

C1

P2X₂ receptor subunit immunoreactive respiratory neurones of the ventral respiratory group in the rat

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The ventrolateral medulla (VLM) functions as a primary central chemoreceptive area, responsible for sensitivity to increases in arterial levels of P_{CO_2} and mediating the ventilatory response to hypercapnia (Loeschcke, 1982). ATP acting via P2X receptors may be involved in mediating changes in the activity of medullary respiratory neurones during hypercapnia, thus playing an important role in central chemoreception (Spyer & Thomas, 2000). The objective of the present study was to determine whether respiratory neurones in the VLM contain P2X₂ and/or P2X₁ receptor subunits.

Experiments were performed in male Sprague-Dawley rats (300–340 g) anaesthetised (pentobarbitone sodium 60 mg kg⁻¹ i.p., then 10 mg kg⁻¹ i.v. as required), injected with gallamine triethiodide (10 mg kg⁻¹ i.v., then 2–4 mg kg⁻¹ h⁻¹ i.v.) and artificially ventilated. All studies were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986. Adequate anaesthesia was ensured by maintaining stable levels of blood pressure, and heart and central respiratory rate. Respiratory neurones located in the area of rostral VLM (stereotaxic co-ordinates: 2.0–2.5 mm rostral to the calamus scriptorius, 1.5–2.0 mm lateral to midline and 2.6–3.0 mm ventral from the dorsal surface of the medulla oblongata) were labelled with Neurobiotin using the juxtacellular method (Pinault, 1996). Animals were transcardially perfused with 4% paraformaldehyde, the brains removed and 50 µm coronal sections of the medulla cut using a vibratome. The filled cells were visualised by incubating the sections in avidin FITC (1:1000, Vector Labs). The sections were then incubated in rabbit polyclonal antibodies raised against either the P2X₂ (1:1000, Dr J.A. Barden, University of Sydney, Australia) or P2X₁ (1:1000; Alomone Labs, Israel) receptor subunits. These were visualised by incubation in a Cy3 conjugated secondary antibody (1:1000, Jackson Immunoresearch) and the sections viewed under a fluorescence microscope.

It was found that (i) out of twelve labelled expiratory neurones, six (50%) were strongly immunoreactive for the P2X₂ receptor subunit; (ii) only two out of seven juxtacellularly labelled pre-inspiratory neurones were immunoreactive for the P2X₂ receptor subunit; (iii) none of the labelled VLM inspiratory neurones ($n = 4$) was detectably immunoreactive for the P2X₂ receptor subunit; (iv) none of the labelled VLM neurones with respiratory-related discharge ($n = 15$) were detectably immunoreactive for the P2X₁ receptor subunit.

This suggests that in the VLM only a relatively small proportion of neurones that display rhythmic respiratory-related activity contain P2X₂ receptor subunit and that it is unlikely that these neurones contain P2X₁ receptor subunit either. P2X₂ receptor subunit immunoreactivity is confined predominantly to the population of the VLM expiratory neurones indicating that extracellular ATP can be an important modulator of their activity.

Loeschcke HH (1982). *J Physiol* **32**, 1–24.Pinault D (1996). *J Neurosci Meth* **65**, 113–136.Spyer KM & Thomas T (2000). *J Auton Nerv Syst* **81**, 228–235.

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All procedures accord with current UK legislation.

C2

Tumour necrosis factor- α inhibits central respiratory output in anaesthetized rats

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The pro-inflammatory cytokine tumour necrosis factor- α (TNF) is produced by astrocytes and microglial macrophages during inflammation and stress (Turnbull & Rivier, 1999). Its role in CNS respiratory control is unknown, although its role in mediating sleep is notable in this context (Krueger, 2001). Recent data suggest that stress attenuates the ventilatory response to hypercapnia (Kinkead *et al.* 2001). Here, the hypothesis that TNF directly inhibits respiratory output has been explored.

Adult Sprague-Dawley rats (270–320 g) were anaesthetized (induction: pentobarbitone 60 mg kg⁻¹ i.p.; maintenance: 30 mg kg⁻¹ h⁻¹ propofol or 100 µg kg h⁻¹ pentobarbitone, i.v. infusion), and mechanically ventilated with neuromuscular blockade (gallamine triethiodide 10 mg kg⁻¹ i.v., then 2–4 mg kg⁻¹ h⁻¹ i.v.). All studies were undertaken in accordance with the UK Animals (Scientific Procedures) Act, 1986. At the end of experiments rats were humanely killed by anaesthetic overdose. Adequate depth of anaesthesia was ensured by maintaining stable levels of blood pressure, heart rate and respiratory output (RO), as recorded from phrenic nerve activity. Homeothermic warming maintained core temperature at 37°C. Artificial cerebrospinal fluid (ACSF) or recombinant rat TNF- α (R&D Systems, USA) were administered either by intracerebroventricular (i.c.v.) injections via a guide cannula placed stereotaxically into the left lateral ventricle ($n = 6$ rats) or by brainstem superfusion ($n = 4$ rats). Intracerebroventricular ACSF had no effect on RO or cardiovascular parameters. 2–15 µl i.c.v. injections of 0.5–100 pg TNF- α produced an $18 \pm 7\%$ fall in RO (mean \pm S.E.M.; $P < 0.05$; Student's paired t test). A 10% fall in peak phrenic activity was seen 32 ± 10 min (mean \pm S.E.M.) after i.c.v. injection. Mean arterial blood pressure declined by 10 ± 2 mmHg from pre-injection levels (mean \pm S.E.M.; $P < 0.05$; Student's paired t test). Bathing the dorsal surface of the brainstem with 10 ng ml⁻¹ TNF- α also inhibited RO in 3/4 rats, with peak phrenic activity reduced by 25% 621 ± 141 s after application. This effect could be reversed by further application of ACSF vehicle.

These data suggest that TNF- α can modulate central respiratory output. During periods of acute major inflammation or stress, CNS expression of TNF may contribute to a reduction in respiratory drive. This may explain the observation that stress results in attenuation of hypercapnic respiratory drive (Kinkead, 2001).

Kinkead R *et al.* (2001). *J Appl Physiol* **90**, 1729–1735.Krueger JM (2001). *Ann N Y Acad Sci* **933**, 211–221.Turnbull AV & Rivier CL (1999). *Physiol Rev* **79**, 1–71.

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All procedures accord with current UK legislation.

C3

Whole-cell patch-clamp recording from neonatal rodent ventral medullary respiratory neurones *in situ*

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The working heart brainstem preparation (WHBP) is a well-established model for investigating integrative and synaptic mechanisms of cardiorespiratory control in the ponto-medullary brainstem of mature and neonatal rats. (Dutschmann & Paton, 2002a). Whilst intracellular recording of ventral medullary respiratory neurones was possible using sharp microelectrodes (Dutschmann & Paton 2002a, b), pharmacological analysis was restricted due to the limited time of stable recordings (~15 min). An alternative approach is whole-cell patch-clamp (w-c-p) which has been performed in the WHBP but from superficial brainstem nuclei (see Paton *et al.* 1999). However, the ventrolateral medullary neurones are out of reach of patch pipettes due to their depth from the dorsal medullary surface. Therefore, in the present study we have developed a new approach to allow the use of w-c-p recording from the ventral medullary respiratory group in the WHBP of neonatal rats.

Neonatal rats of between 3 and 6 days were anaesthetised deeply in a saturated atmosphere of halothane and a WHBP employed (Dutschmann *et al.* 2000). To achieve access to the ventral respiratory group, we exposed the ponto-medullary brainstem laterally by removing the temporal bones of the skull including the bulla. Following this exposure, the ponto-medullary brainstem could be visualised under a dissection microscope. The recording sites were identified by orientating patch pipette tips slightly dorsal (200–400 μm) to the rootlets of the hypoglossal rootlets. Recording success was assisted by stripping off overlying superficial layers of the spinal trigeminal fibre tract/nuclei (300–400 μm), thereby reducing the distance from the medullary surface to the neurones. Patch pipettes (2.5–3 M Ω , tip size 1–2 μm) were filled with the following (mM): 130 potassium gluconate, 10 Hepes, 11 EGTA, 4 NaCl, 2 MgCl₂, 1 CaCl₂, 2 ATP, 0.5 GTP and 5 glucose.

To screen for appropriate co-ordinates, extracellular recordings were performed initially and revealed that ventral respiratory group neurones were located at a depth of 500–800 μm below the lateral surface of the intact brainstem spanning the obex ($n = 20$). Subsequently, w-c-p recordings were made from all major groups of respiratory neurones: inspiratory ($n = 5$), post-inspiratory ($n = 2$), augmenting expiratory ($n = 3$). We achieved 1–2 successful w-c-p recordings per WHBP, which were stable for 20–65 min without obvious changes in resting membrane potential or input resistance.

Thus w-c-p recordings from ventral medullary respiratory neurones can be obtained using a lateral approach in the WHBP. This approach will now permit pharmacological studies to determine cellular mechanisms of respiratory rhythm and pattern generation as well as its modulation by various sensory inputs.

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Dutschmann M & Paton JFR (2002b). *Am J Physiol* **282**, R999–1005.

