating whether these temperatures are affected by environmental temperatures and whether males and females have different body temperatures.
Using isoflurane anesthesia and aseptic techniques in two male and three female pronghorns we implanted small bead thermistors (GE Thermometrics) into the carotid artery, jugular vein, near the hypothalamus, and in the abdominal cavity. Temperature was measured with an accuracy of 0.04°C, recorded every 5 min, and stored on data loggers. After recovery from anesthesia the animals were released into a 100-hectare enclosure at the Sybille Research Station, Wyoming. They were free ranging for 40 to 87 days from November 2004 to February 2005. A 15-channel weather station (Onset HOBO) was used to record black globe temperature and other weather data at the study site.

Over the 3 month recording period, mean ambient temperature was -0.5 ± 8.2°C (mean ± S.D.; range -22 to +9°C). The coldest day (23/12/04) had a mean ambient temperature of -12.4 ± 8.1°C (range -22 to +2°C). Mean brain temperature over the period for all 5 animals was 38.9±0.4°C, mean carotid artery blood temperature was 38.6±0.4°C, and mean jugular vein blood temperature was 38.2±0.5°C. On the coldest day highest ambient temperature occurred at 1200h, and highest body temperatures at 0100h and 1700h. Lowest body temperatures coincided with the lowest globe temperature at 0800h. In one female and one male mean brain temperature on this day was 38.9±0.2°C, mean carotid blood temperature was 38.4±0.3°C, and mean jugular blood temperature was 38.0±0.3°C, which were the same as mean temperatures for all days. The mean brain carotid temperature difference was 0.6±0.3°C. The most frequently recorded arterial blood temperature in the female was 38.2°C and 38.7°C in the male.

Our results show that body temperature of pronghorns is maintained in the winter, and has a nycthemeral rhythm with amplitude less than 1°C. The female had a lower mean body temperature than the male, narrower frequency distribution, and a higher brain-carotid difference. The mean brain temperature in both animals was similar. The male's lower brain-blood difference could have been a consequence of its higher body temperature, or a higher cerebral blood flow combined with cooling of post-carotid rete arterial blood. The data imply that in the winter pronghorn brain temperature is regulated within a narrower range than core temperature.

We thank Drs Mark Nijland, Terry Kreeger, Will Schultz, and the staff at the Wyoming Game and Fish Department's Sybille Research station for their help with this project.

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

PC105

Thermogenesis and heat increment of feeding in the pigeon

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To study the time course of feeding-induced thermogenesis (heat increment of feeding, HIF) in an avian species with a crop, we measured the HIF induced by a bout of feeding in the domestic pigeon (Columba livia). Six birds were fasted for 24 h and then allowed to eat for 40 min by illuminating an otherwise darkened metabolic chamber. Oxygen consumption (MO2) and respiratory quotient (RQ) were measured by indirect calorimetry, deep body temperature (T_b) using intra-abdominal data loggers (iButton Thermochron, DS1921H, implanted 1 week earlier under isoflurane anaesthesia, 4%; post-operatively, buprenorphine was given for analgesia (0.1 mg/kg i.m.)) and shivering thermogenesis by electromyography (r.m.s. of electromyograms) from the pectoral major muscles for 5 h after feeding. All animals were humanely killed at the end of the experiments.

At 23°C, feeding was followed by a significant increase in MO2 from 12 to 16 ml min kg^{-1} and RQ from 0.75 to 0.90 that lasted for several hours (repeated measures ANOVA). At 0.5°C, feeding did not change MO2, although RQ increased as at 23°C. Such results generally indicate that HIF substitutes for facultative heat production (shivering) in the cold. However, we found no difference in shivering between fed birds and non-fed controls in the cold (r.m.s. EMG about 60 μV in both groups). Furthermore, the instantaneous increase in MO2 did not correlate with the amount of food eaten at either temperature, but T_b was higher in fed birds than in controls. Additional experiments showed that 90% of the food ingested was still present in the crop 1 h after feeding indicating a regulated transit of food from the crop to the alimentary tract. We conclude that the additional thermogenesis at 23°C was not necessarily a result of true HIF induced by digestion, but may have resulted from a shift in basal metabolic rate or even an increase in facultative thermogenesis (although shivering was not measured at 23°C). Both of these may be triggered by neuroendocrine signals induced by the mere presence of food in the crop.

