C1

DISRUPTION OF ASTROCYTIC GAP JUNCTIONS AND FAILURE OF ACTIVITY-RELATED DILATATION IN CORTICAL ARTERIOLES AFTER SIMULATED ISCHAEMIA IN BRAIN SLICES

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Interpretation of BOLD signals from functional MRI is based on the assumption that local cerebral blood flow reflects neuronal activity. Scans from patients after recovery from a transient ischaemic attack showed abnormalities suggesting the coupling process was disrupted (1). We have investigated whether activity-related dilatation in cerebral arterioles is compromised after ischaemia and whether gap junctional communication in the neurone-astrocyte-arteriole triad could be affected.

Cortical slices were prepared from urethane-anaesthetised (1.5g kg^{-1} i.p.) adult Wistar rats as described previously (2) and maintained at 33°C. In 10 arterioles, resting internal diameter (ID) 14.6±0.95μm (mean±SEM), addition of 75nM U46619 was used to pre-constrict vessels and induce vasomotion. Superfusion with a glutamate agonist (1μM AMPA for 10min) evoked 9.93±1.6% increase in ID and vasomotion decreased from 12.0±1.3 to 2.3±0.7min^{-1}. 30 min after washout of AMPA, 5 slices were subjected to 5min oxygen and glucose deprivation (OGD, ACSF gassed with 95%N_2/5%CO_2 and glucose replaced by sucrose). OGD induced transient (<10min) dilatation (8.3±1.7% increase in ID, vasomotion decreased to 1.6±0.7min^{-1}). 30min later, a second application of AMPA failed to evoke a significant change in ID (-0.42±1.57%, p>0.05, paired t-test) and vasomotion decreased to only 5.6±0.7min^{-1}. In control vessels (no OGD, n=5) the response to AMPA was unchanged (7.3±1.7% increase in ID, vasomotion decreased to 5.2±1.2min^{-1}, p>0,05 Student's t test).

Immunostaining for connexin 43, the predominant astrocytic gap junctional protein was revealed in 40μm thick sections from fixed slices using antibodies against the phosphorylated (Cx43p) and dephosphorylated (Cx43d) protein (antibodies 71-0700 and 13-8300, Zymed). In neocortex, punctate Cx43p immunoreaction product was interspersed between unstained neuronal somata. Staining for Cx43d was paler but showed a similar distribution. 30min after exposure to 5min OGD, the punctate Cx43p immunostain became paler but patches of a dark, granular immunoreaction product appeared, especially in somatosensory and auditory/visual cortices. In equivalent areas of Cx43d-stained slices, patches of unstained tissue appeared, surrounded by a border of intense punctate staining.

The results indicate that activity-related dilatation in cortical arterioles is disrupted following recovery from a simulated transient ischaemic attack. The concomitant changes in Cx43 immunostaining may reflect a disruption of cellular processes in which communication through astrocytic gap junctions is compromised.

Rossini, PM et al Brain 127 (2004) 99-110

Lovick, TA et al Neuroscience 92 (1999) 47-60

Supported by British Heart Foundation

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

C2

RESPONSES OF CEREBRAL AND FEMORAL VASCULATURES TO CARBON DIOXIDE AND SYMPATHETIC STIMULATION IN HUMANS

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The relative importance of CO_2 and sympathetic stimulation in cerebral and peripheral vascular regulation has not been previously studied in humans. We hypothesized that: 1) increases in sympathetic outflow would elicit cerebral vasoconstriction during isocapnia but not during hypercapnia; 2) sympathetic vasoconstriction would be evident in the peripheral circulation under both conditions.

In 14 healthy males [28.1±3.7 (SD) yrs], we measured blood flow velocity (VP, n=14; transcranial Doppler ultrasound) in the middle cerebral artery during euoxic isocapnia (ISO, end-tidal PCO_2, $PETCO_2$, = +1 Torr above rest) and two levels of euoxic hypercapnia (HC5, $PETCO_2$ = +5 Torr above ISO; HC10, $PETCO_2$ = +10 above ISO). Each $PETCO_2$ level was maintained for 10 min using the technique of dynamic end-tidal forcing, during which increases in sympathetic activity were elicited by a 2-min isometric handgrip (HG) at 30% of maximal voluntary contraction. Femoral blood flow (FBF, n=11; Doppler ultrasound), muscle sympathetic nerve activity (MSNA, n=7; microneurography), and mean arterial pressure (MAP; Portapres) were also measured.

Hypercapnia increased VP and FBF by 5% and 0.6 %.$Torr^{-1}$, respectively, and MSNA by (20-220%). Isometric HG increased MSNA by 50% and MAP by 20% during all conditions. During the ISO HG there was an increase in cerebral vascular resistance (CVR; 12±10%), whilst VP remained unchanged. During hypercapnia, VP increased [9.2±3.3 and 12.6±5.2 $cm.sec^{-1}$ in HC5 and HC10, respectively] but CVR was unchanged. In contrast, HG-induced sympathetic stimulation increased femoral vascular resistance (FVR) during ISO, HC5 and HC10 (21-34%).

HG elicited cerebral vasoconstriction under isocapnic, but not hypercapnic, conditions. In contrast, HG increased FVR during both conditions. Therefore, the cerebral circulation is less responsive to sympathetic stimulation and more responsive to alterations in PCO_2 than the peripheral circulation.

Table 1. Responses during isocapnia, hypercapnia and HG.