Dutschmann M *et al.* (2000). *Auto Neurosci* **84**, 19–29.

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C4

The role of NMDA receptors in mediating central and afferent evoked inspiratory off-switch in the respiratory network of rat

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NMDA receptors play a crucial role in mediating central inspiratory off-switch independent of the sensory feedback from pulmonary stretch receptors (PSR). In vagotomised animals, blockade of NMDA receptors either systemically or locally within the dorsal pons, prevents inspiratory termination and causes apnoeic breathing (see St John, 1998). However, apnoeic breathing should be compensated by PSR input providing afferent inspiratory off-switch. In this study we investigated the afferent mediated inspiratory termination before and after NMDA-receptor blockade.

The working heart–brainstem preparation (WHBP, full details in Paton, 1996) of juvenile rat (age 27–30 days) was employed. Animals were deeply anaesthetised in a saturated atmosphere of halothane. To evoke fictive feedback from PSR, we stimulated repetitively the cervical vagal nerve with stimulus trains (200–300 ms, 20 Hz, 0.5–2 mA) applied at 1 Hz for a minute at 2 min intervals over seven trials. Respiratory cycle length (CL) and inspiratory time (T_i), as calculated from phrenic nerve activity, was measured for all seven trials as well as 1 min before and after stimulation. NMDA receptors were blocked systemically with MK-801 (2–3 μM). All data are expressed as means \pm S.E.M., n = number of WHBP.

Vagal stimulation ($n = 5$) decreased baseline CL from 2.64 ± 0.4 to 1.58 ± 0.2 s and T_i (from 0.56 ± 0.4 to 0.33 ± 0.2 s) during the first trial. However, following the 7th trial, repetitive stimulation progressively decreased CL and T_i to 1.2 ± 0.2 and 0.28 ± 0.02 s, respectively. During this trial repetitive stimulation clearly produced a better entrainment of the respiratory rhythm. This might indicate a learning process of plasticity associated with the integration of fictive PSR input.

Systemic application of MK801 ($n = 6$) increased baseline T_i (from 0.8 ± 0.05 to 1.72 ± 0.08 s), indicative of blockade of the centrally mediated inspiratory off-switch mechanism. CL increased from 2.23 ± 0.4 to 8.04 ± 1.2 s. After MK-801, repetitive vagal stimulation ($n = 5$) caused a reduction of T_i (1.64 ± 0.09 to 0.8 ± 0.1 s) and cycle length (6.18 ± 1.3 to 4.6 ± 0.5 s, 1st trial). In contrast to control, a progressive decrease of the CL and T_i following repetitive stimulation was not observed even at the 7th trial (i.e. 7.8 ± 1.9 and 1 ± 0.1 s, respectively).

These data suggest that NMDA receptor-induced apnoeic and decreased respiratory frequency could only be partially compensated for by afferent vagal feedback. Furthermore, the entrainment of respiratory frequency by vagal nerve stimulation is dependent upon NMDA receptors and may reflect a 'learning process'.

Paton JFR (1996). *Neurosci Meth* **65**, 63–68.

St John WM (1998). *Prog Neurobiol* **56**, 97–117.

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C5

Reduced systolic blood pressure and blood glucose in mice overexpressing insulin-like growth factor binding protein-2

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Insulin-like growth factors (IGF) act in concert with insulin in glucose counter-regulation. Recent studies reveal that both hormones also have important vascular actions. A family of binding proteins (IGFBP) modulate IGF bioavailability at the cellular level. IGFBP-2 is abundant in serum and is expressed in the vascular wall; however, a potential role in vascular homeostasis remains unexplored. We overexpressed IGFBP-2 in transgenic mice in order to determine the role of the protein in vascular physiology.

All studies were conducted in accordance with Home Office regulations for animal experimentation. Transgenic mice were generated on a FVB/N background using a human IGFBP-2 cosmid clone. Transgenic mice overexpressing IGFBP-2 and their wild-type littermates were studied between 30 and 40 weeks of age. Glucose and insulin levels were measured in the fasting state and after feeding with standard laboratory diet. Systolic blood pressure was recorded in conscious, restrained mice by tail cuff plethysmography on three occasions. Animals were then humanely killed and thoracic aortic rings were studied *in vitro* for (i) constriction to phenylephrine (PE, 1 nM–10 μ M), (ii) relaxation to acetylcholine (ACh, 1 nM–10 μ M) and sodium nitroprusside (SNP, 0.1 nM–10 μ M), and (iii) maximal constriction to the NO synthase inhibitor L-NMMA (0.1 mM, 30 min). Data were analysed using analysis of variance repeated measures and are expressed as means \pm S.E.M. $P < 0.05$ was considered significant. Results are summarised in Table 1.

Table 1.

	IGFBP-2	Wild-type	P value
Glucose (fasting)(mM)	6.6 \pm 0.2	7.0 \pm 0.2	0.22
Glucose (fed)(mM)	10.6 \pm 0.4	12.9 \pm 0.7	0.006
Insulin (fasting)(μ g l ⁻¹)	0.14 \pm 0.02	0.13 \pm 0.01	0.86
Insulin (fed)(μ g l ⁻¹)	0.76 \pm 0.08	0.75 \pm 0.15	0.14
Systolic blood pressure (mmHg)	124 \pm 2	135 \pm 1	0.00004
E_{\max} PE (g mg ⁻¹)	1.16 \pm 0.14	1.22 \pm 0.10	0.75
E_{\max} ACh (% relaxation)	82 \pm 12	79 \pm 15	0.85
E_{\max} SNP (% relaxation)	137 \pm 12	132 \pm 7	0.72
Response to L-NMMA (0.1 mM) (% of precontracted tension)	167 \pm 12	171 \pm 14	0.88

Data are means \pm S.E.M. $n = 19$ –24 per group for glucose/insulin, $n = 14$ per group for blood pressure and $n = 6$ per group for aortic rings.

Total IGFBP-2 levels were approximately twofold higher in transgenic mice than wild-type as assessed by Western blotting of serum proteins (data not shown). Fasting glucose and insulin levels were similar in transgenic and wild-type mice. Glucose was significantly lower in IGFBP-2 mice after feeding, despite similar insulin levels. Systolic blood pressure was significantly lower in IGFBP-2 mice than in controls. In aortic rings, dose–response curves for constriction to PE and relaxation to ACh and SNP did not differ between groups (maximal responses are shown in Table 1). The contractile response to L-NMMA was similar in IGFBP-2 and wild-type mice.

Thus over-expression of IGFBP-2 in mice is associated with lower blood pressure and blood glucose, despite normal insulin

levels. These data support a potential role for IGFBP-2 in enhancing insulin sensitivity and lowering blood pressure. Further studies are warranted to explore the mechanisms involved.

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All procedures accord with current UK legislation.

C8

Vagal efferent activity during the pulmonary chemoreflex in anaesthetized rats and cats

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In cats the vagal bradycardia of the pulmonary chemoreflex is unusual in that it is not modulated by central respiration or lung inflation (Daly, 1991). It was proposed that mechanisms acting within the brainstem (preganglionic level) and/or at the cardiac ganglia (postganglionic level) may account for this phenomenon (Jones, 2001). To sample a large number of cardiac vagal preganglionic neurones during the pulmonary chemoreflex we used a suction microelectrode technique to record activity in vagal axons in rats and cats.

Fourteen adult male Wistar rats, weighing 233–371 g, were anaesthetised with urethane (1.5 g kg⁻¹ i.p.). Conduction velocity of the spontaneously active units recorded from the cardiac branch was calculated using spike triggered averaging (STA) of electrical activity in the whole ipsilateral vagus. Of the 225 units recorded and averaged, only 33 discernible averages evolved. Seventeen of these STA latencies corresponded to units in the C-fibre range (conduction velocity < 2 m s⁻¹) and 16 units in the B-fibre range (conduction velocity 3–15 m s⁻¹). Phenylbiguanide (PBG, 20 mg kg⁻¹) was injected into the right superior vena cava to elicit a pulmonary chemoreflex. Increased activity (< 2 s latency) was recorded in 37/192 unclassified fibres, 5/16 B-fibres and 3/17 C-fibres. Of the units responding to PBG, three B-fibres and three C-fibres had central respiratory and/or lung inflation-related activity, but this only became obvious when post-stimulus histograms (PSTHs) were constructed. Since the duration of the reflex response was shorter than the time required to acquire the PSTH data, the rat is an unsuitable model to test our hypothesis. We therefore returned to the cat, the species used by Daly (1991).

Two cats (1.8–2.5 kg) were anaesthetized with chloralose (80 mg kg⁻¹ i.v.). The preparation of the cats was similar to that of the rats except recordings were obtained from the cervical vagus. We recorded and tested only units that exhibited expiratory discharge patterns ($n = 10$). In no case was the respiratory rhythm lost during the pulmonary chemoreflex.

In conclusion, the technical approach of axonal recording developed in the rat, also works very successfully in the cat. Further experiments concentrating on the cardiac vagal branch of the cat will provide an excellent test of the hypothesis that the pulmonary chemoreflex loses respiratory modulation during the final integrative action of cardiac vagal ganglia.

