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## Symptomatic vCJD alters heart rate variability

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*Ante-mortem* diagnosis of variant Creutzfeld Jacob disease (vCJD) is based on the subjective assessment of clinical signs, sometimes combined with invasive biopsy for the presence of infectious prion protein (PrPsc) in the tonsils. *Post mortem* diagnosis of transmissible spongiform encephalopathies in animals routinely depends on the microscopic immuno-histochemical identification of PrPsc in the medulla oblongata of the brainstem (Wells *et al.* 1989), and in particular the nucleus tractus solitarii (NTS) and the dorsal vagal nucleus (DVN), the vagus nerve being a suspected route of infection in some species (Beekes *et al.* 1998). It has already been suggested that symptomatic bovine spongiform encephalopathy is associated with disturbance in heart rate variability (HRV) (Pomfrett & Austin, 1997), possibly occurring as a result of functional changes in NTS and DVN in the presence of PrPsc, and we sought to determine whether this is also the case in humans exhibiting symptoms of vCJD.

Data were collected in accordance with a protocol approved by the North West Multi Centre Research Ethics Committee, including written, informed consent. Three-hundred-second samples of electrocardiogram (ECG) were collected at repeated intervals during a 3-month period from two subjects exhibiting definite clinical signs of vCJD, and who had also been confirmed as carrying PrPsc by tonsil biopsy. Control data were collected from seven healthy volunteers of comparable age not taking medication and with no relevant medical history. The ECG was digitised using a portable monitor (Fathom, Amtec Medical) at 1 kHz frequency and 12 bit resolution. Data were transferred to a PC, translated and analysed using standard software (CED Spike2 v4.02). The ECG waveform was reviewed by eye and artefacts rejected. Tachygrams of instantaneous ECG R-wave frequency were obtained in order to determine power spectra. Interval histograms of the R–R wave intervals were also plotted (see Fig.1). Non-parametric statistics were applied (SPSS v10.1).

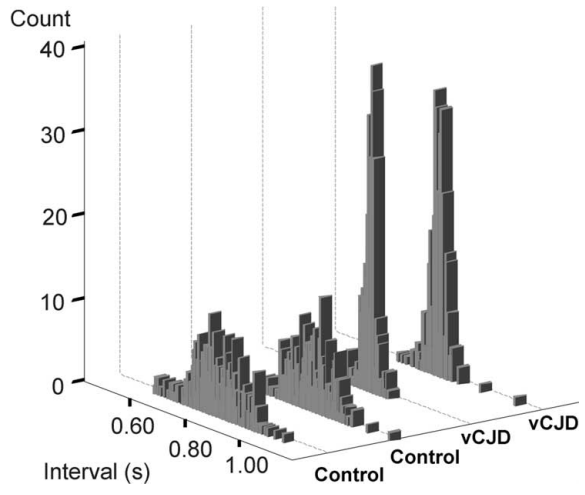


Figure 1. Comparative ECG R–R interval histograms from four subjects.

We observed that symptomatic vCJD disturbed HRV. There was a significant difference in the variances of ECG R wave intervals between controls and vCJD suspects (Kruskal-Wallis $H$ test, $P < 0.001$). Frequency histograms obtained from controls described normal distributions (Kolmogorov-Smirnov test, $P < 0.05$) whereas some frequency histograms of vCJD suspects demonstrated high levels of kurtosis, with significantly greater numbers of ECG R wave intervals in a narrow band between 0.9 and 0.98 s (Kruskal-Wallis test, $P < 0.05$). There was no significant difference between the mean heart rates of the two groups. Power spectral analysis revealed a significant increase in low frequency HRV (0–0.05 Hz) between the vCJD suspects and controls (Mann-Whitney $U$ test, $P < 0.05$).

Further work is needed with a much larger sample size. However, this study allows us to suggest that measurement of HRV has potential as a non-invasive aid to the diagnosis of vCJD.

Beekes M *et al.* (1998). *J Gen Virol* **79**, 601–607.

Pomfrett CJD & Austin AR (1997). *J Physiol* **501.P**, 69*P*.

Wells GA *et al.* (1989). *Vet Rec* **125**, 521–524.

This study was funded by the Department of Health.

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

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## Heart rate and haemodynamic changes differ following voluntary and electrically evoked exercise with maintained chemoreflex activation in humans

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Following a period of isometric exercise, heart rate (HR) falls more rapidly when circulation to the previously exercising muscle is occluded (PECO) as compared to recovery with open circulation (Coote & Bothams, 2001). This may reflect increased baroreflex stimulation of cardiac vagal tone motoneurones when blood pressure is raised above baseline by PECO, at a time when vagal inhibition by the muscle mechanoreceptor (MM) and/or central command (CC) is removed by cessation of voluntary exercise. To separate the roles of MM and CC in this off transient, well established methods were employed (Bull *et al.* 1989) to examine HR changes immediately following 2 min of exercise with PECO. Exercise was either electrically evoked (STIM) and voluntary (VOL) isometric contraction of the human triceps surae performed at 30% of maximal voluntary force.

All protocols were performed according to local ethics committee guidelines. Thirteen healthy subjects of mean age 24.1 ± 1.4 years (11 males) gave informed written consent for participation. HR (ECG) and blood pressure (BP) (Finapres) were continuously monitored throughout the protocol. Haemodynamic indices were measured using impedance cardiography (Minnesota Impedance Cardiograph, Model 304B). All values are presented as 15 s ensemble average changes from rest ± S.E.M. Statistical analysis was performed using two-way ANOVA ($P < 0.05$) and *post hoc* Student's paired $t$ test with Bonferroni correction for multiple comparisons.

During exercise the heart rate changes in STIM and VOL were not significantly different. However over the first 15 s of PECO, the change in HR from rest was significantly different between the VOL (2.9 ± 1.25 beats min$^{-1}$) and STIM (−3.7 ± 1.29 beats min$^{-1}$) conditions ($P < 0.01$). Also the change from rest in pre-ejection period (PEP), an index of the inotropic state of the heart, was significantly greater in VOL (−12 ± 1.4 ms) than

STIM (−5 ± 1.1 ms). BP was not different between conditions during PECO.

Lower HR and longer PEP over the 15 s following stimulated exercise are compatible with the idea that cessation of MM activation allows a greater recovery of cardiac vagal tone (Lewis *et al.* 2001) than occurs on cessation of both MM and the CC associated with VOL (Coote & Bothams, 2001).

Bull RK *et al.* (1989). *J Physiol* **411**, 63–70.

Coote JH & Bothams VF (2001). *Exp Physiol* **86**, 811–815.

Lewis ME *et al.* (2001). *J Physiol* **534**, 547–552.