Condition	VP	CVR	MAP	MSNA	FBF	FVR
ISO	60.2±8.9	1.49±0.23	90±10	100.0±0.0	534±228	0.17±0.09
ISO + HG	63.6±11.2	1.80±0.32*	115±14*	147.2±41.9*	478±229*	0.24±0.11*
HC5	72.7±11.3*	1.28±0.20	93±11	120.3±32.1*	526±233	0.18±0.08
HC5 + HG	81.9±11.6*[a]	1.37±0.32	112±16*[a]	176.4±40.2*[a]	507±219	0.22±0.10[a]
HC10	87.8±15.0*	1.13±0.24*	100±12*	165.0±83.0*	567±264	0.18±0.09
HC10 + HG	100.4±13.9*[b]	1.20±0.20*	120±15*[b]	189.8±111.9*[b]	568±242	0.21±0.10[b]

* significantly different from * ISO, [a] HC5 and [b] HC10 at P < 0.05. VP, ($cm.sec^{-1}$); CVR,($mmHg/ cm.sec^{-1}$); MAP, mmHg; MSNA, %; FBF, ($ml.min^{-1}$); FVR, ($mmHg/ ml.min^{-1}$).

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

C3

ROLE OF OXIDATIVE STRESS ON THE RENAL MICROVASCULATURE OF ANAESTHETISED WISTAR AND STROKE PRONE SPONTANEOUSLY HYPERTENSIVE RATS (SHRSP)

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A range of autocrine and paracrine factors influence basal tone of the renal microvasculature. Important amongst these are the reactive oxygen species (ROS) and include super oxide anions, hydrogen peroxide (H2O2) and nitric oxide (NO), which are generated to differing degrees in various situations. This study aimed to investigate the impact of ROS on perfusion of blood through the renal cortex and medulla normally and in a hypertensive state.

Eight groups (n= 6-7) of male Wistar and SHRSP, 250-300g, were anaesthetised with an I.P injection of 1 ml chloralose/urethane, 16.5/250 mg/ml. The right femoral vein was cannulated for infusion of saline (154mM NaCl) at 3ml/h and supplemental doses of anaesthetic. The right femoral artery was cannulated for measurement of blood pressure (BP). The left kidney was exposed via a flank incision, placed in a holder and a small cannula was inserted 4.5mm into the kidney for intramedullary (i-m) infusion of saline or drugs at 0.6-1.0 ml/h. Two Laser-Doppler microprobes (each 0.5 mm diameter) were inserted 1.5 and 4.0 mm into the kidney to measure cortical and medullary blood perfusion, respectively (100 perfusion units (PU) = 1 V). After 90min, baseline measurements were taken, then either vehicle, tempol, a super oxide dismutase (SOD) mimetic, 30 µmol/kg/min, diethyl-dithio-carbamate (DETC), a SOD inhibitor, 2 mg/kg/min, or a combination of tempol 30µmol/kg/min plus Catalase (an enzyme degrading H2O2) 5000 I.U were infused i-m for 60 min. At the end of the experiments the animals were killed with an anaesthetic overdose. Data ± SEM were subjected to the Student′s t-test and significance taken at $P<0.05$.

In the Wistar rats, baseline levels of BP were 101±15 mmHg, for cortical perfusion (CP), 210±24 PU and medullary perfusion (MP) 119±15 PU and for the SHRSP, BP was 131±9 mmHg, CP was 269±48 PU, and MP was 90±20 PU. Administration of tempol had no effect on CP in either Wistar or SHRSP but increased MP in the Wistar rats by 13±5% ($P<0.05$) and in the SHRSP by 26±10% ($P<0.05$). Intramedullary DETC decreased CP and MP 10±2% and 13±3%, respectively ($P<0.05$) in the Wistar rats while in the SHRSP CP fell by 11±3% ($P<0.05$) and MP by 21±4%. Catalase + Tempol infused i-m, increased CP in both Wistar and SHRSP, by 16±7% and 18±9%, respectively, and MP rose to a greater degree, by 39±6% and 50±9% respectively (both $P<0.05$), in the Wistar and SHRSP, respectively.

These results indicate that ROS can determine the level of tone in the renal microvasculature to a greater extent in the medulla than cortex. Moreover, the impact of ROS in the renal medulla of the SHRSP is relatively greater, suggesting a raised degree of oxidative stress.

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

C4

BOTH α4β2 AND α7 NEURONAL ACH RECEPTORS CAUSE THE RELEASES OF VASOPRESSIN IN ANAESTHETISED RATS

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Activation of central α4β2 and α7 nACh receptors with TC–2559 and Astra IV, respectively, causes a rise in mean arterial blood pressure (MAP), sympathoexcitation, but no change in heart rate (HR) in anaesthetized rats (Moore *et al.* 2004). However, nicotine i.c.v. although causing a rise in MAP, causes sympathoinhibition. Nicotine i.c.v. has been shown to cause a rise in MAP associated with the release of vasopressin (Iitake *et al.* 1986). This release of vasopressin would explain the sympathoinhibition, as it would cause a peripherally mediated rise in BP which would activate the baroreceptor reflex. Experiments were carried out to examine the effects of these agonists, in the presence (I.V.) of a V1 receptor antagonist, on the above cardiovascular variables.

Male Sprague Dawley rats (250-350g) were anaesthetized (I.V.) with α-chloralose (100 mg kg^{-1} and maintained with 15 mg kg^{-1} when necessary), artificially ventilated and neuromuscular blocked (3 mg kg^{-1}; decamethonium). Recordings were made of MAP, HR and renal nerve activity (RNA). Depth of anaesthesia was assessed by the stability of MAP, HR and RNA following a noxious stimulus. Changes were compared with saline (5 µl; i.c.v.) in the presence of the V1 receptor antagonist by two-way ANOVA and the least significant difference test. All values are means ± S.E.M. At the end of the experiment animals were killed by an overdose of pentobarbitone.

In presence of V1 receptor antagonist ([β-Mercapto-β,β-cyclopentamethylenepropionyl[1],O-Me-Tyr[2],Arg[8]]-Vasopressin; 30 µg kg^{-1}, I.V.), cardiovascular effects of nicotine (0.3 µmol kg^{-1}; i.c.v.; n=5) were now completely blocked. TC-2559 (3 µmol kg^{-1}; i.c.v.; n=5) also now caused no significant change in MAP and HR but RNA still significantly increased (30 ± 6% at 3 min) but compared to TC–2559 alone it was significantly decreased. The effects of Astra IV (3 µmol kg^{-1}; i.c.v.; n=5) on MAP were also blocked along with the sympathoexcitation.