Daly M de Burgh (1991). *J Physiol* **439**, 559–577.

Jones JFX (2001). *Exp Physiol* **86**, 807–811.

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C10

Effect of hypothermia on baroreflex control of heart rate and renal sympathetic nerve activity in anaesthetised rats

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The arterial baroreflex (Jordan, 1995) regulates blood pressure (MABP) by changing sympathetic vasomotor tone and heart rate (HR). Acute hypothermia at a core temperature (T_b) of 25°C caused a greater fall in HR than MABP, and decreased the pulsatility of renal sympathetic nerve activity (RSNA) (Sabharwal *et al.* 2001). The present study aimed to determine whether these changes were due to the influence of the hypothalamic thermoregulatory centre on central drive or a direct hypothermic effect on the periphery. This was done by generating baroreflex curves for HR and RSNA at $T_b = 37^\circ\text{C}$, on cooling to $T_b = 25^\circ\text{C}$ and rewarming to 37°C.

Male Wistar rats, 290–320 g, were anaesthetised with fluothane (2.5% in O_2) and α -chloralose/urethane (32/450 mg kg^{-1} i.v.). MABP and HR were measured via a femoral artery. A renal nerve bundle to the left kidney was placed on recording electrodes to measure integrated RSNA (%). Deep oesophageal T_b was regulated by means of a thermostatted plate. Baroreflex curves were generated using bolus doses of phenylephrine (10 μg) and sodium nitroprusside (10 μg) and the responses in HR and RSNA to a change in MABP were recorded and fitted to logistic function curves (Kent *et al.* 1972). Responses were compared between normothermic ($n = 6$) and cold-acclimated ($n = 7$) rats (Sabharwal *et al.* 2002). Rats were killed with an overdose of sodium pentobarbitone. Data (means \pm S.E.M.) were analysed using ANOVA and significance taken at $P < 0.05$.

In both groups of animals, the baroreflex–HR curve was completely suppressed at $T_b = 25^\circ\text{C}$ with a reduction of ~30% ($P < 0.01$) in maximum response from 440 ± 3 b.p.m., ~95% ($P < 0.01$) in response range from 75 ± 10 b.p.m., and ~10% in midpoint pressure from 123 ± 17 mmHg at $T_b = 37^\circ\text{C}$. The reduction in maximum gain at $T_b = 25^\circ\text{C}$ was 96 and 60% in normothermic and cold-acclimated rats from -0.9 ± 0.2 and -1.6 ± 0.7 b.p.m. mmHg^{-1} , respectively ($P < 0.01$). The baroreflex–RSNA curve, with maximum response at $70 \pm 14\%$, response range at $154 \pm 18\%$, and maximum gain at $-2.3 \pm 0.7\%$ mmHg^{-1} at $T_b = 37^\circ\text{C}$, were reduced by 26 and 70% ($P < 0.05$), 12 and 40% ($P < 0.05$) and 13% ($P < 0.05$) and 40% ($P < 0.01$) at $T_b = 25^\circ\text{C}$ in normothermic and cold-acclimated rats, respectively. All parameters returned towards precooling levels on rewarming. The suppression of baroreflex control of sympathetic nerve activity may contribute to the modest hypotension observed during hypothermia, whereas the marked bradycardia may be due to cooling of the sinus node and increased vagal tone. The data suggest that baroreflex control of HR during hypothermia is mediated peripherally rather than centrally.

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Sabharwal R *et al.* (2001). *J Physiol* **531.P**, 216P.

Sabharwal R *et al.* (2002). *J Physiol* **544.P**, 88P.

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C11

Convergence of cardiorespiratory inputs on nucleus tractus solitarius (NTS) neurones at different stages in the baroreceptor reflex pathway in anaesthetized rats

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Cardiorespiratory sensory afferent inputs terminate in the NTS where information is integrated before transmission to neuronal pools controlling autonomic outflow. Some convergence between different cardiorespiratory inputs has been shown in NTS (e.g. Boscan *et al.* 2002). We used stimulation of the aortic depressor nerve (AN) to identify barosensitive neurones in the NTS and examined their convergence with lung inflation inputs.

Sprague-Dawley rats (320–380 g) were anaesthetized (pentobarbital sodium, 60 mg kg^{-1} , i.p.), neuromuscularly blocked (α -bungarotoxin, 140 μg kg^{-1} , i.v.) and artificially ventilated (Jones *et al.* 2002). Anaesthesia and neuromuscular block were monitored and maintained (both i.v.) until humane termination with anaesthetic (i.v.). Putative barosensitive neurones were identified on the basis of their response to ipsilateral AN stimulation. Responses to phenylephrine (PE, 5–10 μg , i.v.) were recorded and correlations of neuronal activity with tracheal pressure (TP) and blood pressure made. Neurones were categorised on the standard deviation of the latency of their response to AN stimulation (Doyle & Andresen, 2001). Group A neurones (presumed to be 2nd order) had s.d. values < 0.5 ms. Group C neurones had s.d. values > 1 ms (presumably higher order). Those with s.d. values 0.5–1 ms were designated Group B. Following identification some neurones were labelled juxtacellularly, visualised and where possible their axons traced (Pinault, 1996; Jones *et al.* 2002).

In total 101 NTS neurones responding to AN stimulation were recorded. 44 were classed as Group A, 19 Group B and 38 Group C. PE excited 19 neurones and inhibited three. Only two were unaffected, indicating that the majority of the neurones were barosensitive. Group A neurones were significantly more active (8.51 ± 2.14 Hz, $n = 23$; mean \pm S.E.M.) than Group C (3.15 ± 0.59 Hz, $n = 22$, $P < 0.05$; Student's unpaired *t* test) with Group B falling between (5.30 ± 2.98 Hz, $n = 8$). Activity in 71% Group A neurones, 63% of Group B neurones but only 27% of Group C neurones were strongly correlated with TP. Cessation of artificial ventilation inhibited firing in 11/12 tests. Pulse related modulation was identified in 71% of Group A, 63% of Group B and 59% of Group C neurones. These populations showed considerable overlap with those synchronised to TP.

Thus convergence of lung inflation with aortic nerve inputs appears to occur most commonly at presumed 2nd order neurones within the NTS.

Boscan P *et al.* (2002). *Exp Physiol* **87**, 259–266.

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All procedures accord with current UK legislation.

C12

Differential modulation of baroreflex autonomic outputs by noxious stimulation: a role for substance P in the nucleus of the solitary tract

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We have reported that noxious pinch attenuates the cardiac baroreflex (Boscan *et al.* 2002). This nociceptive attenuation of the baroreflex bradycardia is blocked by NK-1 receptor antagonists and mimicked by substance P (SP) microinjected to the nucleus of the solitary tract (NTS). Here, we examine the effect of noxious pinch and NTS microinjections of SP on baroreflex sympathoinhibition.

Using a novel variant of the working heart-brainstem preparation (WHBP; Paton, 1996), a decerebrated (under halothane anaesthesia) artificially perfused rat, sympathetic nerve activity was recorded from the lumbar (L2–3) and thoracic chain (T8–9), and the adrenal nerve. In the WHBP, cardiac sympathetic and vagal branches were also recorded. Noxious stimuli were delivered to the paw with a calibrated mechanical pincher. Data are means \pm S.E.M. and Student's paired *t* test was used.

Sympathetic nerve activity showed respiratory modulation, peaking during early post-inspiration. Perfusion pressure ramps demonstrated baroreflex sympathoinhibition in all outflows. Hindlimb pinch evoked increases in sympathetic activity ($212 \pm 32\%$, $n = 6$), accompanied by increased pressure (8 ± 2 mmHg), tachycardia (8 ± 2 b.p.m.) and tachypnoea ($192 \pm 27\%$). Noxious pinch attenuated the cardiac vagal baroreflex gain (-1.71 to -0.74 b.p.m. mmHg $^{-1}$, $P < 0.01$, $n = 6$). In contrast, the baroreflex sympathoinhibition was unaffected (-69 ± 4 vs. $-77 \pm 6\%$).

NTS microinjection of SP (0.5 pmol, 50 nl) produced a reversible inhibition of cardiac baroreflex gain (-1.81 to -0.68 b.p.m. mmHg $^{-1}$, $P < 0.005$, $n = 6$) but no change in the baroreflex sympathoinhibition (-78 ± 4 vs. $-76 \pm 6\%$). Recordings from the inferior cardiac nerve showed a similar lack of effect of SP on baroreflex sympathoinhibition. However, baroreflex-evoked activity on the cardiac vagal nerve was attenuated by SP. No change was seen in the baroreflex sympathoinhibition at doses of SP up to 50 pmol even when microinjected at two rostro-caudally distinct sites bilaterally within the NTS. By comparison microinjection of the GABA_A agonist (isoguvacine, 500 pmol, 50 nl) reversibly attenuated both components of the baroreflex.

These data indicate that noxious pinch and SP selectively modulate the vagal component of the baroreflex by an action within the NTS. Further this implies that there are distinct and differentially regulated baroreflex pathways with outputs to the para- and sympathetic nervous systems within the NTS.

Boscan P *et al.* (2002). *Eur J Neurosci* **16**, 1–17.