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

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## Modulation of the autonomic control of the kidney by leptin acting in the brain of anaesthetised rats

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A complex neural network exists in the hypothalamus determining food intake and thermogenesis to ensure energy balance and to maintain body fat levels. A number of neuromodulators are involved which control the degree of sympathetic nervous activity to various organs, including the kidney, to ensure metabolic rate is optimised against food intake. The afferent limb of this complex reflex is leptin, which is a peptide hormone released from white adipocytes that activates receptors in the hypothalamus. Central administration of leptin has been shown to cause a slowly developing stimulation of the sympathetic nervous system. The question addressed in this study was whether leptin within the brain might modify cardiovascular reflex regulation of sympathetic outflow. To this end, cardiopulmonary receptors were stimulated and the responses in renal sympathetic nerve activity (RSNA) and nerve-dependent function evaluated normally and following I.C.V. leptin administration.

Male Wistar rats, 310 ± 7 g, were anaesthetised (32/450 mg $kg^{-1}$ chloralose/urethane, I.V.). Blood pressure (BP) and heart rate (HR) were monitored from a femoral artery and saline (150 mM NaCl) was infused via a femoral vein at 3 ml $h^{-1}$. A cannula was placed into the right cerebroventricle to infuse saline or leptin (5 $\mu$g bolus plus 5, 10 or 20 $\mu$g at 7.7 $\mu$l $h^{-1}$). The left kidney was exposed via a flank incision and either the renal sympathetic nerves were isolated and sealed on to recording electrodes or the right and left ureters were cannulated, the left kidney was denervated and a flow probe was placed on its artery. Inulin was infused to allow measurement of glomerular filtration rate (GFR). After a 2 h recovery period, 15 min clearances were taken, two before and two during either saline or leptin I.C.V. infusion and two after 20 min of phenylbiguanide (PBG) infusion I.V. at 32 $\mu$g $min^{-1}$ to stimulate the cardiopulmonary receptors. The animals were killed with an overdose of anaesthetic. Data are means ± S.E.M. and were tested using ANOVA and Student's paired *t* test with significance at $P < 0.05$.

Infusion of saline I.C.V. ($n = 6$) had no effect on any variable, but leptin (5+20 $\mu$g $h^{-1}$) I.C.V. ($n = 6$) increased RSNA by 11% to 11.0 ± 2.6 $\mu$V $s^{-1}$ ($P < 0.05$) without change in BP, 99 ± 2 mmHg, or HR, 6.6 ± 0.4 Hz. When PBG was given I.V. with saline I.C.V., BP was unchanged, HR increased by 15% ($P < 0.05$) while RSNA decreased by 50% ($P < 0.05$), but PBG infusion with leptin I.C.V. decreased RSNA by only 18% ($P < 0.05$), less than that with saline I.C.V. ($P < 0.05$). In the functional studies, during saline I.C.V. ($n = 9$) PBG had no effect on fractional sodium excretion ($FE_{Na}$) in the denervated (DNX) kidneys at 1.51 ± 0.36%, but it was increased some 3-fold in the innervated (INN) kidney, from 0.54 ± 0.14 to 1.70 ± 0.52 ($P < 0.05$). During infusion of leptin I.C.V. ($n = 9$, 5+20 $\mu$g $h^{-1}$), PBG had no effect on $FE_{Na}$ in the DNX kidney, at 1.46 ± 0.3% or in the INN kidney, at 0.77 ± 0.23%. A similar blockade of response in $FE_{Na}$ was obtained when the two lower doses of leptin were given I.C.V.

These findings show that activation of cardiopulmonary receptors depressed RNSA causing a renal nerve-dependent natriuresis and diuresis but that this response could be blocked by leptin acting in the brain. The elevated leptin levels in obesity could contribute to the associated hypertension by preventing normal regulation of cardiovascular reflexes.

*All procedures accord with current UK legislation.*

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## GluR1 localisation and responses to 5-fluorowillardiine in sympathetic preganglionic neurones of rat thoracic spinal cord

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AMPA receptors are composed of GluR1–4 subunits that form homomers or heteromeric complexes of any two of these four subunits. The pharmacology of these receptors is dependent on the subunit composition and one agonist, 5-fluorowillardiine (5-F-Will) has a much higher affinity for GluR1 ($K_i$ 14.7 nM) and 2 ($K_i$ 25.1 nM) than GluR4 (305 nM). Here we sought to determine whether the GluR1 subunit is localised and functional in sympathetic regions of the spinal cord.

We first used immunohistochemistry to determine whether GluR1 receptor subunits were located in the spinal cord. Male Wistar rats (200 g) were injected intraperitoneally with Fluorogold (0.1 ml of 1% in 0.9% NaCl, Fluorochrome Int.) 5–7 days prior to being humanely killed by anaesthetizing with sodium pentobarbitone (60 mg $kg^{-1}$, I.P.) and perfusing transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer. Vibrating microtome sections (50 $\mu$m) of upper thoracic spinal cord (T2–T5) were incubated in rabbit anti-GluR1 (1:150, Chemicon) for 24 h prior to visualisation with Cy3-conjugated donkey anti-rabbit (1:800, Jackson Immunoresearch). GluR1 immunoreactivity was detected in approximately 50% of Fluorogold-labelled sympathetic preganglionic neurones (SPNs) in the intermediolateral cell column (IML) while fluorogold-negative neurones in the IML and lamina X were also immunopositive.

Using visualised whole cell patch clamp techniques the responses of identified SPNs in the IML to different concentrations of 5-F-Will were determined. Rats (12–15 days) were anaesthetized with urethane (2 g $kg^{-1}$, I.P.) and after removal of the upper thoracic spinal cord were humanely killed by decapitation. Slices (250 $\mu$m) were superfused with oxygenated aCSF (composition (mM): NaCl (124); $NaHCO_3$ (26); KCl (3); $MgSO_4$ (2); $NaH_2PO_4$ (2.5); $CaCl_2$ (2); glucose (10)) at room temperature. Eight of 21 neurones depolarised to bath applied 15–30 nM 5-F-Will (13.8 ± 2.2 mV; mean ± S.E.M.) indicating that these neurones contained GluR1/2 subunits. The remaining 13 SPNs were unaffected by this concentration but were depolarised by 300 nM 5-F-Will (20.2 ± 2.5 mV) consistent with the presence of GluR4 but not the GluR1/2 subunits. Since parallel investigations indicate that GluR2-immunoreactive SPNs are located more caudally and the proportion of SPNs responding to 15–30 nM 5-

F-Will corresponds with the percentage of GluR1-immunopositive SPNs, the current data suggest that GluR1 subunits are indeed present and functional in SPNs of the upper thoracic spinal cord.

We acknowledge the support of the British Heart Foundation.