This study confirms that i.c.v. nicotine causes a rise in MAP due to vasopressin release and is consistent with the view that the renal sympathoinhibition is secondary to this rise. The data indicate that vasopressin release can be caused by either α4β2 and α7 nACh receptor activation. Interestingly, the renal sympathoexcitation caused by the nACh receptor agonists is also mediated by V1 receptors.

Moore C. *et al.* (2004). *Br. J. Pharmacol.* Abstract at the July BPS meeting in Bath.

Iitake *et al.* (1986). *Am. J. Physiol.* **251**, E146-E150.

Christopher Moore has a BBSRC collaborative award with Eli Lilly.

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

C5

BRAIN ANGIOTENSIN II (AII) AND THE BAROREFLEX CONTROL OF RENAL SYMPATHETIC NERVE ACTIVITY (RSNA) IN THE CONSCIOUS RAT

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The aim of the present study was to determine whether the role of brain AII in mediating/modulating the baroreflex control of RSNA was present in the conscious state. The angiotensin AT1 receptors within brain were manipulated by feeding a high Na diet in the post-weaning period, and by intracerebroventricular (icv) administration of antisense oligodeoxy-nucleotides (ASODN) directed against the AT1 receptor gene.

Male Wistar rats (4 weeks old) were fed a regular (0.25%) or a high (3.1%) Na diet for 7 weeks. The rats were anaesthetised with pentobarbital sodium (60 mg/kg ip). The left carotid artery and right jugular vein were cannulated for monitoring blood pressure (BP) and heart rate (HR), and giving drugs. The left kidney was exposed and electrodes sealed onto the renal nerve. Using a sterotaxic frame, a guide cannula was implanted into the right lateral cerebroventricle. The animals recovered for 3 days before entering the study. BP, RSNA and HR were measured during iv phenylephrine hydrochloride and sodium nitroprusside (both at 10μg) before, 1 and 2 days after the icv infusion of the ASODN (5′TAACTGTGGCTGCAA) for the promoter region of the AT1 receptor gene, at 50μg. Animals were killed with an anaesthetic overdose. Baroreflex curves for both RSNA and HR were constructed (Miki et al, 2003). Data (means±SEM) were subjected to Student's t test and significance taken as $P<0.05$.

In rats fed the regular diet (n=5), BP was 112±3 mmHg, HR was 479±14 beats min-1 (bpm) and RSNA 0.33±0.10 mVs-1. Logistic model parameters describing the baroreflex curves for RSNA, i.e. A1 (range), A2 (slope), A3 (mid-point BP) and A4 (minimum RSNA or HR), were 185.5±13.4%, 0.080±0.01% mmHg-1, 105.4±4.3 mmHg, and 11.0±8.3%, respectively and those for HR were 222±31 bpm, 0.082±0.012 bpm mmHg-1, 120.4±2.7 mmHg and 305±21 bpm, respectively. They were unchanged on days 1 and 2 following the icv ASODN. In rats fed a high Na diet (n=5), basal values of BP, HR, RSNA and baroreflex curve parameters were comparable to those in the rats on a normal sodium intake. However, A2 for RSNA, the sensitivity of the baroreflex curve, increased by 39.7% ($P<0.05$) 1 day after the icv ASODN, but returned to basal levels by day 2.

These data showed that the ASODN to the angiotensin AT1 receptor gene caused a long acting increase in baroreflex sensitivity for RSNA. This would suggest that the involvement of endogenous AII within the brain in mediating/modulating the baroreflex control of RSNA in the conscious rat became more important after feeding a high Na diet in the post-weaning period.

Miki K, Yoshimoto M & Tanimizu M (2003). J Physiol 548.1, 313-322.

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

C6

DIFFERENCES IN THE ARTERIAL PRESSURE RESPONSIVENESS OF THE SYMPATHETIC AND PARASYMPATHETIC BARORECEPTOR REFLEX.

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The baroreceptor reflex is a key regulator of arterial blood pressure. Its main effects are mediated by reciprocal modulation of the sympathetic and parasympathetic nervous systems. However, there is some evidence to suggest that these outflows of the baroreflex can be controlled independently (e.g. Pickering et al., 2003). To identify autonomic differences, we tested whether the sympathetic and parasympathetic baroreceptor reflex outflows have comparable arterial pressure thresholds.

Studies were performed on male Wistar rats (80g, 4-5 weeks) using the working heart brainstem preparation (Paton, 1996). Rats were deeply anaesthetised with halothane (until loss of withdrawal reflex), transected sub-diaphragmatically, decerebrated precollicularly and perfused retrogradely via the descending aorta with a Ringer's solution plus ficoll (1.25%) at 32ºC. Flow through the preparation was computer controlled with a peristaltic pump to alter baseline arterial pressure or by infusion of vasopressin (2-400pM). Baroreceptors were stimulated with pressure pulses. Recordings were made of arterial pressure, heart rate, phrenic nerve and thoracic sympathetic chain (T8-12) activity. Values quoted are mean±SEM.

Pressure pulses of approximately 30mmHg for 1 second evoked graded baroreflex-mediated sympathoinhibition. (30-100%) from a wide range of baseline pressures (30-80mmHg). The peak gain of the baroreflex sympathoinhibition was 2.2±0.09%inhibition/mmHg (n=15). However the cardiac component of the baroreflex was only seen clearly with pulses that crossed a higher pressure threshold (70-90mmHg). The cardiac baroreflex gain changed on crossing this threshold from -0.29±0.04 to -1.57±0.24bpm/mmHg (n=15, $p<0.0005$, paired t-test). Plots of the arterial pressure-baroreflex gain relationship confirmed the cardiac baroreflex is right shifted by approximately 25mmHg compared to the sympathoinhibition. Dual recordings of sympathetic chain and cardiac vagal nerve support this differential pressure responsiveness. To determine whether the difference in cardiac gain is due to the vagus we added a β-blocker (atenolol 0.5-1mg; n=6), which further increased the pressure threshold for the bradycardia by 8-10mmHg, confirming that the parasympathetic component has a higher threshold.