Supported by the Academy of Finland.

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PC106

Heat shock proteins (Hsp70) in ectothermic toads do not correlate with ambient temperature

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Heat shock proteins (in particular Hsp70) and other components of this system were investigated in the nucleated erythrocytes of toads that are ectothermic and are exposed to a wide range of ambient temperatures. We have studied the heat shock proteins in two species that differ profoundly in their sensitivity to low temperatures, a cold resistant (Bufo viridis) and a cold sensitive (Bufo regularis) toad. Using mammalian anti Hsp70 (Sigma) on Western blot it was found in more than 20 specimens of each species that Hsp70 in red blood cells of the two species is expressed constitutively at high levels in control, room temperature conditions. It was expressed variably and comparable in the two species following thermal stress. The formation of HSF1-HSE binding complex, which is required for the expression of the stress protein, was unconnected to thermal conditions. Although the toads possess the components involved in the cellular stress response, the relationships and regulation of this system seem to be substantially different from the regular response of this system in mammals.

It is hypothesized that the high resting level of Hsp70 in toad erythrocytes provides the basis for the survival in the ambient stress conditions. This hypothesis was tested on blood of two examplary species, an ectothermic lizard (Agama stelio) and an endothermic chicken (Gallus domesticus). In the chicken blood Hsp70 responded directly and reversibly on temperature change, whereas the lizard responded similar to the toads. It is suggested that a cellular strategy that involves a permanently high level of Hsp70 is predominant in vulnerable species, and makes them prepared to unpredictable stress stimuli that might interfere with appropriate cellular functions in the organism.

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

PC106a

The microtrauma caused by the insertion of a microdialysis catheter into human skeletal muscle changes the expected vasodilatation into vasoconstriction during intravenous adrenaline infusion

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In a recent study (Widegren U, Hickner RC, Lindström A, Ungerstedt U, Hjemdahl P, Jorfeldt L & Henriksson J, unpublished observations), we found that i.v. infusion of adrenaline resulted in a significant decrease in the blood flow of human gastrocnemius muscle when blood flow was measured by the microdialysis ethanol technique (Hickner et al. 1992). This contrasted with the significant increase in blood flow, which was detected when blood flow was instead measured by the ^{133}Xe clearance technique or by venous occlusion pletysmography. We hypothesized that the observed discrepancy in the results may be secondary to the more extensive local trauma involved in the microdialysis ethanol technique compared with the ^{133}Xe clearance and the pletysmography methods. In the present study, this hypothesis was tested by using ^{133}Xe clearance to measure blood flow close to an already inserted microdialysis catheter, both in the basal state and during i.v. infusion of adrenaline (0.1 nmol kg^{-1} min^{-1}) or vehicle only. This was done by administering the ^{133}Xe through a fine tube that had been inserted along with the microdialysis catheter. Values (n=8), given as means ± S.E.M., were statistically tested using two-way repeated measures ANOVA with two within factors, treatment (adrenaline - placebo infusion) and effect (basal - infusion values). Although adrenaline caused a significant increase in heart rate, and a widening of the pulse pressure, the blood flow close to the microdialysis catheter was decreased (to an average value of 72.8 ± 5.4% of basal; $p<0.01$). This decrease was significantly larger ($p<0.05$) than the change that occurred during i.v. infusion of vehicle only (to 87.7 ± 2.3% of basal, $p<0.01$). The decrease in the control experiment was possibly due to the cold temperature of the infusion solutions. In conclusion, the vasodilatation in skeletal muscle normally observed in response to intravenously infused adrenaline is converted into vasoconstriction by the influence of an inserted microdialysis catheter. We propose that a small local trauma may change the balance between vasoconstrictive and vasodilatatory effects of adrenergic activation in skeletal muscle.

Hickner RC et al. (1992). Acta Physiol Scand 146, 87-97.

This work was supported in part by grants from the Swedish Research Council (Projects 07917 and 04139), the Swedish Heart Lung Foundation and the Swedish National Center for Research in Sports.

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