*All procedures accord with current UK legislation.*

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## Evidence for the existence of GABAergic and glutamatergic neurones within the nucleus intermedius with synaptic connections to the nucleus of the solitary tract in rat

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In an unpublished study we noted that injections of retrograde tracers into the nucleus of the solitary tract (NTS) in the medulla oblongata resulted in labelled neurones in the nucleus intermedius (InM; T.F.C. Batten, personal communication). Here we utilise *in situ* hybridisation and electrophysiological studies to investigate the connections from the InM to the NTS.

For *in situ* hybridisation, rats ($n = 5$, 150 g) were humanely killed by anaesthetising with sodium pentobarbitone (60 mg kg$^{-1}$, I.P.) followed by transcardial perfusion with 4 % paraformaldehyde in 0.1 M phosphate buffer. Brainstem slices (30 $\mu$m) were hybridised with digoxigenin-UTP-labelled GAD65, GAD67 and VGlut2 sense and antisense RNA probes using a modified version of the manufacturer's protocol (www.biochem.roche.com). mRNA for GAD65, GAD67 or VGlut2 was visualised in a number of regions in the medulla oblongata but neurones within the InM were notably mRNA positive for both inhibitory and excitatory markers. For electrophysiology, male Wistar rats (15–21 days) were terminally anaesthetized with sodium pentobarbitone (120 mg kg$^{-1}$, I.P.) and 300 $\mu$m coronal slices of medulla oblongata prepared. Whole cell patch clamp recordings were made from 15 neurones located within dorsal and dorsomedial ($n = 12$) or medial ($n = 3$) regions of the NTS, at room temperature. These NTS neurones had an action potential amplitude of 61.3 ± 1.66 mV (mean ± S.E.M.), an action potential duration of 4.8 ± 0.35 ms and an AHP amplitude of 15.7 ± 1.7 mV.

Electrical stimulation of the InM, using bipolar stimulating electrodes (voltage 9–18 V, duration 100 $\mu$s), elicited excitatory and/or inhibitory synaptic potentials in 12/15 NTS neurones. EPSPs had an amplitude (AMP) of 6.01 ± 0.5 mV, width at half-amplitude (HW) of 52.1 ± 5.12 ms and 10–90 % rise time (RT) of 0.73 ± 0.09 mV ms$^{-1}$ ($n = 10$), and were blocked by 1 mM kynurenic acid indicating that they were mediated by excitatory amino acids. IPSPs (AMP = 6.01 ± 0.41 mV, HW = 51.3 ± 1.88 ms, RT = 0.53 ± 0.04 mV ms$^{-1}$($n = 9$)) in seven NTS neurones were GABAergic as they were blocked by bicuculline (10 $\mu$M) while the remaining two were mediated by both GABA and glycine as they were abolished by co-application of bicuculline (10 $\mu$M) and strychnine (2 $\mu$M). Further to this, recordings were also made from InM neurones where stimulation of the NTS resulted in antidromic action potentials in these neurones ($n = 2/2$). The function and the pharmacology of these excitatory and inhibitory synaptic pathways are currently being investigated.

The University of Leeds and The Wellcome Trust supported this work

*All procedures accord with current UK legislation.*

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## Simultaneous optical and electrical recording from respiratory-rhythmic neurones *in situ* using micro-optoelectrodes

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Detection of intracellular calcium transients in mammalian neurones is restricted to *in vitro* slice preparations or dissociated cells. In the present study we attempted to record calcium transients from neurones within an intact brainstem. We have studied respiratory-rhythmic neurons using a calcium-sensitive dye in conjunction with a micro-optoelectrode. This approach was designed to allow simultaneous recording of extracellular electrical activity and changes in fluorescence corresponding to transient changes in intracellular calcium ion composition.

Lengths of optical fibre (outer diameter 125 $\mu$m, core diameter 62.5 $\mu$m) were flame-pulled to a taper; the tips were then broken back to achieve core diameters ranging from 5 to 10 $\mu$m. This end of the fibre was placed into a saline (2 M NaCl) filled glass microelectrode (0.5 MΩ) along with a silver wire, and sealed with dental wax. The other end of the fibre led to a photomultiplier tube for signal detection. The calcium-sensitive dye Fluo-4-AM was infused into the hypoglossal motor nucleus (5–15 $\mu$l, 50–200 $\mu$M at 0.2 $\mu$l min$^{-1}$), which is inspiratory modulated, of the working heart–brainstem preparation (Paton, 1996). This *in situ*, unanaesthetized, arterially perfused, decerebrate rat preparation was established under halothane anaesthesia which was withdrawn when decerebration produced insentience. (Animals were killed humanely.) Phrenic nerve discharge was monitored continuously as an output of central respiratory drive. Excitatory 488 nm laser light was guided into a 600 $\mu$m optical fibre and positioned on the surface of the medulla for illumination of the dye-loaded region. The micro-optoelectrode was then tracked down through the area using a stepper motor.

Transient changes in fluorescence in phase with phrenic nerve discharge and correlated to mass extracellular electrical activity from inspiratory-modulated hypoglossal motoneurones were observed ($n = 10$). These remained stable for hours. No transient fluorescent changes were detected either above or below the hypoglossal motor nucleus where respiratory activity was not detected electrically. During elevations in central respiratory drive following stimulation of peripheral chemoreceptors (arterial injection of sodium cyanide, 50 $\mu$l, 0.03 %), the frequency of these transients increased; there was also a rise in baseline fluorescence ($n = 44$). Overdosing with sodium pentobarbitone (600 mg kg$^{-1}$) abolished both the respiratory-related optical and electrical signals ($n = 6$).

This technique is being applied to investigate the relative roles of transmembrane *versus* intracellularly released calcium transients during different functional output states of the respiratory network.

Paton JFR (1996). *J Neurosci Meth* **65**, 63–69.

P.M.J.B. is funded by a UOB Scholarship. Royal Society and British Heart Foundation funded research.

*All procedures accord with current UK legislation.*

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## Baroreflex control of heart rate following autonomic blockade during acute hypothermia

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We have shown that baroreflex control of renal sympathetic nerve activity (RSNA) was slightly reduced during acute hypothermia, but by contrast baroreflex control of heart rate (HR) was completely suppressed (Sabharwal *et al.* 2003). The aim of this study was to investigate the extent of sympathetic and vagal contributions towards baroreflex control of HR during acute hypothermia. This was done by generating baroreflex curves for HR at deep oesophageal core temperature ($T_b$) of 37 °C and on cooling to 25 °C in the presence of atropine (muscarinic cholinergic blocker) or propranolol ($\beta$-adrenergic blocker).