We conclude that the sympathetic and cardiac vagal components of the arterial baroreceptor reflex have different pressure sensitivities suggesting a hierarchy of baroreflex autonomic response. It remains to be determined where within the baroreflex arc this difference is generated.

Paton, J.F.R. (1996). J Neurosci Methods 65, 63-68.

Pickering, A. E. *et al.* (2003). J Physiol. 551, 589-599.

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

C7

IMPORTANCE OF THE LATENCY IN THE BAROREFLEX-MEDIATED VASCULAR RESISTANCE RESPONSE IN POSTURALLY RELATED SYNCOPE

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The delay, and not only the gain, of the baroreflex response (baroreflex sensitivity) play an important role in the maintenance of cardiovascular system stability. Additionally when postural changes induce sudden drops in blood pressure, a delayed response may fail to maintain sufficient cerebral perfusion pressure.
We tested the hypothesis that the delay of the carotid baroreceptor reflex might be impaired in subjects with poor orthostatic tolerance.
All the subjects involved in the study gave their written informed consent and the protocol was approved by the local ethical committee. An orthostatic test with 60 deg head-up-tilt, and progressive lower-body-negative-pressure was performed on 25 patients with histories of unexplained syncope and 11 control subjects (age 29 ± 8.6). Test was stopped at the onset of presyncope and time to presyncope was taken as a measure of orthostatic tolerance. Twelve patients had normal tolerance (normal patients, age=46±13), thirteen patients showed to have low orthostatic tolerance (early fainters, age=43±18). We measured beat-to-beat blood pressure (Finapres) and brachial artery blood flow velocity (Doppler ultrasonography).
Before the test, we determined the response of forearm vascular resistance (mean arterial pressure/ mean brachial artery velocity) to loading/unloading of carotid baroreceptors by the application of neck suction/pressure (-/+30mmHg) to a chamber fitted overlying the carotid sinus. We measured the gain in the response (maximum percentage change from baseline value in vascular resistance divided by the neck collar pressure) and the latency in the response (delay in heartbeats of the maximum change in vascular resistance after neck-collar stimulation). Results are reported as mean±S.E.M. and differences were determined by repeated measures ANOVA. In the three groups there were no differences in the sensitivity of the vascular resistance response after baroreceptors loading/unloading. Following baroreceptors unloading the latency of the response was 15.2 ± 1.3 heartbeats in early fainters, 10.2 ± 0.9 heartbeats in normal patients and 11.4 ± 1.3 heartbeats in controls. The latency in blood pressure rise was 12.1 ± 1.2 heartbeats in early fainters, 8.7 ± 1.0 heartbeats in normal patients and 8.0 ± 1.0 in controls. The results following baroreceptors loading were more scattered. The early fainters had still the tendency to show prolonged latency.
These results suggest that the delay in the baroreflex response may play an important role in posturally related syncope. When postural changes induce sudden drops in blood pressure (simulated by the baroreceptors unloading), a delayed response may lead to a failure to maintain sufficient cerebral perfusion pressure, even in the presence of a normal baroreflex gain.

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

C8

DOMINANT ROLE OF AORTIC BARORECEPTORS IN THE CARDIAC BAROREFLEX OF THE RAT

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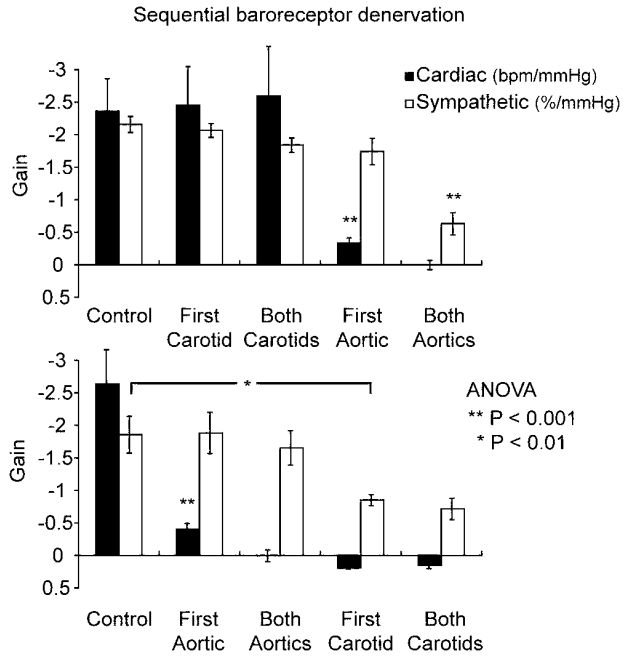
The arterial baroreflex receives its afferent input from sensors located in the carotid bifurcation and aortic arch. In this study we examined the role of the individual afferent sites in determining the overall baroreflex response.
Studies were performed using decerebrate, artificially perfused rats (Pickering et al., 2003). Briefly, Wistar rats (90g) were anaesthetised with Halothane until loss of paw withdrawal reflex and the intestines were removed. The animal was decerebrated precollicularly and perfused, with a carbogenated Ringers solution. Sympathetic nerve activity was recorded from the greater splanchnic nerve and the thoracic chain. For the isolated carotid sinus experiments a double lumen cannula was inserted into the common carotid and connected to a separate perfusion circuit. Data are mean±sem.
Systemic perfusion pressure ramps demonstrated baroreflex sympathoinhibition and bradycardia. By isolating one carotid sinus and applying pressure ramps we showed baroreflex sympathoinhibition, apnoea and vascular resistance changes. However, despite vigorous pressure stimulation (up to 200mmHg, n=8), we were unable to evoke baroreflex bradycardias. Direct injection of sodium cyanide (20ug) to the isolated carotid sinus provoked a chemoreflex response indicating that the sinus nerve was intact.
We therefore undertook a series of baroreceptor denervations, sequentially cutting the afferent nerves from the aortic arch (aortic depressor, ADN) and the carotid sinus (glossopharyngeal). Irrespective of the order of denervation we were able to demonstrate pronounced sympathoinhibition from a single baroreceptor site (figure, n=9). In contrast cutting a single ADN was sufficient to reduce the cardiac gain by 85% and section of the contralateral nerve completely abolished the bradycardia (gain from -2.6±0.5 to 0±0.09bpm/mmHg, n=6). The cardiac baroreflex appeared unchanged by loss of input from the carotids (n=5). These data indicate the aortic arch inputs in the rat predominate in mediating the cardiac baroreflex. The sympathetic component of the baroreflex was less sensitive to the sequential loss of afferent inputs. These different response properties explain our difficulty (and that of others (Dworkin et al., 2000)) in obtaining a bradycardia from the isolated carotid sinus. This may give some functional significance to the differential target distribution, within the nucleus of the solitary tract, of baroafferents carried from the aortic arch versus the carotid sinus (Chan et al., 2000).