Paton JFR (1996). *J Neurosci Meth* **65**, 63–68.

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C13

Nitric oxide and GABA are involved in the angiotensin II-mediated depression of neurones responsive to baroreceptor inputs in the nucleus tractus solitarii (NTS)

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We hypothesised that depression of the cardiac baroreceptor reflex by angiotensin II (ANGII) in the NTS is mediated via activation of nitric oxide synthase (NOS) and subsequent release of GABA (Paton *et al.* 2001). To test this hypothesis, we have recorded from identified baroreceptive NTS neurones, and determined how their barosensitivity is altered by ANGII alone or in the presence of either a NOS inhibitor or GABA_A receptor antagonist.

Experiments were performed in the *in situ* working heart-brainstem preparation of the rat (Paton, 1996). Rats were anaesthetized deeply in a saturated atmosphere of halothane and failed to respond to a noxious pinch of the tail. NTS neurones were recorded using a triple-barrelled microelectrode. The recording barrel was filled with NaCl (2 M) and the remaining barrels with 10 μ M ANGII and either the NOS inhibitor L-NAME (20 mM) or the GABA_A receptor antagonist bicuculline (100 μ M). Drugs were ejected using pressure. Baroreceptive NTS neurones were identified by their firing response to distension of the aortic arch, using a balloon tipped catheter, or inflation of a carotid sinus by injection of Ringer solution via a double lumen cannula that allowed measurement of carotid sinus pressure. Additional NTS neurones were identified that responded to peripheral chemoreceptor activation by aortic injection of sodium cyanide (7–30 μ g). Data are expressed as means \pm S.E.M. Statistical significance was determined using Student's paired *t* test.

Baroreceptor stimulation increased neuronal activity from 3.3 ± 0.9 to 10.8 ± 1.2 Hz ($n = 28$, $P < 0.01$). Of these neurones, ANGII either inhibited baroreceptor activation by $56.9 \pm 6\%$ ($n = 20$) or increased baroreceptor-induced activation ($146.4 \pm 12.9\%$ control; $n = 4$) or produced no change ($96.9 \pm 3.4\%$ control; $n = 4$). L-NAME did not alter the baroreceptor-evoked activation of NTS neurones ($91.7 \pm 7.6\%$; $P = 0.4$), but prevented the ANGII-induced depression ($n = 7$, $P = 0.94$). Similarly, bicuculline prevented the depressant effects of ANGII ($n = 4$; $P = 0.47$). In contrast, AII potentiated the chemoreceptor-evoked firing responses in 9 of 10 neurones ($146.2 \pm 17.4\%$, $P < 0.05$). L-NAME had no effect on the chemoreceptor-evoked response or its potentiation by ANGII. Bicuculline potentiated the chemoreceptor-evoked response, although co-application with ANGII did not further potentiate this response ($n = 2$).

Our results support the hypothesis that in the NTS ANGII-induced depression of the baroreceptor reflex depends on the activation of NOS and release of GABA acting on GABA_A receptors. ANGII-evoked potentiation of chemoreceptive NTS neurones is not dependent on NO synthase.

Paton JFR (1996). *J Neurosci Meth* **65**, 63–68.

Paton JFR *et al.* (2001). *J Physiol* **531**, 445–458.

Australian Heart Foundation and British Heart Foundation funded research.

All procedures accord with current UK legislation.

C14

Role of nitric oxide from endothelial nitric oxide synthase in the nucleus tractus solitarius for arterial pressure control in the spontaneously hypertensive rat

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C15

Effect of progesterone in the rat middle cerebral artery

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The role of progesterone (P_4) as a protector of pregnancy is well established. This action is thought to be the result of inhibition of uterine contraction, probably mediated through inhibition of smooth muscle contraction. However, whether P_4 can inhibit the vascular smooth muscle (VSM) contraction, and the underlying mechanism of the inhibition, are largely unknown.

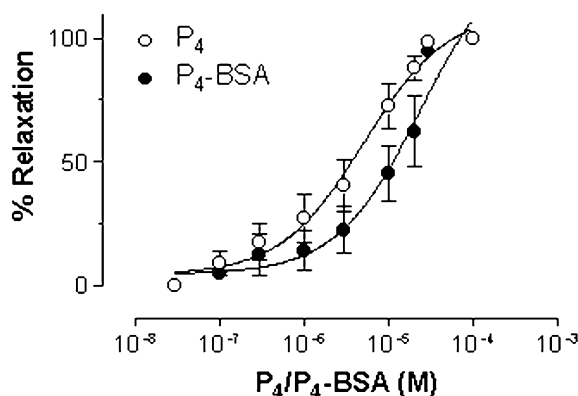
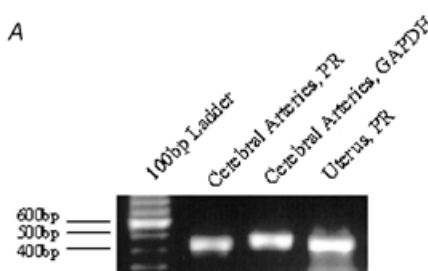


Figure 1. The relaxant effect of P_4 and $P_4\text{-BSA}$ in endothelium-intact rings preconstricted by U46619. Data are means and S.E.M. of 6–7 experiments.

This study was approved by the Animal Research Ethics Committee of the Chinese University of Hong Kong. Female Sprague-Dawley rats were killed by cervical dislocation. Middle cerebral arteries with or without endothelium were used. Isometric tension was measured on rings mounted on small vessel myograph. P_4 induced concentration-dependent relaxations in U46619-preconstricted rings. Removal of endothelium had little effect. P_4 produced less relaxant response in high K^+ -preconstricted rings. This indicates that P_4 produces its relaxant effect partially through inhibition of voltage-gated calcium channels. BSA-conjugated P_4 also induced vasorelaxation, indicating a role of membrane-associated progesterone receptor (PR) in P_4 -induced relaxation. Incubation of RU 486, a classical genomic PR antagonist, shifted the concentration–response curve to the right, showing the possible involvement of classical genomic PR as well. RU 486 and ICI 187780 (a classical oestrogen receptor antagonist) did not affect oestrogen-induced and P_4 -induced relaxation, respectively, confirming the specificity of PR-mediated relaxation. The presence of classical

PR and 25-Dx (a candidate for membrane-associated PR) (Krebs *et al.* 2000) mRNA were detected by RT-PCR. Immunohistochemistry showed the presence of PR on the VSM layer. Confocal fluorescent microscopy study indicated that fluorescein isothiocyanate-BSA- P_4 bound only to the surface membrane of VSM. The present results indicate that P_4 -induced cerebral relaxation may be partly mediated by classical PR and also by membrane-bound PR. Further experiments are needed to characterize the pharmacological action of P_4 in the cerebral arteries.

A



B

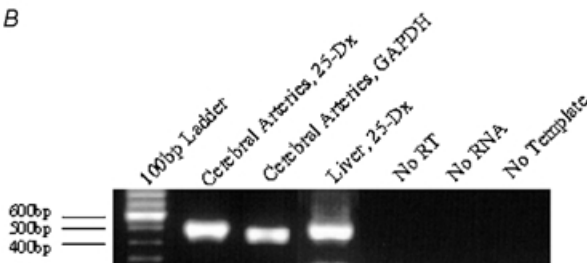


Figure 2. A, RT-PCR of classical PR mRNA in cerebral artery and uterus in female rats. B, RT-PCR of 25-Dx mRNA in cerebral artery and liver. Uterus and liver were used as positive control for the experiment. No signal is shown for controls for RT-PCR (no RT, no RNA and no template).

Krebs CJ *et al.* (2000). *Proc Natl Acad Sci USA* **97**, 12816–12821.

This work was supported by a RGC grant.

All procedures accord with current local guidelines.

C16

Influence of experimental reduction of rat arterial media:lumen ratio on noradrenaline-stimulated contractions

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The objective of this study was to further investigate the relationship between the arterial media thickness:lumen diameter (M:L) ratio and the arterial contractile response to noradrenaline (NA) under isobaric conditions. Femoral resistance arteries from normally perfused hindlimbs of spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats were used as well as those taken from hindlimbs subject to partial ligation of the proximal external iliac artery.

A silk ligature (0.37 mm i.d.) was placed around one external iliac artery of male SHR and WKY rats at 5 weeks of age under

anaesthesia (inhalation of 5 % halothane in air). At 20–24 weeks of age rats were humanely killed by stunning followed by cervical dislocation and branches (~180–250 μm i.d.) of the femoral artery from either the unligatured (Unlig) or ligatured (Lig) hindlimb were dissected post-mortem and mounted onto two glass microcannulae in an arteriograph. Arteries were pressurised to their estimated *in vivo* pressure (SHR Unlig, 116 mmHg; SHR Lig, 64 mmHg; WKY Unlig, 90 mmHg; WKY Lig, 53 mmHg) and bathed in physiological salt solution at 37°C, pH 7.4. Once pressurised, the vessels developed spontaneous myogenic tone and were then challenged with increasing concentrations of NA (1 nM–10 μM). The bathing solution was then replaced with a calcium-free physiological saline solution, in which the vessels relaxed completely and structural measurements were made using a calibrated micrometre eyepiece and light microscopy. Data are presented as means \pm S.E.M. (*n*). Statistical comparisons were made by ANOVA followed by Student's unpaired *t* test modified by the False Discovery Rate test for multiple comparisons.