Male Wistar rats, 290–320 g, were anaesthetised with fluothane (2.5 % in $O_2$) and $\alpha$-chloralose/urethane (32/450 mg kg$^{-1}$ I.V.). Mean arterial blood pressure (MABP) and HR were measured via a femoral artery. $T_b$ was regulated by means of a thermostatted plate connected to a temperature control unit. HR baroreflex curves were generated using bolus doses of phenylephrine (10 $\mu$g) and sodium nitroprusside (10 $\mu$g) and the responses in HR to a change in MABP were recorded and fitted to logistic function curves (Kent *et al.* 1972). Responses were compared among control ($n = 6$) and animals treated I.V. with atropine methylnitrate (2 mg kg$^{-1}$; $n = 5$) or propranolol (1 mg kg$^{-1}$; $n = 7$). Rats were killed with an overdose of sodium pentabarbitone. Data (means ± S.E.M.) were analysed using ANOVA and significance taken at $P < 0.05$.

Basal levels of HR at $T_b = 37$ °C were 4 % higher ($P < 0.01$) and 18 % lower ($P < 0.001$) in rats treated with atropine and propranolol, respectively, compared with control (424 ± 1 beats min$^{-1}$). MABP was 4 % lower ($P < 0.01$) in rats given propranolol compared with control (101 ± 1 mmHg) at $T_b = 37$ °C. In the presence of atropine, baroreflex–HR parameters (Kent *et al.* 1972) were: $A_1 = 27 \pm 14$ beats min$^{-1}$, $A_2 = 0.06 \pm 0.04$ mmHg$^{-1}$, $A_3 = 85 \pm 1$ mmHg and $A_4 = 442 \pm 9$ beats min$^{-1}$ at $T_b = 37$ °C, while in the presence of propranolol $A_1 = 46 \pm 6$ beats min$^{-1}$, $A_2 = 0.08 \pm 0.18$ mmHg$^{-1}$, $A_3 = 106 \pm 6$ mmHg and $A_4 = 334 \pm 16$ beats min$^{-1}$ at $T_b = 37$ °C. Atropine depressed the maximal gain by ~50 % ($P < 0.05$) compared to control values (Sabharwal *et al.* 2003) whereas it was unchanged with propranolol at $T_b = 37$ °C. Hypothermia decreased MABP (~10 %, $P < 0.01$) and HR (~35 %, $P < 0.001$) in all groups. Baroreflex–HR curves were markedly attenuated, in all groups of animals, at $T_b = 25$ °C with a decrease in maximal gain by ~85 % *vs.* control at $T_b = 37$ °C (−0.93 ± 0.2 beats min$^{-1}$ mmHg$^{-1}$; $P < 0.001$).

These results suggest that reduced levels of both sympathetic and vagal components contribute to the impairment of baroreflex control of HR during acute hypothermia.

Kent BB *et al.* (1972). *Cardiology* **57**, 295–310.

Sabharwal R *et al.* (2003). *J Physiol* **547.P**, C10.

*All procedures accord with current UK legislation.*

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## Arterial chemoreceptors in the superior laryngeal nerve of the rat

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McDonald & Blewett (1981) have argued that in the rat, peripheral arterial chemoreceptor tissue is diffusely distributed along the main trunks and branches of the IXth and Xth cranial nerves. We have confirmed that the superior laryngeal nerve (SLN) of the rat contains abundant paraganglia. The question arises as to whether these SLN paraganglia are oxygen sensitive.

Five male Wistar rats (224–260 g in weight) were killed humanely by a sharp blow to the head followed by transection of the spinal cord (according to local animal ethics committee guidelines). The right SLN was quickly dissected and placed in a chamber containing Hepes-buffered Tyrode solution at pH 7.4 (Sigma T2145). The chamber had a capacity of 2 ml and was water-jacketed to maintain temperature at 35°C. The bath contained two glass microelectrodes; one for single axon recording and the other for pressure micro-ejection of sodium cyanide (NaCN; 250–500 ng in 5 $\mu$l) in the proximity of glomus tissue at the bifurcation of the SLN. A small WPI probe monitored $P_{O_2}$ continuously. Two reservoirs were used to superfuse the preparation (5 ml min$^{-1}$) and were bubbled with oxygen or nitrogen in order to alter the bath $P_{O_2}$. It was found that this preparation was very robust, probably due to the small size of the glomi in the nerve. Single chemoreceptor units could be held for hours and as many as 15 NaCN excitatory responses made without deterioration of the preparation. It proved to be difficult, however, to lower the $P_{O_2}$ below 90 mmHg because of the gas-permeable tubing used in the experimental rig. Therefore, we tried to demonstrate oxygen sensitivity of NaCN excitation. The duration of the NaCN response, and the number of spikes in the 30 s after micro-ejection of cyanide, were compared during normoxia and hyperoxia using the Wilcoxon signed rank test in five animals. The criterion for statistical significance was $P < 0.05$. All values are means ± S.D.

Hyperoxia significantly shortened the response duration from 169 ± 52.9 s to 62.6 ± 46.2 s ($P = 0.03$) and reduced the number of spikes (30 s after application of NaCN) from 231 ± 173 to 123 ± 92. This latter reduction, however, was not statistically significant ($P = 0.3$). A linear regression was calculated for the data points of $P_{O_2}$ and response duration and the correlation was found to be strong, negative and significant ($R = -0.82$; $P = 0.004$).

In conclusion, we now have convincing morphological and electrophysiological data for the presence of arterial chemoreceptors in the SLN of the rat.

McDonald DM & Blewett RW (1981). *J Neurocytology* **10**, 607–643.

We wish to acknowledge support of University College Dublin.

*All procedures accord with current local guidelines.*

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## Role of central 5-$HT_7$ as well as 5-$HT_{1A}$ receptors in cardiopulmonary reflex control in anaesthetised rats

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Central 5-hydroxytryptamine-containing neurones, *via* activation of 5-$HT_{1A}$ receptors, control parasympathetic outflow to the heart, airways (see Ramage, 2001) and bladder (Conley *et al.* 2001). Furthermore, blockade of central 5-$HT_7$ receptors can inhibit the micturition reflex (Read *et al.*, 2003). The present experiments compare the roles of central 5-$HT_7$ and 5-$HT_{1A}$ receptors on the cardiopulmonary reflex, using the selective 5-$HT_7$ receptor antagonist SB-269970 (Hagan *et al.* 2000) and the selective 5-$HT_{1A}$ receptor antagonist WAY-100635.