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Dworkin BR, et al (2000) Am J Physiol 279, R1910-1921.

Pickering AE, (2003) J Physiol 551, 589-599.

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

C9

REGION SPECIFIC RESPONSES OF NEURONES IN THE NUCLEUS TRACTUS SOLITARIUS (NTS) ON APPLICATION OF MU OPIOID AGONIST DAMGO.

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There are three major subtypes of opioid receptor, mu (μ), kappa(κ) and delta (δ), located in various CNS regions. The mu-opioid receptor (MOR) has been shown experimentally to have the most potent effects in relation to cardiovascular and gastrointestinal functions. Microinjection of the MOR agonist, DAMGO, into the NTS significantly increased food intake, kappa and delta opioid agonists were without effect (Kotz et al.1997). At the cellular level, DAMGO has pre- and postsynaptic effects on neurones within the NTS, inducing a hyperpolarisation of the membrane and a decrease in evoked EPSP amplitude (Rhim et al.1993). The specific location of DAMGO responsive neurones was not determined, which is pertinent since immunohistochemical studies have demonstrated intense staining for the MOR within the commissural and the dorsal medial subnucleus of the NTS, with only moderate staining of the lateral and ventral medial subdivisions (Nomura et al. 1996). Since these receptors could be located either pre- or post-synaptically, the aim of these studies was to evaluate the region specificity, within the NTS, of pre- and post-synaptic responses to DAMGO. Male Wistar rats (18 day) were humanely killed by anaesthetising with Sagatal (60mg/Kg i.p.), coronal medullary slices (300μm) prepared and whole cell patch clamp recordings made from visually identified NTS neurones using electrodes containing Lucifer yellow for post-recording localisation of the neurones (Bouryi & Lewis, 2003). To evoke synaptic potentials, a stimulating electrode was placed in the solitary tract.

DAMGO (100nM) elicited a membrane hyperpolarisation of 6.9±0.91mV (mean±S.E.M.) and a decrease in input resistance (305.6±61.8MΩ) in 11 out of 22 medial neurones with 6 commissural, 2 ventral medial, 1 dorsal, 1 ventral and 7 intermediate neurones being unresponsive. DAMGO also decreased the peak amplitudes of pairs of EPSPs,(61±6.8% decrease 1st EPSP; and 46 ±7.9% decrease 2nd EPSP), resulting in an increase in the paired pulse ratio from 0.632±0.05 to 0.981±0.13 ($P<0.01$, paired t test, n=10). These presynaptic effects were blocked by the MOR antagonist CTOP (1μM) Neurones responsive to the presynaptic actions of DAMGO were located throughout the NTS.

These data suggest that whilst the presynaptic actions of DAMGO are ubiquitous throughout the NTS, postsynaptic responses are restricted to specific subdivisions of the nucleus. It may be that these neurones have specific roles in autonomic function.

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This work is funded by the BBSRC.

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

C10

EXCITATION OF RAT NUCLEUS TRACTUS SOLITARIUS (NTS) NEURONES BY VAGAL AFFERENTS INVOLVES CENTRAL 5-HT$_7$ AND AMPA RECEPTORS

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In anaesthetised rats, intracisternal application of 5-HT$_7$ receptor antagonists inhibits the bradycardias evoked during the cardiopulmonary, chemoreceptor and baroreflex (Kellett *et al.* 2003, 2004) This occurs within 5 min of administration, but the site of action is unknown. The present experiments investigate the effect of the 5-HT$_7$ antagonist SB-269970 (Hagan *et al.*, 2000), applied topically, and of the AMPA receptor antagonist DNQX, applied iontophoretically, on vagus-evoked NTS neuronal activity.

Male Sprague-Dawley rats (280 - 380 g) were anaesthetised with pentobarbitone sodium (60 mg kg^{-1} I.P. followed by 20 mg kg^{-1} h^{-1} I.V), neuromuscularly blocked (gallamine 30 mg kg^{-1} I.V. followed by 6 mg kg^{-1} h^{-1}), mechanically ventilated, and instrumented to record BP and HR. Depth of anaesthesia was assessed by the stability of BP and HR following a noxious stimulus. NTS and dorsal vagal nucleus (DVN) neurones that responded to stimulation of the ipsilateral vagus nerve (1 Hz, 1 ms pulse, 50 - 500 μA) were recorded extracellularly with single glass microelectrodes (impedance 5 - 15 MΩ) or compound recording/iontophoresis electrodes. At the end of experiments, animals were killed by an overdose of anaesthetic.