M:L (%) of SHR Unlig (11.91 ± 0.77 (9)) were greater ($P < 0.01$) than WKY Unlig (8.68 ± 0.73 (10)) and significantly reduced ($P < 0.01$) in arteries distal to the ligature within both rat strains (SHR Lig, 6.10 ± 0.40 (10); WKY Lig, 4.16 ± 0.46 (10)). Maximal contractile responses to NA (μM) were not significantly different between the groups (SHR Unlig 116 ± 9 (9), SHR Lig 133 ± 13 (10), WKY Unlig 129 ± 16 (10), WKY Lig 109 ± 17 (10)). Thus in no comparison did the arteries with greater M:L contract to a greater extent in response to noradrenaline.

These data provide further evidence that the increased M:L in hypertension does not impart an exaggerated contractile function in response to vasoconstrictor agonists.

All procedures carried out on the animals were performed according to Institutional Guidelines and under the Cruelty to Animals Act (1876) as amended by SI 17/94 to comply with the EC Directive 86/609/EEC.

This work was funded by the Health Research Board of Ireland.

All procedures accord with current National and local guidelines.

PC76

Validity and reliability of a commercial metabolic analyser

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The purpose of this study was to examine a commercially available metabolic measuring system, which previously had no published data on its validity and reliability. After obtaining ethical approval and informed consent, 20 healthy subjects (10 males, 10 females, aged 32 ± 10 years, mean \pm S.D.) completed, in random order, a series of three incremental exercise tests to volitional fatigue using the protocol described by Bruce (1973). During two of the tests (MG1 and MG2) expiratory gas analysis was performed repeatedly using the CardiO₂ online metabolic system (Medical Graphics Corp., St Paul, MN, USA) with respiratory gases sampled on a breath-by-breath basis. During the other test (DB), a Douglas bag was used to collect the expirate and an electronic gas analyser (1440C, Servomex Group Ltd, Crowborough, UK) measured gas fractions. This test (DB) served as the criterion method, with gas samples collected throughout the final minute of each stage and through the final minute of exercise (peak exercise). Validity of the system was assessed by comparison of the results from MG1

and DB using a non-parametric paired *t* test (Wilcoxon). Reliability was assessed by comparing the results from MG1 and MG2 using the same statistical procedures. In terms of validity, the results from MG1 and DB ($n = 20$) were significantly different ($P < 0.05$) in both oxygen consumption rate (\dot{V}_{O_2} , Bruce Stage 3: 2.51 vs. 2.23) and carbon dioxide production rate (\dot{V}_{CO_2} , Bruce Stage 3: 2.23 vs. 2.11). Differences between measurements of expired oxygen ($F_{\text{E,O}_2}$) and carbon dioxide fractions ($F_{\text{E,CO}_2}$) were non-significant ($P > 0.05$), whereas expiratory flow (\dot{V}_{E}) measurements were significantly different ($P < 0.05$, Bruce Stage 3: 51.4 vs. 47.9). Pearson product moment correlation between \dot{V}_{O_2} as measured by MG1 vs. DB, at each stage and at peak exercise, was high ($r = 0.80$ – 0.98) but Bland-Altman analysis indicated weak agreement between the two. More importantly, the difference between \dot{V}_{O_2} values as measured by the two methods was that DV was 11% higher, which is well outside the 4% level usually considered acceptable. Reliability analysis ($n = 10$) indicated that the CardiO₂ system provides acceptable reliability ($P > 0.05$) on all measured variables. These data suggest that the CardiO₂ system provides a valid means of assessing $F_{\text{E,O}_2}$ and $F_{\text{E,CO}_2}$ across a range of exercise intensities. Significant error exists, however, in the system's measurement of expiratory flow. Further investigation, and discussion with the manufacturer, has led us to suspect that this is due to a timing error in the system's calibration valve. The manufacturer is currently issuing a software update that should eliminate this error.

Bruce RA *et al.* (1973). *Am Heart J* **85**, 546–562.

All procedures accord with local guidelines and the Declaration of Helsinki.

PC77

Peak cardiac power output: physiological range and relationship to peak oxygen uptake in healthy adults

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Cardiac power output (CPO) is a descriptor of cardiac function, calculated from the product of mean arterial blood pressure and cardiac output. Previous studies (Bain *et al.* 1990) have suggested that maximum cardiac power output (CPO_{peak}) and the ability to increase cardiac power output on stimulation are good descriptors of functional cardiac reserve. The normal range for CPO_{peak} has yet to be determined and the purpose of this study is to establish this in healthy adults. The study also examined the relationship between CPO_{peak} and peak oxygen consumption rate ($\dot{V}_{\text{O}_{2,\text{peak}}}$) in the same population. After obtaining ethical approval and informed consent, 59 healthy adults (31 males and 28 females, means \pm S.D.: age 43 ± 13 years, mass 74 ± 13 kg) completed an incremental exercise test to volitional fatigue using the protocol described by Bruce *et al.* (1973). $\dot{V}_{\text{O}_{2,\text{peak}}}$ was assessed on a breath-by-breath basis by online expiratory gas analysis (CardiO₂, Medical Graphics Corp., St Paul, MN, USA). Having established each subject's $\dot{V}_{\text{O}_{2,\text{peak}}}$ and following a 40 min recovery period, cardiac output was measured at peak exercise (Q_{cpeak}) using the non-invasive CO₂ rebreathing method described by Defares (1958). During this second procedure, subjects attained a mean oxygen consumption rate of $101 \pm 7\%$ of the $\dot{V}_{\text{O}_{2,\text{peak}}}$ achieved in the previous test. CPO_{peak} in Watts (W), was then computed using the equation described by Cooke *et al.* (1998). Mean \pm S.D. values for the variables investigated were: $\dot{V}_{\text{O}_{2,\text{peak}}} = 2.47 \pm 0.7 \text{ l min}^{-1}$, $\text{Q}_{\text{Tpeak}} = 18.3 \pm 4.5 \text{ l min}^{-1}$, and $\text{CPO}_{\text{peak}} = 4.7 \pm 1.3 \text{ W}$. The relationship between $\dot{V}_{\text{O}_{2,\text{peak}}}$ and CPO_{peak} was assessed by the Pearson product moment correlation

coefficient. Analysis revealed a strong ($r = 0.91$, $P < 0.01$) correlation between the two variables. CPO_{peak} ranged from 3.80 to 7.94 W in men and 2.53 to 5.57 W in women. Although the sample size remains moderate, the cardiac power output values attained were normally distributed and these values provide a useful indication of the normal range for CPO_{peak} in healthy adults.

Bain RJ *et al.* (1990). *Eur J Appl Phys* **61**, 112–118.

Bruce RA *et al.* (1973). *Am Heart J* **85**, 546–562.

Cooke GA *et al.* (1998). *Heart* **79**, 289–294.

Defares JG (1958). *J Appl Physiol* **13**, 159–164.

All procedures accord with current local guidelines and the Declaration of Helsinki.

PC78

Medullary control of cutaneous vasoconstrictor activity during a fever-like state in the anaesthetised rat

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PC79

Regional differences in age-related changes in the density of the sympathetic nerve supply of arterial vessels of the rat

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The effects of ageing on the sympathetic nerve supply of arterial vessels are unclear. For example, in the rabbit, a decrease in the density of the nerve supply of the carotid and femoral arteries occurred between 6 weeks and 6 months and between 6 months and 3 years, whereas that of the basilar artery was well maintained over this period (Cowan *et al.* 1982). By contrast, in Wistar rats, sympathetic nerve density of the basilar and internal carotid arteries reached a peak at 4 weeks and decreased from 8 weeks to 27 months of age (Mione *et al.* 1988). We have compared sympathetic nerve densities of the middle cerebral, basilar, femoral and caudal ventral arteries (MCA, BA, FA and CVA, respectively) in male Wistar rats of 4, 8 and 40 weeks ($n = 8, 7$ and 7 , respectively).

Arteries were taken after the rats had been killed by cervical dislocation according to UK legislation. They were prepared as whole mount stretch preparations by using the glyoxylic acid method for demonstrating noradrenergic nerves (Cowan *et al.* 1982). Photomicrographs were taken with epi-illumination and standard filters for fluorescence and analysed quantitatively using image analysis. Nerve densities were assessed as fluorescent area, expressed as percentage surface area of vessel, and as surface density, expressed as number of nerve fibre intercepts mm^{-1} on a squared grid placed on the vessel image. The results obtained with the two methods were similar.