Male Sprague-Dawley rats (300–350 g) were anaesthetised with $\alpha$-chloralose (80 mg $kg^{-1}$ I.V. and maintained with 15 mg $kg^{-1}$ when necessary), atenolol pretreated (1 mg $kg^{-1}$ I.V.), neuromuscularly blocked ($\alpha$-bungarotoxin 150 $\mu$g $kg^{-1}$ I.V.; Jones *et al.* 2002), mechanically ventilated, and instrumented to record BP, phrenic (PNA) and renal nerve activity (RNA), and ECG (R–R interval). Depth of anaesthesia was assessed by the stability of BP, HR and PNA following a noxious stimulus. Cardiopulmonary afferents were stimulated by right atrial injection of phenylbiguanide (PBG; 3–15 $\mu$g $kg^{-1}$). After three control reflexes, SB-269970 (30, 100, or 300 $\mu$g $kg^{-1}$), WAY-100635 (100 $\mu$g $kg^{-1}$), or saline was given intracisternally (10 $\mu$l over 20 s), and the reflex repeated after 5, 15, 25 and 35 min (Table 1). At the end of experiments, animals were killed by an overdose of pentobarbitone.

| | Control | 5min | 15min | 25min | 35min |
|---|---|---|---|---|---|
| SB-269970 30 μg kg$^{-1}$ | 43±7 | 27±3* | 24±3* | 24±6 | 36±6 |
| SB-269970 100 μg kg$^{-1}$ | 41±7 | 23±7* | 12±3** | 17±4** | 23±4 |
| SB-269970 300 μg kg$^{-1}$ | 52±11 | 7±2** | 4±2** | 5±3** | 8±3** |
| WAY-100635 100 μg kg$^{-1}$ | 48±7 | 20±3* | 30±4 | 43±3 | -- |
| Saline control pH 5.8 | 41±4 | 40±4 | 39±3 | 39±3 | 37±3 |

Table 1. Effect of intracisternal SB-269970 and WAY-100635 on changes in R-R interval (ms; mean ± S.E.M.) evoked by intra-atrial PBG. * $P < 0.05$, ** $P < 0.001$, 2-way ANOVA followed by least significant difference test.

Neither drug significantly altered baseline BP, R-R interval or RNA, but after 15 min SB-269970 (300 $\mu$g $kg^{-1}$) significantly inhibited PNA by 76 ± 12 %, and the reflex decrease in RNA by 97 ± 2 % (means ± S.E.M.).

These data demonstrate that both central 5-$HT_7$ and 5-$HT_{1A}$ receptors are activated during the cardiopulmonary reflex to cause an increase in cardiac vagal outflow in anaesthetised rats.

Conley RK *et al.* (2001). *Br J Pharmacol* **133**, 61–72.

Hagan JJ *et al.* (2000). *Br J Pharmacol* **130**, 539–548.

Jones GA *et al.* (2002). *Auton Neurosci* **98**, 12–16.

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

Read KE *et al.* (2003). *Br J Pharmacol* **138**, 4*P*.

D.O.K. is a BHF PhD student. We are grateful to GSK (UK) for the gift of SB-269970.

*All procedures accord with current UK legislation.*

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## TRH modulates electrotonic coupling between rat hypoglossal motoneurones *in vitro*

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Thyrotropin-releasing hormone (TRH) projections to hypoglossal motoneurones originate from the raphe pallidus and obscurus (Lynn *et al.* 1991). TRH-evoked excitatory responses in these motoneurones are the combination of both pre- and postsynaptic actions: a direct membrane depolarisation (Bayliss *et al.* 1992; Bouryi & Lewis, 2002) and an increased release of glutamate from the terminals of caudal raphe projections (Bouryi & Lewis, 2002). Here we provide evidence of an additional action of TRH on hypoglossal motoneurones, the opening of gap junctions between adjacent neurones and hence electrotonic coupling.

Male Wistar rats (12–14 days) were terminally anaesthetised with sodium pentobarbitone (120 mg $kg^{-1}$, I.P.) and 300 $\mu$m coronal slices of the appropriate region of the medulla oblongata prepared (Bouryi & Lewis, 2001). Whole cell voltage clamp recordings were made from visually identified hypoglossal motoneurones within the ventral region of the nucleus, with pharmacological agents being applied in the superfusate as required.

Superfusion of TRH (10 $\mu$M) resulted in the induction of oscillatory activity or spikelets, characterised as a fast inward current followed by a slower outward current in 28 hypoglossal motoneurones. These spikelets were not observed at rest or when the neurones were depolarised by increased extracellular $K^+$ (10 mM, $n = 19$). They remained during the co-application ($n = 12$) of 2,3-dioxo-6-nitro-1,2,2,3-tetrahydrobenzo[f]-quinxaline-7-sulphonamide disodium (NBQX, 20 $\mu$M), strychnine (4 $\mu$M) and bicuculline (10 $\mu$M) demonstrating that they were not synaptic potentials. They were, however, abolished by the gap junction blocker, carbenoxolone (200 $\mu$M, $n = 6$). With a superfusate containing high $K^+$, NBQX, strychnine and bicuculline, inclusion of GTP-$\gamma$-S (100 $\mu$M) in the patch pipette resulted in the gradual appearance of spikelets following attainment of the whole cell configuration. These GTP-$\gamma$-S-evoked spikelets were abolished when $K^+$ levels were reduced to 3.1 mM, reappearing when $K^+$ was returned to 10 mM.

These data suggest that TRH, as a result of the modulation of G-protein-mediated pathways within hypoglossal motoneurones, can open gap junctions between adjacent motoneurones. The resultant electrotonic coupling would facilitate the co-ordinated activation of distinct sub-populations of hypoglossal motoneurones.

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Bouryi VA & Lewis DI (2001). *Neuroreport* **12**, 1709–1713.

Bouryi VA & Lewis DI (2002). *J Physiol* **544.P**, 30*P*.

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The support of The Wellcome Trust is gratefully acknowledged.

*All procedures accord with current UK legislation.*

C57

## Vasomotor properties of rat coronary and accessory arteries

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The determination of the direct effects of autonomic control of coronary resistance arteries is complicated by concomitant changes in myocardial metabolism and extravascular compression. In the rat, cardiac muscle extends into the cranial venae cavae and receives its blood supply from an accessory circulation provided by branches of the subclavian arteries. This circulation is a potential experimental surrogate for the coronary circulation because the vessels are not subject to myocardial compression and are more accessible. We studied the responses of coronary and accessory arteries to autonomic vasomotor stimuli by the application of agonists to isolated arterial segments *in vitro*.

Male Wistar rats (331 ± 33 g, $n = 13$) were killed humanely by stunning followed by cervical dislocation in accordance with institutional guidelines. The heart and superior vena cava were rapidly excised and placed in ice-cold physiological salt solution (PSS). A branch from the septal coronary artery or a segment of the accessory artery was dissected free and mounted onto two glass microcannulae in an arteriograph. The segment was pressurized to 80 mmHg and bathed in PSS at 37°C, pH 7.4 and permitted to generate spontaneous myogenic tone. Internal diameter was continuously recorded using a video imaging system. Concentration–response curves (1 nM to 10 mM) to noradrenaline (NA), acetylcholine (ACh) and phenylephrine (PE) were determined in random order. Responses were taken as peak changes in internal diameter (mm) from the myogenic baseline. Fully relaxed internal diameters in calcium-free PSS were then determined. All values are expressed as means ± S.D. ($n$ = number of animals).