Topical application of SB-269970 (100 μg kg^{-1}; 10 μl) significantly reduced vagus-evoked NTS neuronal activity compared

to topical saline (see Figure 1; 24 ± 8 vs. 47 ± 2 spikes (50 sweeps)$^{-1}$ at 7 min; $P < 0.01$, 2-way ANOVA; $n = 5$). The time course of this inhibition was similar to the effect of intracisternal SB-269970 on reflex bradycardias. Furthermore, the same dose did not significantly affect ongoing discharge of DVN neurones ($n = 6$). Baseline BP and HR were unchanged. Iontophoretic application of DNQX (at currents selective for AMPA receptors: 30 - 100 nA) also significantly reduced vagus-evoked NTS neuronal activity (from 36 ± 8 to 15 ± 2 spikes (20 sweeps)$^{-1}$; $P < 0.01$, Mann-Whitney test, $n = 12$).

These data demonstrate that SB-269970 can act at the level of the NTS, where both 5-HT$_7$ and AMPA receptors are activated by vagal afferents. The precise synaptic circuitry remains to be determined.

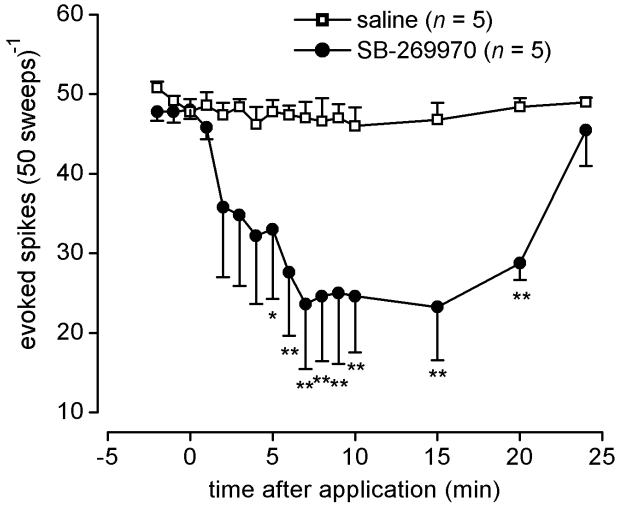

Figure 1. Graph of mean (± s.e.m) effects of topical SB-269970 (100 µg kg-1) and saline (10 µl) on vagus-evoked NTS activity. *P<0.05, ** P<0.01, 2-way ANOVA followed by least significant difference test.

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DOK is a BHF PhD student

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

C11

CAROTID BAROREFLEX REGULATION OF VASCULAR RESISTANCE IN LOWLAND AND HIGH ALTITUDE ANAESTHETIZED DOGS.

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In this study we compared carotid baroreflex function in dogs living at high altitude (4338 m, PB = 450 mmHg; n = 8) with that in lowland animals (n = 6). Dogs were anaesthetized with α-chloralose (100 mg kg^{-1} i.v.) and artificially ventilated with a gas mixture to produce arterial oxygen tension typical of the prevailing ambient barometric pressure i.e. either normoxia (lowland) or hypoxia (high altitude). Following vagotomy to eliminate other baroreceptor reflexes, the pressure perfusing the vascularly isolated carotid sinuses was changed in a stepwise manner. Vascular responses were determined from changes in perfusion pressure to a vascularly isolated hind limb (constant flow). Stimulus-response curves were defined during carotid perfusion with blood equilibrated (a) with gases similar to ambient (lowland or high altitude) and (b) hyperoxic gases. Sigmoid functions were applied to the curves and indicators of carotid baroreflex function were determined: i.e. the maximal slope (equivalent to peak gain) and the corresponding carotid pressure (equivalent to 'set point'). Blood samples were taken at regular intervals for determination of carotid and systemic arterial blood gases and pH. Animals were killed by exsanguination following a lethal dose of anaesthetic.

The results are summarized in Table 1. The maximum slopes of the relationship between perfusion pressure change and carotid sinus pressure were not different either between groups of dogs (high altitude versus lowland) or during different carotid perfusates (ambient or hyperoxic). The 'set point' was lower in the high altitude than in lowland dogs, during perfusion with ambient blood gases. Also in the high altitude dogs changing the perfusate from ambient (hypoxic) blood to hyperoxic blood caused a significant increase in the 'set point'.

The results indicate that compared to lowland dogs, in the high altitude animals the carotid stimulus-response curve is displaced to the left, i.e. to lower pressure. The effect may be partly attributed to perfusion of the carotid sinuses with hypoxic blood because unlike in the lowland animals in the high altitude dogs hyperoxia significantly increases the 'set point'.

Table 1. Group average values.

	Lowland dogs		High altitude dogs	
	Ambient air	Hyperoxia	Ambient air	Hyperoxia
$Pa0_2$ (mmHg)	106.2±8.5	294.6±27.2	47.4±2.1	245.6±13.7
Max. slope	-1.8±0.5	-1.5 ±0.5	-2.1±0.9	-2.6±0.8
Set Point (mmHg)	116.6±6.5	119.8±6.3	90.4±7.9*	101.5±6.7#

* P < 0.04 versus lowland ambient air (Unpaired t test); # P < 0.03 versus high altitude ambient air (Paired t test)

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

C12

RESPONSES OF ISOLATED MESENTERIC ARTERIES OF DOGS AT HIGH ALTITUDE TO VASOACTIVE AGENTS

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The effects of hypobaric hypoxia are widespread and complex. In this study we compared the contractile and relaxation properties of mesenteric arteries from dogs living at high altitude (4338 m; PB = 450 mmHg; HA, n = 8) with those from lowland

animals (SL; n = 5). Dogs were anaesthetized with α-chloralose (100 mg kg^{-1}i.v.), the abdomens opened and intestinal segments removed and placed in cold physiological saline solution (PSS) at 4°C. Animals were killed by exsanguination following a lethal dose of anaesthetic. Lengths of fourth order mesenteric arteries (approx diameter 400 μm) were dissected free and cannulated in a pressure myograph. They were perfused (at 70 mmHg) and superfused with PSS at 37°C and pH 7.4, which was gassed with CO_2 (35 mmHg) in oxygen. Changes in the luminal diameter were determined using a video tracking device. The vessels were allowed to equilibrate for 60-90 min., after which concentration-response curves were determined for noradrenaline (NA). Relaxation responses to acetylcholine (ACh) were determined following preconstriction with NA to 50 % of the resting diameter and expressed as percent of the preconstriction. Tests were done before and after L-NAME (10^{-4} M), an inhibitor of endothelial nitric oxide synthase activity.