For MCA, nerve density increased from 0.274 ± 0.012 at 4 weeks to 0.355 ± 0.027 at 8 weeks and then fell to 0.272 ± 0.053 intercepts mm^{-1} at 40 weeks (means \pm S.E.M.; *, ***, $P < 0.05$, 0.0001, respectively, 4 or 40 weeks vs. 8 weeks, ANOVA with

Fisher's *post-hoc* test). A similar pattern was observed in BA. By contrast, in CVA, nerve density increased from $0.324 \pm 0.021^*$ at 4 weeks to 0.390 ± 0.023 at 8 weeks and increased further to $0.432 \pm 0.051^*$ intercepts mm^{-1} at 40 weeks. Moreover, in FA, nerve density was unchanged between 4 and 8 weeks (0.192 ± 0.011 and 0.184 ± 0.016 intercepts mm^{-1} , respectively), but increased substantially between 8 and 40 weeks (to $0.372 \pm 0.030^{***}$).

These results suggest that there are regional differences in the effects of ageing on the density of the sympathetic nerve supply to arterial vessels, with cerebral vessels showing a decrease in nerve density from adult to older age and peripheral arteries supplying muscle and cutaneous circulations showing an increase in nerve density with age.

Cowan T *et al.* (1982). *J Auton Nerv Syst* **5**, 317–336.

Mione MC *et al.* (1988). *Brain Res* **460**, 103–113.

All procedures accord with current UK legislation.

PC80

Does endothelin (ET) contribute to the muscle vasodilator response evoked by acute systemic hypoxia in the anaesthetised rat?

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ET has been implicated in hypoxic pulmonary vasoconstriction. It is not clear whether it is involved in the systemic vascular response to acute hypoxia.

Experiments were performed on rats anaesthetised with a continuous infusion of Saffan ($7\text{--}12 \text{ mg kg}^{-1} \text{ h}^{-1}$ i.v.) in accordance with the HO Animals (Scientific Procedures) Act, 1986. At the end of the experiment the animal was killed with an overdose of anaesthetic. In Group 1 ($n = 4$), ET infused at 1 nmol kg^{-1} i.v. over 5 min evoked a gradual increase in arterial pressure (ABP) and increase, followed by a decrease in femoral vascular conductance (FVC: femoral blood flow divided by ABP). One hour later when baselines had stabilised, the ET receptor antagonist PD 145065, which is non-selective between ET_A and ET_B receptors, was given at 300 ng kg^{-1} , a dose which reduced the increase in ABP induced in the rat by chronic intermittent hypoxia (Kanagy *et al.* 2001). This dose had no effect on baseline ABP or FVC, but reduced the initial decrease in FVC evoked by subsequent infusion of ET at 1 nmol kg^{-1} , from 1.06 ± 0.17 conductance units (CU, D in integrated FVC) (mean \pm S.E.M.) to $0.19 \pm 0.21^*$, $P < 0.05$, Student's paired *t* test). In Group 2 ($n = 8$), systemic hypoxia (breathing 8% O_2 for 5 min) evoked an increase in FVC (4.13 ± 0.64 CU): this response was reduced when retested after PD 145065 (300 ng kg^{-1}) to $2.96 \pm 0.42^*$ CU).

These results contrasted with those obtained in Group 3. In these rats ($n = 4$), the nitric oxide (NO) synthesis inhibitor (L-NAME, 10 mg kg^{-1} i.v.) increased baseline ABP and decreased baseline FVC and the increase in FVC evoked by 8% O_2 was reduced from 5.11 ± 0.99 to $1.93 \pm 0.49^*$ CU, showing that the muscle vasodilator response is NO dependent (Skinner & Marshall, 1996). However, when PD 145065 (300 ng kg^{-1}) was given in the presence of L-NAME, there was no effect on baseline values of ABP or FVC, but the increase in FVC evoked by 8% O_2 was accentuated (to $2.52 \pm 0.50^*$ CU).

These results indicate that exogenous ET can exert both vasodilator and vasoconstrictor influences on hindlimb skeletal muscle. The effects of the ET receptor antagonist PD 145065 suggest that endogenously released ET predominantly exerts a vasodilator influence on skeletal muscle during acute systemic hypoxia, but that when the synthesis of NO is blocked, then a vasoconstrictor influence of ET is revealed.

Kanagy NL *et al.* (2001). *Hypertension* **37**, 511–515.

Skinner MR & Marshall JM (1996). *J Physiol* **495**, 553–560.

All procedures accord with current UK legislation.

PC81

Central nervous system site of ATP action on body temperature during fever in rats

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P2 purinoreceptors are present in hypothalamic and brainstem nuclei that are involved in the regulation of body temperature (T_b) and development of fever (Kanjhan *et al.* 1999). Recently, using the intracerebroventricular (i.c.v.) injections of ATP analogues and P2 receptor antagonists we have shown that ATP acting on certain P2 receptors may play an important role in thermoregulation (Gourine *et al.* 2002). However, the site of ATP action in relation to regulation of T_b has not been investigated.

Experiments were performed in adult male Wistar rats (280–350 g) and were approved by the Institutional Animal Care and Use Committee (in Minsk). Rats were anaesthetised (ketamine 87.0 mg kg⁻¹ + xylazine 13.0 mg kg⁻¹), a steel guide cannula was implanted into the third cerebral ventricle or anterior hypothalamus (AH), and a telemetry transmitter was implanted into the abdomen for monitoring of T_b . After a 7 day recovery period, fever was induced by intraperitoneal injection of *E. coli* lipopolysaccharide (LPS; 50 µg kg⁻¹). Effects of i.c.v. and intrahypothalamic administration of the ATP analogue α,β -methyleneATP (α,β -meATP, 0.2 µmol) or artificial cerebrospinal fluid (ACSF) on T_b during fever were determined. The rat was humanely killed by overdose of anaesthetic at the end of the experiment. In addition, activity of the hypothalamic thermosensitive neurones was recorded in brain slices (400 µm) containing AH. Firstly, the thermosensitivity of the recorded neurones was determined by changing the bath temperature and relating the firing rate of the neurone to the slice temperature. Then, the effect of ATP on the activity of these cells was determined.

It was found that following i.p. injection of LPS, fever reached a maximal T_b (around 39°C) 2.5 h after injection. α,β -MeATP injected i.c.v. or into the AH at the peak of fever caused a profound decrease in febrile T_b . Ten minutes after i.c.v. injection of α,β -meATP, T_b of febrile rats decreased to 37.84 ± 0.19°C, some 0.9°C lower than that of the T_b of febrile rats 10 min after i.c.v. treatment with ACSF (38.74 ± 0.19°C, mean ± S.E.M., $P < 0.05$, Student's unpaired *t* test). The decrease in T_b of febrile rats induced by intrahypothalamic injection of α,β -meATP developed more slowly and lasted longer compared with the response evoked by this ATP analogue injected into the third cerebral ventricle. Forty minutes after injection of α,β -meATP into the AH, T_b of febrile rats decreased to 37.43 ± 0.38°C,

mean ± S.E.M., some 1.0°C lower than that of the T_b of febrile rats 40 min after intrahypothalamic administration of ACSF (38.42 ± 0.10°C, mean ± S.E.M., $P < 0.05$, Student's unpaired *t* test). It was also found that application of ATP increases the firing rate of 60 % of warm-sensitive neurones in the AH *in vitro*.

These data indicate that a population of warm-sensitive neurones in the AH is likely to be the site of action of ATP on T_b during fever, supporting our previous hypothesis that extracellular ATP acting on P2X receptors may be involved in the mechanisms of fever development, limiting the magnitude of the febrile response.

Gourine AV *et al.* (2002). *Br J Pharmacol* **135**, 2047–2055.

Kanjhan R *et al.* (1999). *J Comp Neurol* **407**, 11–32.

This work was supported by The Wellcome Trust.

All procedures accord with current local guidelines.

PC82

Stochastic Ca²⁺-dependent beat-to-beat fluctuations of the non-linear pacemaker potential component determine the chronotropic state of sinoatrial nodal cells

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Local Ca²⁺ releases during the diastolic depolarization (CRDD) in sinoatrial nodal cells (SANC) activate Na⁺/Ca²⁺ exchange current and modulate the membrane potential. Since CRDD is a stochastic process we hypothesized that it produces spontaneous, beat-to-beat pacemaker potential fluctuations that characterize the SANC chronotropic state.

All procedures accorded with local guidelines. Rabbits were humanely killed with an overdose of anaesthetic and isolated SANCS loaded with fluo-3 AM were studied using confocal microscopy combined with a perforated patch-clamp technique. Pipettes were filled with (mM): 120 potassium gluconate, 20 KCl, 5 NaCl, 5 Hepes and 5 MgATP (pH 7.2, 34 °C).

The diastolic depolarization (DD), i.e. spontaneous depolarization between action potentials (AP) measured by whole-cell current clamp, displays two components: an initial linear one followed by a non-linear one that extends to the next AP upstroke. DD fluctuations, extracted by subtracting the DD averaged over several beats (bold curve in Fig. 1) from the DD of a given beat increased with time following a prior AP, achieving a maximum at 20–60 ms before the subsequent AP upstroke (Fig. 2). The evolution of CRDD occurrence, measured by confocal Ca²⁺ imaging, exhibited a similar time course, indicating a close link between CRDDs and DD fluctuations (Fig. 2). Strong positive correlations were observed among the changes in amplitudes of DD fluctuations, the average non-linear DD component amplitude, and beating rate in response interventions that alter CRDD magnitude (Bogdanov *et al.* 2001; Vinogradova *et al.* 2002). Ryanodine receptor blockade, intracellular Ca²⁺ chelation (BAPTA-AM), inhibition of sarcolemmal L-type Ca²⁺ channels (nifedipine), or β -adrenergic receptor stimulation (isoprenaline) that produced a 3-fold range of beating rates (70–200 b.p.m.), paralleled by a 3-fold variation of the average amplitudes of DD fluctuations (0.5–1.5 mV). In contrast, these perturbations did not significantly affect the initial, linear DD slope.