Relaxed internal diameters of the coronary and accessory arteries were 229 ± 73 (5) and 150 ± 23 mm (8), respectively. After generation of myogenic tone, arteries achieved baseline diameters approximately 50 % of the fully relaxed diameter. ACh effected a dilator response in all coronary arteries (93 ± 48 mm (5)) and all accessory arteries (70 ± 23 mm (6)) and PE effected a constrictor response in all coronary arteries (20 ± 10 mm (4)) and all accessory arteries (49 ± 18 mm (6)). All coronary arteries dilated in response to NA (65 ± 55 (5)). On average, accessory arteries responded to NA with a small vasoconstrictor response (3 ± 50 mm (8)) but two distinct subgroups were apparent: one responded with dilatation (39 ± 13 mm (4)) and the other with constriction (46 ± 28 mm (4)).

These data show that coronary and accessory arteries respond similarly to ACh and PE. However, accessory arteries could be classified differently according to the nature of their response to NA. The neglected accessory circulation of the rat heart shows promise as an experimental surrogate for the coronary circulation.

This work was funded in part by the Health Research Board.

*All procedures accord with current National and local guidelines.*

PC36

## Comparison of gastrocnemius and tibial nerve sympathetic discharge frequency components in anaesthetised rats

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Previously we have demonstrated that population and single unit cutaneous vasoconstrictor discharges have a dominant rhythm (~0.4–1.2 Hz: T-rhythm). We propose that it arises from a family of oscillators whose graded and dynamic synchronisation may be important in sympathetic control (see Gilbey, 2001). Currently we are analysing muscle sympathetic activity to examine the idea that our concepts have equal application to mechanisms underlying muscle vasoconstrictor rhythms. As an initial stage we have compared population gastrocnemius (GN) (muscle) and tibial nerve (TN) (recorded at a point distal to the main muscle branches – 'plantar' branch) sympathetic activity.

Thirteen male Sprague-Dawley rats (290–350 g) were anaesthetised (60 mg kg$^{-1}$ sodium pentobarbitone I.P.; supplemented with 5–10 mg $\alpha$-chloralose I.V. as required) vagotomised, ventilated artificially, given a pneumothorax and neuromuscularly blocked (pancuronium bromide, 1 mg kg$^{-1}$). Anaesthesia and neuromuscular block were monitored and maintained (both I.V.) until humane killing with anaesthetic (I.V.) (see Johnson & Gilbey, 1996). Some animals additionally underwent sino-aortic denervation (SAD; ibid.). Using glass suction electrodes, recordings were made from either TN or a GN (medial or lateral head) simultaneously with that from an ipsilateral GN (filtered, 80–1000 Hz; rectified and smoothed, $\tau = 20$ ms). Phrenic nerve discharge was recorded as an index of central respiratory drive. Auto and coherence spectra were calculated as described by Smith & Gilbey (2000) (300 s data sets; sampling rate 100 Hz; FFT block size 2048, 50 % overlap, 28 blocks).

During central apnoea in baroreceptor-intact animals (mmHg, means ± S.E.M.: $P_{a,CO_2}$ 32 ± 2, $P_{a,O_2}$ 157 ± 17, BP 106 ± 5, $n = 8$) GN spectra (11 recordings in 8 animals including 3 pairs) had a peak at heart rate frequency (fHR) whereas TN spectra did not ($n = 5$). Conversely, in these same recordings TN spectra had a peak in the range 0.6–0.9 Hz (T-rhythm) whereas GN spectra did not. Consistent with these differences in spectral profile GN–GN (3 pairs) coherence was significant (>0.1) at all frequencies examined (< 10 Hz), whereas GN–TN coherence at T-rhythm frequency reached significance (>0.1) in 1/5 cases. In SAD rats ($n = 5$, GN–TN pairs, $P_{a,CO_2}$ 26 ± 4, $P_{a,O_2}$ 146 ± 17, BP 101 ± 7) a peak at fHR was absent and GN–TN coherence was significant (>0.1) in 1/5 cases (at T-rhythm frequency).

Our data show that the coherence spectrum of GN and TN sympathetic activities rarely reach significance (>0.1) at T-rhythm frequency. We conclude that at this frequency the drive to these nerves is not obligatorily linearly coupled.

Gilbey MP (2001). *Clin Exp Pharmacol Physiol* **28**, 130–137.

Johnson CD & Gilbey MP (1996). *J Physiol* **497**, 241–259.

Smith JE & Gilbey MP (2000). *J Physiol* **523**, 449–457.

This work was supported by BHF grant no. PG/2001054.

*All procedures accord with current UK legislation.*

PC37

## Increase in contralateral lower limb vascular conductance at the onset of voluntary and electrically evoked isometric exercise in man is not explained by a decreased muscle sympathetic nerve activity

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We have recently reported a transient increase in conductance of the contralateral lower limb occurring 10–15 s after the onset of either voluntary or electrically evoked isometric exercise of the human calf muscles (Fisher & White, 2003). One possible explanation of this observation is that muscle sympathetic nerve activity (MSNA) is decreased soon after exercise onset.

With local ethical committee approval and after all subjects gave informed written consent seven healthy subjects, mean age 24 ± 1.4 years (6 males) performed electrically evoked (STIM) and voluntary (VOL) isometric contractions at 30 % maximum voluntary contraction force, using the protocols described in Fisher & White, (2003). In the last 5 s of the pre-exercise rest period and throughout exercise, circulation through the exercising limb was occluded by thigh cuff inflation to 200 mmHg. HR (ECG) and blood pressure (MAP) (Finapres) and MSNA, recorded from the peroneal nerve of the contralateral lower limb, were continuously monitored throughout the protocol (Hansen & Sander, 2003). On a separate occasion, using the same protocol, contralateral lower limb blood flow was measured every 15 s using venous occlusion plethysmography. Vascular conductance was calculated from blood flow/MAP. In three subjects the effects of thigh cuff inflation for 1 min without exercise on contralateral limb MSNA were investigated. All values are presented as 15 s ensemble average changes from rest ± S.E.M. Statistical analysis was performed using repeated measures ANOVA ($P < 0.05$) and Student's paired $t$ tests with Bonferroni correction for multiple comparisons.

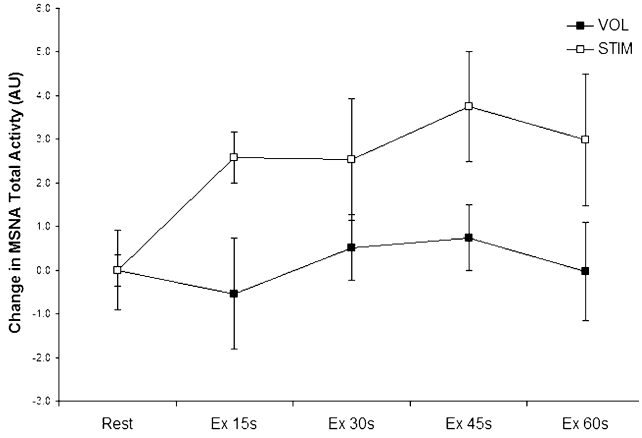


Figure 1. Change in MSNA from rest during exercise. AU, arbitrary units; VOL, voluntary protocol; STIM, stimulated protocol.