SL and HA vessels constricted in response to NA (Fig. 1A). The responses of HA vessels, however, were significantly smaller at doses of 3 x 10^{-7} to 10^{-6} M (two way repeated measures ANOVA $P < 0.05$). ACh before L-NAME, caused relaxation in both groups (Fig. IB). After L-NAME, responses in vessels from SL animals were greatly reduced 100.3 ± 1.6 % to 25.5 ± 12.2 (mean ± s.e.m.; paired t test, $P > 0.05$). In HA vessels, however, the maximal responses were not significantly changed after L-NAME, although the responses were significantly smaller at ACh concentrations up to an including 10^{-6}M.

These results demonstrate that mesenteric arteries from highland dogs are much less responsive to NA. Furthermore, the finding that in the highland dogs Lα-NAME does not alter the maximum vasodilatory response to ACh implies that endothelial function is different. It suggests that, in addition to the endothelial nitric oxide NO pathway, vasodilation in highland animals is mediated by other mechanism(s).

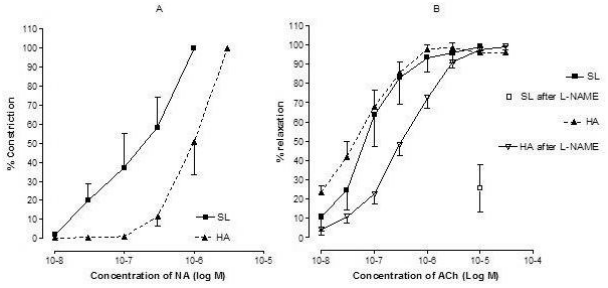

Fig. 1. Responses to cumulative doses concentrations of NA (A) and ACh (B)

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

C13

BARORECEPTOR REFLEX CONTROL OF THE CARDIAC VAGUS DURING AND FOLLOWING EXERCISE IN HUMANS

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During isometric contraction of limb muscles, heart rate (HR) and vasoconstrictor tone are differentially regulated (Gladwell & Coote, 2002). At the onset of contraction, HR increases before a blood pressure (BP) change. At the end of contraction, if the circulation remains occluded (post-exercise circulatory occlusion, PECO) HR recovers rapidly to control whereas BP remains elevated (Bull et al., 1989). This is in part due to baroreceptor reflex control of cardiac vagal tone. In this study we attempted to test this by reducing the input from the carotid baroreceptors with neck pressure (NP) applied bilaterally to the carotid bifurcation (Raine & Cable, 1999). We proposed that NP at the initiation of a voluntary contraction of triceps surae would reduce the excitatory drive from the baroreceptors to the cardiac vagal neurones (CVN), thus increasing the HR response to the contraction. Further, we considered that if NP was applied during PECO, immediately at the cessation of contraction, the usual rapid fall in HR would be diminished.

Following South Birmingham Health Authority ethics committee approval, in agreement with the Declaration of Helsinki, healthy human volunteers were instrumented to record BP (FINAPRES), ECG, respiration and force of contraction during two studies. The effect of the 2 conditions (with and without NP) was statistically tested using paired t-tests ($p \leq 0.05$). Values given as mean±standard deviation. In study 1, (n=5, mean age 29±6 years) NP was applied at the initiation of 30% MVC. The change in HR to contraction without NP applied was very small (-0.6±1.6 beats/min (bpm)) whereas with NP the HR increase was significantly greater ($p \leq 0.02$) (4.0 ±0.4 bpm). In study 2, (n=6, mean age 26±7 years), the application of NP at the cessation of 40% MVC with PECO, significantly altered the fall of HR from peak value at end of contraction with 3.2 ±1.5 bpm, compared to7.9±1.4 bpm with no NP ($p=0.003$). There were no significant differences in SBP or DBP in the two conditions in either of the studies ($p \geq 0.5$). These data support the idea that during contraction that central command and/or muscle mechanoreceptors are the main factors that control baroreceptor excitation of CVN.

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Bull, R.K., Davies, C.T., Lind, A.R., and White, M.J. (1989). J.Physiol. 411

Raine, N.M and Cable, N.T. (1999). Am. J. Physiol. 277

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

C14

THE CARDIOVASCULAR RESPONSE TO HUMAN CALF MUSCLE STRETCH IS INDEPENDENT OF THE LEVEL OF CONCURRENT MUSCLE METABOREFLEX STIMULATION

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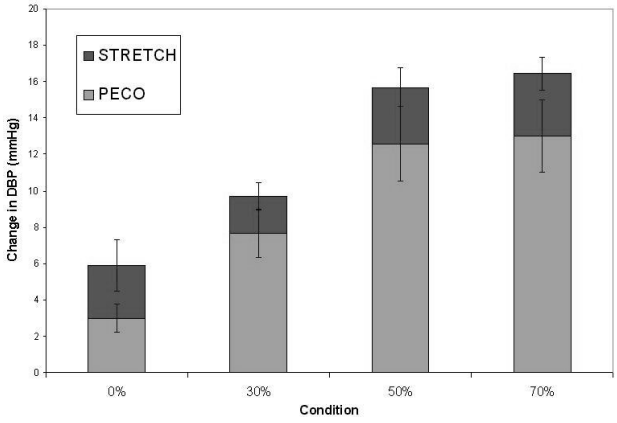
The cardiovascular response to external calf muscle compression, believed to stimulate muscle mechanoreceptors, is progressively augmented by increasing levels of muscle metaboreflex stimulation during post exercise circulatory occlusion (PECO) (White & Bell, 2003}. The cardiovascular response to muscle mechanoreceptor stimulation by passive stretch of calf muscles (STRETCH) differs from that of external compression (Gladwell & Coote, 2002). However, the interaction between STRETCH and muscle metaboreflex activation is unknown.