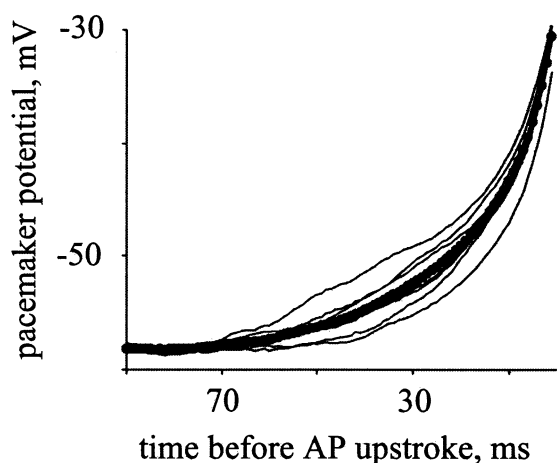


Figure 1. Superimposition of seven individual pacemaker potentials and their mean (bold).

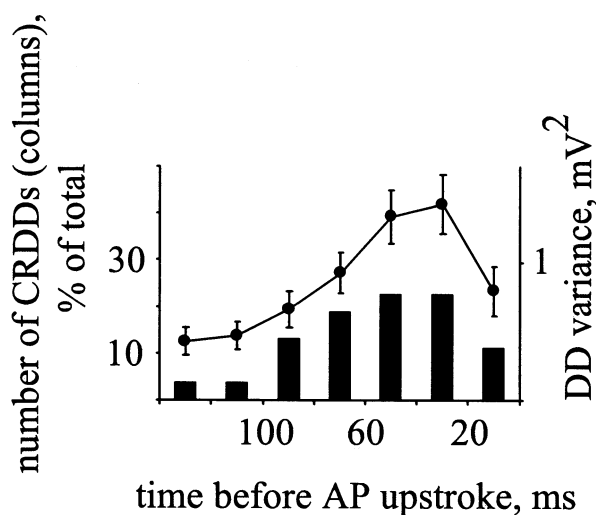


Figure 2. Mean time course of CRDD occurrence and DD variance ($n = 31$). Means \pm S.E.M.

These observations support the idea that variations in CRDD magnitude produce fluctuations in the later, non-linear part of the DD, which modulate its amplitude and thus the time at which the subsequent AP fires. Thus spontaneous Ca^{2+} -dependent DD fluctuations modulate the SANC chronotropic state.

Bogdanov KY *et al.* (2001). *Circ Res* **88**, 1254–1258.

Vinogradova TM *et al.* (2002). *Circ Res* **90**, 73–79.

All procedures accord with current National and local guidelines.

PC83

Identification of medullary raphe sites involved in control of vagal drive to the heart in anaesthetised rats

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Nuclei containing cardiac vagal preganglionic neurones (CVPNs), the nucleus ambiguus (nA) and dorsal vagal nucleus, are densely innervated by 5-HT immunoreactive terminals, some of which make synaptic contact with CVPNs (Izzo *et al.* 1993). Reflex activation of CVPNs can be attenuated by antagonising central 5-HT_{1A} receptors in the nA (Wang & Ramage, 2001). However, the location of the 5-HT-containing cell bodies involved in this control is unknown. Since 5-HT-containing neurones projecting to the nA are located in raphe magnus, obscurus and pallidus (Haxhiu *et al.* 1993), the present experiments have been carried out to identify sites within the medullary raphe that may be involved in modulating cardiac vagal drive.

Male Sprague-Dawley rats (300–400 g) were anaesthetised with isoflurane (induction) and α -chloralose (80 mg kg⁻¹ i.v.; maintenance), neuromuscularly blocked (α -bungarotoxin 150 μ g kg⁻¹ i.v.; Jones *et al.* 2002), atenolol pretreated (1 mg kg⁻¹ i.v. for cardiac sympathoadrenal blockade), and instrumented for measurement of BP, HR, integrated phrenic (PNA) and integrated renal sympathetic nerve activity (RNA). Cardiovascular variables were used to monitor the depth of anaesthesia. Distinct areas of the medullary raphe were stimulated using a 3-barrel glass micropipette containing 50 mM DL-homocysteic acid (DLH), pontamine sky blue, and either 5 mM DLH, saline, or Wood's metal for electrical stimulation. At the end of experiments, animals were killed by an overdose of pentobarbitone.

Electrical stimulation (10–50 Hz, 0.2 ms, 50–100 μ A, 10 s) produced variable results. Chemical stimulation (2.5 nmol DLH in 50 nl) of the raphe 0.5 mm rostral to obex evoked significant (Student's paired *t* test; $P < 0.05$) changes in baseline PNA ($+116 \pm 21\%$; mean \pm S.E.M.) and RNA ($-45 \pm 8\%$; $n = 6$). At 1.5 mm rostral, RNA reduction was smaller ($-29 \pm 8\%$; $n = 7$). At 2.5 mm rostral (raphe magnus/pallidus), DLH evoked significant changes in HR (-42 ± 6 beats min⁻¹), PNA ($-60 \pm 6\%$) and RNA ($+46 \pm 6\%$; $n = 7$). These DLH effects were dose dependent and lateral (0.5–1 mm) microinjection evoked much smaller responses. Saline microinjection had no effect. The 5-HT_{1/2/7} receptor antagonist methiothepin (3 mg kg⁻¹ i.v.) had no significant effect on the evoked bradycardia ($n = 4$).

The present data are consistent with the hypothesis that the caudal raphe have an excitatory action on CVPNs.

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D.O.K. is a BHF PhD student.

All procedures accord with current UK legislation.

PC84

Reduced L-S-nitrosocysteine vasodilatation after induction of tachyphylaxis to peroxynitrite in anaesthetized rats

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S-nitrosothiols such as the putative endothelium-derived relaxing factor, L-S-nitrosocysteine, possess biological activity that is independent of their decomposition to nitric oxide (NO). Peroxynitrite is a powerful oxidant of protein and non-protein sulphhydryls and readily nitrates free and protein-associated tyrosine residues. Systemic injections of peroxynitrite elicit pronounced vasodilator responses in anaesthetized rats, which are subject to tachyphylaxis (Benkusky *et al.* 1998; Graves *et al.* 1998). Interestingly, the haemodynamic responses elicited by a variety of G protein-coupled receptor agonists are diminished after induction of tachyphylaxis to peroxynitrite. We have provided evidence that L-S-nitrosocysteine recognition sites may contain cysteine residues that are subject to oxidation (i.e. disulphide-bond formation) (Hoque *et al.* 1999, 2000). Accordingly, the aim of this study was to determine whether the vasodilator actions of L-S-nitrosocysteine are modified after induction of tachyphylaxis to peroxynitrite.

This study determined whether induction of tachyphylaxis to peroxynitrite (induced by giving ten i.v. injections of a 10 mmol kg⁻¹ dose) alters the haemodynamic actions of L-S-nitrosocysteine (12.5–100 nmol kg⁻¹, i.v.), in pentobarbitone-anaesthetized rats. Animals were humanely killed at the end of each experiment. L-S-nitrosocysteine elicited dose-dependent reductions in mean arterial blood pressure and in hindquarter and mesenteric vascular resistances. These responses were substantially attenuated after administration of peroxynitrite. For example, maximum fall in blood pressure to L-S-nitrosocysteine changed from $-40 \pm 5\%$ (control) to $-13 \pm 3\%$ (peroxynitrite treated; $P < 0.05$) and maximum fall in hindquarter resistance changed from $-55 \pm 7\%$ (control) versus $19 \pm 3\%$ (peroxynitrite treated; $P < 0.05$, *t* test, $n = 6$). We have previously reported that the hypotensive and, and vasodilator actions of the NO donor (Z)-1-(N-methyl-N-(6(N-methylammoniohexyl)amino))diazene-1-ium-1,2-diolate (MAHMA NONOate) are not attenuated by induction of tachyphylaxis to peroxynitrite (Benkusky *et al.* 1998).

This study demonstrates that peroxynitrite diminishes L-S-nitrosocysteine-induced hypotension and vasodilatation although peroxynitrite does not impair NO-mediated hypotension and vasodilatation. These data support the concept that peroxynitrite reduces the vasodilator actions of L-S-nitrosocysteine via oxidation and/or nitration of S-nitrosothiol recognition sites.

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All procedures accord with current National guidelines.