In both STIM and VOL, conductance had increased significantly ($P < 0.05$) after 10 s of exercise, declining thereafter (Fisher & White, 2003). At this time HR had increased by 3.08 ± 1.61 and 6.29 ± 1.79 beats min$^{-1}$ for STIM and VOL respectively ($P < 0.05$) falling back to resting levels by 30 s of exercise. During both protocols exercise MAP increased ($P < 0.05$). There were no differences between STIM and VOL for any of the above variables, which changed as has previously been reported (Fisher & White, 2003). Thigh cuff inflation for 1 min without exercise caused a 47 ± 7.5 % ($P < 0.05$) reduction in contralateral limb MSNA, which recovered when circulation was restored. However, when cuff inflation and the associated MSNA decrease was followed by STIM and VOL exercise, MSNA did not fall further (Fig. 1).

These data do not support the hypothesis that the increase in conductance seen at the onset of STIM or VOL exercise is due to reduction in total MSNA at this time.

Fisher JP & White MJ (2003). *J Physiol* **546.1**, 315–323.

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

PC38

## Effect of non-selective cyclooxygenase inhibition on renal function in ischaemia–reperfusion injury in the anaesthetised normotensive and hypertensive rat

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Prostaglandins have a regulatory role in the normal functioning of the kidney, but their involvement in the renal response to ischaemia is little understood. We investigated the effect of non-selective inhibition of cyclooxygenase (COX) enzymes on renal function up to 2.5 h after a period of ischaemia in Wistar and stroke-prone spontaneously hypertensive rats (SPSHRs).

Two groups of male Wistar and two groups of male SPSHRs ($n$ = 5–7, 250–350 g) received polyethylene glycerol vehicle or aspirin (53.8 mg kg$^{-1}$ day$^{-1}$) orally for 7 days. On day 7 the rats were anaesthetised with chloralose–urethane (12 and 180 mg ml$^{-1}$; 1 ml I.P. initially, 0.05 ml I.V. when necessary). A tracheostomy was performed and cannulae were placed in the right femoral vein for the infusion of inulin (1.5 g (100 ml)$^{-1}$ 0.9 % saline; at 3 ml h$^{-1}$) and anaesthetic, and in the right femoral artery for BP, heart rate monitoring and taking plasma samples. The left ureter was cannulated for the collection of urine and the rat was left to stabilise for 1–2 h. Two baseline collections of urine and plasma were taken, and a non-traumatic clamp was placed on the renal artery for 30 min. Further urine collections were taken at 15 and 30 min, and 1, 1.5 and 2 h, and plasma samples at 0 and 30 min and 1.5 h. Animals were humanely killed with an anaesthetic overdose. Data are means and S.E.M., and were compared using ANOVA, with significance taken at $P \leqslant 0.05$.

The mean arterial pressure returned to near basal levels 2 h after the period of ischaemia in all groups. The vehicle-treated SPSHRs had the highest BP of 140–160 mmHg, the vehicle-treated Wistar group was at 120 mmHg, the aspirin-dosed SPSHRs was at 110 mmHg and the lowest BP at 90–100 mmHg was the aspirin-treated Wistar group, thus indicating that the inhibition of vasoconstrictor prostaglandins by aspirin counteracted the SPSHR's genetic predisposition to hypertension. The vasodilatory effect of aspirin was also apparent in the renal blood flow (RBF) data. In both aspirin-treated groups the RBF was between 50 and 65 ml min$^{-1}$ kg$^{-1}$, which was significantly higher than both vehicle-treated groups, which were between 15 and 20 ml min$^{-1}$ kg$^{-1}$. The glomerular filtration rate (GFR) fell by at least 50 % in all groups after the ischaemic period, indicating that the inhibition of COX enzymes afforded little protection to GFR. However aspirin ameliorated the

diuretic effect of ischaemia seen in the vehicle-treated groups, as the aspirin-treated groups had significantly lower urine flows. The beneficial effect of non-selective COX inhibition was also indicated by the decreased natriuretic effect of the aspirin-treated groups in comparison to the vehicle. These results suggest COX inhibition could have a protective effect during ischaemia–reperfusion injury.

*All procedures accord with current UK and Eire legislation.*

## PC39

### Localisation of steroid receptor coactivator-1 in lumbosacral spinal cord neurons of male and female Wistar rats

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Steroid receptors belong to a superfamily of transcription factors that modulate hormone-responsive genes and are critically involved in regulating homeostasis and development. Circulating steroids operate in the brain by binding to intracellular neuronal steroid receptors to form a steroid–receptor complex. This complex binds to a hormone response element located on DNA where it co-ordinates gene transcription and subsequently neuronal function. Transcription is enhanced by the interaction between steroid receptors and other proteins termed nuclear receptor coactivators, including steroid receptor coactivator-1 (SRC-1), which increase their binding and effect at the hormone response element. SRC-1 has been shown to interact with a variety of nuclear receptors including thyroid receptors but particularly those for sex steroids (Onate *et al*. 1995). Consequently the localisation and levels of SRC-1 in the brain serve as a marker of general steroid levels and can be used to compare both age- and sex-related changes in distribution of steroid receptors. However few studies, to date, have investigated the immunolocalisation of SRC-1 and scant information is available on its distribution within sexually dimorphic nuclei in lumbosacral spinal cord. Our current study attempts to address this omission.

Three- and 24-month-old Wistar rats of both sexes were humanely killed by terminal anaesthesia (Euthatal, 200 mg $kg^{-1}$ I.P.) then perfused with 4% paraformaldehyde. Spinal cord segments L5–S1 were removed and sectioned at 45 $\mu$m before immunocytochemical processing. To localise SRC-1, sections were incubated for 72 h in anti-SRC-1 antibodies (Santa Cruz Biotechnology) at a dilution of 1:333. In addition two male rats underwent bilateral orchidectomy, under halothane anaesthesia (4% in $O_2$) 5 days prior to immunoprocessing.

In all rats SRC-1 immunostaining was seen in the nuclei of many neurones distributed throughout all laminae. However labelling was predominant in laminae I/II of the dorsal horn and within the motornuclei of lamina IX including the sexually dimorphic nuclei. Labelling was also notable in the region of the sacral parasympathetic nucleus. The distribution was similar in both sexes with the exception of the spinal nucleus of the bulbocavernosus which is absent in females. Labelled nuclei had a similar distribution in the aged rats though they contained fewer positive cells. In orchidectomised rats, in which circulating testosterone was abolished, SRC-1 reactivity was unchanged in motorneurons of sexually dimorphic nuclei which are known to express receptors to other steroids including glucocorticoids.

