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 immunohistochemical 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).

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.


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

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
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 µg bolus plus 5, 10 or 20 µg at 7.7 µl 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 artery, inulin was infused to allow measurement of glomerular filtration rate (GFR). After a 2 h recovery period, 15 min clearances were taken, of identified SPNs in the IML to different concentrations of 5-F-Will (20.2 ± 2.5 mV) consistent with the presence of GluR4 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 µm) were superfused with oxygenated aCSF (composition (mM): NaCl (124); NaHCO₃ (26); KCl (3); MgSO₄ (2); NaH₂PO₄ (2.5); CaCl₂ (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-immunonegative SPNs are located more caudally and the proportion of SPNs responding to 15–30 nM 5-F-Will to...
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.
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_e) of 37°C and on cooling to 25°C in the presence of atropine (muscarinic cholinergic blocker) or propranolol (β-adrenergic blocker).

Male Wistar rats, 290–320 g, were anaesthetised with fluothane a femoral artery. Mean arterial blood pressure (MABP) and HR were measured via a femoral artery. T_e 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 µg) and sodium nitroprusside (10 µg) 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 methyllyscitate (2 mg kg^{-1}; n = 5) or propranolol (1 mg kg^{-1}; n = 7). Rats were killed with an overdose of sodium pentabarbitorute. Data (means ± S.E.M.) were analysed using ANOVA and significance taken at P < 0.05.

Basal levels of HR at T_e = 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_e = 37°C. In the presence of atropine, baroreflex–HR parameters (Kent et al. 1972) were: A_1 = 27 ± 14 beats min^{-1}, A_2 = 0.06 ± 0.04 mmHg^{-1}, A_3 = 85 ± 1 mmHg and A_4 = 442 ± 9 beats min^{-1} at T_e = 37°C, while in the presence of propranolol A_1 = 46 ± 6 beats min^{-1}, A_2 = 0.08 ± 0.18 mmHg^{-1}, A_3 = 106 ± 6 mmHg and A_4 = 334 ± 16 beats min^{-1} at T_e = 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_e = 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_e = 25°C with a decrease in maximal gain by ~85% vs. control at T_e = 37°C (~93 ± 0.2 beats min^{-1} mmHg^{-1}, P < 0.0001).

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


All procedures accord with current UK legislation.

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 Hapes-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 µl) 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 P_O_2. It was found that this preparation was very robust, probably due to the small size of the glomus 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.


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All procedures accord with current local guidelines.
Role of central 5-HT7, as well as 5-HT1A receptors in cardiopulmonary reflex control in anaesthetised rats

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Central 5-hydroxytryptamine-containing neurones, via activation of 5-HT1A receptors, control parasympathetic outflow to the heart, airways (see Ramage, 2001) and bladder (Conley et al. 2001). Furthermore, blockade of central 5-HT1 receptors can inhibit the micturition reflex (Read et al., 2003). The present experiments compare the roles of central 5-HT7 and 5-HT1A receptors on the cardiopulmonary reflex, using the selective 5-HT receptor antagonist SB-269970 (Hagan et al. 2000) and the selective 5-HT1A receptor antagonist WAY-100635.

Male Sprague-Dawley rats (300–350 g) were anaesthetised with α-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 (α-bungarotoxin 150 μg 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 phenylbiguanide (PBG; 3–15 μg kg−1). After three control reflexes, SB-269970 (30, 100, or 300 μg kg−1), WAY-100635 (100 μg kg−1), or saline was given intracisternally (10 μl 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.

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<th>Control</th>
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<td>SB-269970 30 μg kg−1</td>
<td>43±7</td>
<td>27±3*</td>
<td>24±3*</td>
<td>24±6</td>
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<tr>
<td>SB-269970 100 μg kg−1</td>
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<td>23±3*</td>
<td>12±3**</td>
<td>17±4**</td>
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<tr>
<td>SB-269970 300 μg kg−1</td>
<td>52±11</td>
<td>7±2**</td>
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<td>5±3**</td>
<td>8±3**</td>
</tr>
<tr>
<td>WAY-100635 100 μg kg−1</td>
<td>48±7</td>
<td>20±3*</td>
<td>30±2*</td>
<td>43±3</td>
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<td>40±2</td>
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Table 1. Effect of intracisternal SB-269970 and WAY-100635 on changes in R–R interval (ms; mean ± S.E.M.) evoked by intra-atral PBG. *P < 0.05, **P < 0.001, 2-way ANOVA followed by least significant difference test.

Table data demonstrate that both central 5-HT7 and 5-HT1A receptors are activated during the cardiopulmonary reflex to cause an increase in cardiac vagal outflow in anaesthetised rats.

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.


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


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All procedures accord with current UK legislation.
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 micromanipules 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.

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All procedures accord with current National and local guidelines.
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) were anaesthetised with chloralose–urethane (12 and 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 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 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.

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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 μm 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 were incubated for 72 h in anti-SRC-1 antibodies (Santa Cruz). 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 bulbocavernous 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 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 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 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 a similar distribution in the aged rats though they contained fewer positive cells. 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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.

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All procedures accord with current UK legislation.

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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 on 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, 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 also 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.


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 µm) 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.


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All procedures accord with current UK legislation.