PC85

Hypoxic pulmonary vasoconstriction in conscious rats: lack of effects of endothelin-1 receptor blockade

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PC86

The necessary role of transforming growth factor β in aspirin-mediated antiproliferative effect

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Vascular smooth muscle cell (VSMC) proliferation seems to be an important factor in the development of atherosclerosis, and acetylsalicylic acid (ASA) has been demonstrated to stop this proliferation (Kodama *et al.* 2000). On the other hand the pleiotropic cytokine transforming growth factor β (TGF- β) shows similar properties (Blobe *et al.* 2000). Nevertheless its transcription is increased in high-proliferating cells (Satoh *et al.* 2001).

In order to assess how ASA affects TGF- β function we performed a primary cell culture of VSMC extracted from the thoracic aorta of a rat which was killed humanely with an overdose of pentobarbitone and decapitation. We studied cell proliferation in serum-free medium (PDGF-BB was added as a mitogenic stimulator). Statistical analysis was performed using Student's unpaired *t* test. Data are expressed as means \pm S.E.M. and $P < 0.05$ was considered significant. ASA inhibited cell proliferation in a dose-dependant manner at 0.5, 1 and 2 mM (78.87 ± 0.0215 , 41.7 ± 0.0103 and $36.3 \pm 0.0041\%$, respectively). No cytotoxicity was observed at these concentrations (LDH increases were not significant). Addition of 50 $\mu\text{g ml}^{-1}$ of monoclonal anti-TGF- β 1 to 2 mM of ASA reversed this inhibition by 33.25 %, which proves ASA-mediated antiproliferative effect involves the molecule TGF- β .

In Northern blot experiments we found a decrease of TGF- β 1 transcription at a dose of 2 mM ASA. In our ELISA measurements of TGF- β 1 in conditioned medium we did not find a significant increase in the treated group (48 h of incubation with 2 mM ASA in a serum-free medium) compared with the control group.

Our data suggest an important role of TGF- β in ASA-mediated antiproliferative effect on VSMC.

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All procedures accord with current National guidelines.

PC88

smad7 modulates heme oxygenase-1 induction in human vascular smooth muscle cells

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Increased levels of reactive oxygen species (ROS) in the vessel wall contribute to atherogenesis and restenosis (Ross, 1999). The stress protein heme oxygenase-1 (HO-1) is induced by ROS and catabolises heme to generate the antioxidant biliverdin and vasodilator carbon monoxide, which protects against oxidative injury (Siow *et al.* 1999). Transforming growth factor- β 1 (TGF- β 1) promotes migratory and proliferative responses in smooth muscle cells (SMC) during vascular remodelling and can induce HO-1 expression (Kutty *et al.* 1994). We have investigated whether oxidative stress agents hydrogen peroxide, generated by glucose oxidase (GO), or diethylmaleate (DEM), an electrophilic agent that depletes glutathione, can induce TGF- β 1 generation and HO-1 expression in SMC, and studied the effects of adenoviral overexpression of smad7, an endogenous inhibitor of TGF- β 1 signalling (Nakao *et al.* 1997).

Human aortic SMC obtained with local ethics approval were cultured from explants and transfected with adenoviruses co-ordinating expression of either smad7 or β -galactosidase as a control. Cells were then treated for 24 h with GO (10 mU ml⁻¹) or DEM (100 μ M). TGF- β 1 production by SMC was determined by an enzyme-linked immunosorbant assay of the conditioned culture medium and HO-1, smad7 and phosphorylated smad2 protein expression by Western blot analysis.

Expression of HO-1 was markedly induced in SMC treated with the stress agents, concomitant with a significant ($P < 0.01$, Student's unpaired t test) increase in medium TGF- β 1 levels ($n = 4$, mean \pm S.E.M., pg ml⁻¹) from 120 ± 10 in control cells to 375 ± 18 and 280 ± 15 in DEM and GO-treated cells, respectively. Adenoviral overexpression of smad7, but not β -galactosidase, attenuated DEM or GO-mediated HO-1 induction, but did not alter TGF- β 1 generation by SMC. Inhibition of TGF- β 1 signalling in cells by smad7 overexpression was confirmed by a decrease in smad2 phosphorylation induced by recombinant TGF- β 1. We have demonstrated for the first time that TGF- β 1 signalling can modulate HO-1 induction by ROS, thus providing further insights into mechanisms involved in SMC dysfunction in vascular diseases.

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All procedures accord with current local guidelines.

PC89

'Optrode' measurement of fluorescence in living rodent brain: application for detection of intracellular calcium signalling and *in vivo* gene transfer

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Fluorescent indicators have become useful tools for the study of intracellular function. However, conventional imaging techniques are usually limited to *in vitro* preparations. We have developed a fibre optic probe or 'optrode' to record real-time changes in fluorescence corresponding to intracellular processes within the intact central nervous system.

The optrode consists of two 125 μ m optical fibres placed side by side along with a tungsten microelectrode for simultaneous extracellular recording. 488 nm laser light was launched down one fibre, whilst the other fibre collected the resulting green fluorescence. This was long-pass filtered to remove residual blue light and focused onto the face of a photo-multiplier tube for detection.

Three experiments have been performed. First, cellular activity was recorded from brainstem slices ($n = 4$) loaded with 15 μ M Oregon Green-BAPTA-1AM (OG-I), a cell-permeant calcium-sensitive dye. Increases in fluorescence F ($\Delta F/F = 250\%$, S.E.M. = 34.4%) were observed from the ventrolateral medulla during perfusion with a high potassium medium (30 mM) to depolarise cells. Second, we extended this study to examine calcium transients in respiratory modulated hypoglossal motoneurons in the *in situ* working heart-brainstem preparation of rat (WHBP; Paton, 1996). Following local microinjection of OG-I (20 μ l at 0.4 μ l min⁻¹), recordings show transient changes in fluorescence that correlate with mass extracellular activity and phrenic nerve discharge ($n = 2$). Third, the ability to monitor changes in gene expression in the brains of freely moving animals should be useful in linking neuronal gene function to physiological parameters and behaviour. The hippocampi of rats were microinjected with a replication-deficient adenovirus expressing enhanced GFP under control of the tetracycline system (tet-off; see Harding *et al.* 1998). Using this system eGFP expression can be switched off and on by administration and withdrawal of doxycycline to drinking water, respectively. In anaesthetised *in vivo* rats (53.33 mg kg⁻¹ ketamine + 0.33 mg kg⁻¹ medetomidine), eGFP expression was detected in the hippocampi of animals without dox treatment *vs.* undetected in dox treated ($n = 2$). Animals were humanely killed with an overdose of pentobarbitone.

These preliminary data support the use and further refinement of a single optical fibre probe for the detection of fluorescence from discrete regions of intact brain.

Harding TC *et al.* (1998). *Nat Biotech* **16**, 553–555.

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PC90

Carotid baroreflex responsiveness to head-up tilt induced central hypovolaemia: effect of aerobic fitness

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The purpose of this investigation was to examine the interaction between carotid baroreflex (CBR) responsiveness and maximal aerobic fitness during head-up tilt (HUT)-induced central hypovolaemia. Eight averagely fit (AF) men with a group mean bicycle ergometer maximal oxygen uptake ($V_{O_{2peak}}$) of 50 ± 1.0 ml O_2 kg^{-1} min^{-1} (mean \pm S.E.M., range 45.4–55) and eight highly fit (HF) men with a $V_{O_{2peak}}$ of 62 ± 1.0 ml O_2 kg^{-1} min^{-1} (range 57.2–65.9) volunteered as subjects in the investigation. All procedures were approved by the review board of the Fredricksberg Municipality. After a 30 min period of rest in the supine position each subject was tilted to a 30 deg HUT position for 5 min and then tilted further to 60 deg HUT for another 5 min. During the final 5 min of the supine rest and each position of HUT, transthoracic impedance (TI) and CBR responsiveness, using a rapid pulse (500 ms) train of neck pressure (NP) and neck suction (NS) ranging from +40 to –80 Torr, were measured. Throughout the experiment heart rate (HR) and directly measured brachial mean arterial blood pressure (MAP) were recorded on a beat-to-beat basis using a customized data acquisition system. CBR responsiveness of HR (G_{max} -HR) and MAP (G_{max} -MAP) were obtained by fitting the HR and MAP responses to the NP/NS stimuli to a logistic function curve. Statistical analysis of comparisons between HF and AF during the HUT conditions were performed using two-factor ANOVA with repeated measures across HUT conditions. During HUT the G_{max} -HR of the AF subjects measured ($P < 0.08$), while the G_{max} -HR of the HF subjects was unchanged. The G_{max} -MAP of the AF subjects increased ($P < 0.03$) during HUT, although the G_{max} -MAP of the HF subjects did not increase. Regression analysis identified a significant relationship between G_{max} -HR and G_{max} -MAP with Δ TI in the AF subjects. These relationships were not significant in the HF subjects (Δ TI, 30–60 deg, AF = 1.8 ± 0.8 Ω , $P < 0.05$; HF = 1.2 ± 0.5 Ω , $P > 0.05$). These data indicate that unloading of the cardiopulmonary (CP) baroreceptors by HUT increased the CBR responsiveness of the AF subjects because of a greater unloading of the CP baroreceptors. The CBR responsiveness of the HF subjects was unchanged from that observed in the supine position as a result of an insignificant decrease in central blood volume during the tilt.

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All procedures accord with current local guidelines and the Declaration of Helsinki.