These results suggest that the distribution of SRC-1-containing cells closely follows that described previously for steroid receptors and that differences in the distribution patterns between sexes reflects their sexual dimorphism.

Onate SA *et al.* (1995). *Science* **270**, 1354–1357.

This work was supported by an ERA grant from BBSRC.

*All procedures accord with current UK legislation.*

## PC40

### Blood pressure responses to external compression of the human lower leg during calf muscle chemoreflex stimulation of varying intensity

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The cardiovascular response to isometric exercise and its control depends to a variable extent upon the afferent information transduced from metabolic and mechanical stimuli generated within the exercising muscle (Kaufman & Rybiki, 1987). During a phase of post-exercise circulatory occlusion (PECO) blood pressure remains elevated above baseline due to continued activation of the muscle chemoreflex (Alam & Smirk, 1937). Application of external compression to the muscle at this time can further increase blood pressure (White *et al.* 1998). This could result because of activation of muscle mechanoreceptive afferents or sensitisation of active muscle chemoreceptive afferents. The present study was designed to investigate further these two possibilities. If a standardised external compression stimulus applied during PECO increased blood pressure independent of the level of muscle chemoreflex activation then a constant mechanoreceptive reflex stimulation would be suggested. However if the level of response to compression varied with increasing muscle chemoreflex activation then sensitisation, or even saturation, of the reflex response could be inferred.

With local ethics committee approval eight male subjects (mean ± S.E.M. age, 26.3 ± 2.2 years, height, 177.2 ± 1.6 cm, weight, 74.6 ± 2.8 kg) gave written informed consent to perform five trials randomly assigned over two visits separated by a rest period of at least 20 min. Using previously published techniques (Bull *et al.* 1989) subjects sustained in random order either 30%, 40%, 50%, 60% or 70% of maximal voluntary contractile (MVC) force of the calf muscles for 90 s in a dynamometer. Circulation through the lower leg was occluded throughout exercise and for the following 3 min by inflation of a thigh cuff. After 1 min of PECO a second cuff, placed around the calf muscles, was rapidly inflated to 300 mmHg (Hokansen) and was deflated 1 min later. Blood pressure was measured throughout the protocol (Finapres) and heart rate was recorded from ECG. Data were sampled at 1000 Hz by an A–D converter (CED 1401plus) and PC.

Exercise at 30, 40, 50, 60 and 70% MVC progressively increased mean arterial blood pressure (MAP) during PECO ($P < 0.001$) by a mean (± S.E.M.) of 3.9 ± 1.4, 9.5 ± 3.4 and 16.2 ± 5.7 mmHg, respectively, above resting levels (MANOVA). External compression caused MAP to rise further with each condition (MANOVA, $P < 0.001$) reaching 9.2 ± 1.9, 8.6 ± 1.9, 15.9 ± 2.4, 21.9 ± 1.7 and 24.9 ± 2.8 mmHg respectively, above resting values. During compression the mean increases in MAP above those in PECO were 2.4 ± 1.6, 4.7 ± 1.2, 6.4 ± 0.9, 5.6 ± 1.1 and 5.1 ± 2.0 after 30, 40, 50, 60 and 70% MVC, respectively, and were not significantly related to the preceding exercise intensity. Heart rates were not significantly altered from baseline levels during PECO or compression and were not influenced by preceding exercise intensity. These data show that muscle chemoreflex activation during PECO is influenced by the

intensity of preceding exercise and that blood pressure is further elevated by forceful external compression applied at this time.

On the basis of these preliminary data it could be concluded that 300 mmHg external compression activates a population of mechanoreceptive afferents, independent of the level of muscle chemoreflex activation. However study of a wider range of data is required before this can be said with certainty.

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White MJ *et al.* (1998). *J Physiol* **513.P**, 87*P*.

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

PC41

## Kv3.4 potassium channel subunit immunoreactivity in presynaptic terminals in the brainstem and spinal cord of rats

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Voltage gated potassium channel subunits of the Kv3 subfamily (Kv3.1–Kv3.4) play a vital role in action potential repolarisation in neurones (Rudy & McBain, 2001). Whereas Kv3.1–Kv3.3 appear to encode delayed rectifier currents which facilitate fast firing, the role of Kv3.4 subunits in neurones is less clear. Cellular and sub-cellular differences in the pattern of ion channel expression are important in defining specific roles of channels and since little is known regarding the possible presence of this subunit in the spinal cord and medulla, we used immunohistochemistry to study its distribution in these regions.

Rats (100–200 g, $n = 5$) were injected intraperitoneally with 0.1ml 1 % Fluorogold (Fluorochrome Inc.) and 7 days later were humanely killed by Sagatal (100 mg kg$^{-1}$ I.P.) and perfused transcardially with 4 % paraformaldehyde–0.1–0.5 % glutaraldehyde. Slices (50 $\mu$m) of medulla and thoracic spinal cord were cut and incubated in anti-Kv3.4 antibody (Alomone; 1:1K) followed by a Cy3-conjugated secondary antibody. Sections were also incubated in antibodies raised against SV2, VGluT1, VGluT2 and GlyT2 (Iowa Uni Hybridoma Bank, 1:500; Chemicon, 1:20K, 1:5K and 1:5K, respectively), which were visualised using a biotinylated conjugated secondary antibody and Streptavidin Alexa.

Kv3.4 immunoreactivity was observed in punctate structures throughout the spinal cord and medulla. Particularly dense labelling was observed in regions involved in autonomic control such as the intermediolateral cell column (IML) in the spinal cord and the dorsal vagal nucleus (DVN) in the medulla. These punctate structures, suggestive of terminals, closely apposed Fluorogold labelled autonomic neurones and motoneurones. Co-localisation of Kv3.4-IR with the synaptic vesicle protein, SV2, provided confirmation of presynaptic labelling. Furthermore, co-localisation of Kv3.4-IR with the glutamate vesicle transporters, VGluT1 and VGluT2, and the glycine transporter, GlyT2, demonstrated that these subunits are present in both excitatory and inhibitory presynaptic terminals.

This study indicates that Kv3.4 subunits are found in presynaptic terminals throughout the spinal cord and brainstem, with strong labelling in regions involved in autonomic control. These results suggest that Kv3.4-containing channels, probably by modulating the shape of invading action potentials, regulate neurotransmitter release from excitatory and inhibitory terminals.

Rudy B & McBain CJ (2001). *Trends Neurosci* **24**, 517–526.

This work was funded by the British Heart Foundation

*All procedures accord with current UK legislation.*