To examine this interaction eight (7 male) active young (22 ±1yr) subjects were recruited. With local ethics committe approval subjects were seated semi-supine in a Biodex System 3 with the right knee flexed at 150 degrees and the foot attached to the ankle attachment. Heart rate and blood pressure was measured using ECG and Finapres. Phase of respiratory cycle was detected by a strain gauge placed around the chest. Following 115s of rest a cuff was inflated around the right thigh to 200mmHg. Subjects then performed 90s of isometric calf plantarflexion at either 0, 30, 50 and 70% of maximum voluntary contraction (MVC). With the thigh cuff still inflated subjects rested for 90s (the last 60s of period taken as PECO1), then the Biodex moved the foot to full dorsiflexion where it was held for 60s of STRETCH. After a further 60s of rest (PECO2) the thigh cuff was deflated. Subjects performed 2 trials at each exercise intensity and breathed to a metronome at their eupnoeic frequency. Statistical analysis was performed using MANOVA and repeated measures ANOVA, with significance taken as $P<0.05$.

During PECO1 and PECO2 blood pressure was progressively elevated above baseline following the 0-70% MVC trials ($p<0.05$). Figure 1 shows that STRETCH produced a further significant ($p<0.05$) blood pressure rise, the magnitude of which was not significantly different between trials. HR increased to the same extent during the first 3 cardiac cycles of the STRETCH period in all trials. However it recovered rapidly, such that overall there was no significant elevation above baseline during STRETCH.

We conclude that the cardiovascular response to calf muscle stretch is unaffected by the level of muscle metaboreflex stimulation in that same muscle group, suggesting that the stretch activated muscle mechanoreflex is not sensitised by the metabolic conditions within the muscle. This is in contrast to the cardiovascular responses to muscle compression, which appear to be augmented when the metabolic conditions within the muscle are elevated.

Figure 1.



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Gladwell & Coote, (2001) J.Physiol. 540.3, 1095-1102.

This work was supported by BHF grant PG/03/148/16352

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

C15

SHORT LATENCY OF HUMAN CARDIOVASCULAR CHANGES DURING AEROBIC TRAINING

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Chronic exercise lowers resting blood pressure, reduces pressor responses to laboratory stress tests and increases microvascular dilator capacity. While these changes are most pronounced after prolonged training, 5-6 weeks of moderate aerobic training is sufficient to induce significant effects (O'Sullivan & Bell, 2001; OSullivan, 2003). We have now studied the latency of onset of these cardiovascular changes during a shorter training period.

With institutional ethics approval, 15 sedentary non-obese male subjects (age 21.9 ± 0.4 years, mean ± SEM) were recruited into two groups. Eight subjects undertook moderate aerobic training (cycle ergometry, 30 min at 60% VO_{2max} 3-4 times/week) for 4 weeks. The remaining 7 subjects acted as controls. Before training commenced and at weekly intervals during the training period, all subjects attended the laboratory where beat-to-beat blood pressure and heart rate were measured at rest and during isometric handgrip to fatigue at 30% maximal power, and forearm reactive hyperaemia was measured by venous occlusion plethysmography following 3 min arterial occlusion.

The total 4-week training period increased VO_{2max} by 10% from 47 ± 1 to 52 ± 1 [ml.kg^{-1}].min^{-1} and increased circulating endothelial cells from 42 ± 5 to 49 ± 4 cells.ml^{-1} ($P<0.05$ each, paired Students *t* test). These changes are similar in magnitude to those that we have reported previously after 5 weeks training. Resting blood pressure remained at pre-training values over the first week of training but after 2 weeks had fallen from 90 ± 2 to 76 ± 2 mmHg and remained at this level over the ensuing weeks ($P<0.001$, repeated measures ANOVA). Both systolic and diastolic pressures fell to similar extents. Resting heart rate was not reduced. Prior to training, isometric handgrip caused a rise in heart rate of 29 ± 1 bpm. This fell to 17 ± 3 bpm ($P<0.01$) at

week 3 and remained reduced. Pressor responses to handgrip did not vary significantly during the training period but peak rate-pressure product during exercise was reduced after 3 or more weeks training (pre- 154 ± 12, 3 weeks 125 ± 9 bpm.mmHg^{-2}, $P< 0.05$). Prior to training, peak forearm conductance during reactive hyperaemia was 0.12 ± 0.01 [ml.100 ml^{-1}].min^{-1}.mmHg^{-1}. After 2 weeks training the reponse was increased ($P<0.001$) to 0.23 ± 0.01 [ml.100 ml^{-1}].min^{-1} mmHg^{-1} and stabilised at this value. No changes in any measured parameter were seen in the control group.

In summary, our results indicate that a number of circulatory effects of intermittent, moderate exercise programmes reach near-maximal levels within 2-3 weeks. We are currently investigating how long these changes persist after cessation of training.

O'Sullivan SE & Bell C (2001) *Auton Neurosci* 91, 76-84.

O'Sullivan SE (2003) *Int J Sports Med* 24, 404-409.

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

C16

EFFECT OF EARLY TO MID GESTATION NUTRIENT RESTRICTION ON UNCOUPLING PROTEIN-2 MRNA ABUNDANCE IN THE OVINE FETAL PLACENTA AND ADOLESCENT LUNG.

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Maternal nutrient restriction has marked effects on the developing fetus, including upregulation of glucocorticoid receptor (GCR) mRNA in a range of fetal tissues, including the lung (Whorwood *et al.* 2001). Uncoupling protein (UCP)-2 is a member of the inner mitochondrial protein superfamily, whose exact function is not known. Postulated roles include the modulation of reactive oxygen species (ROS) and apoptosis, and immune regulation (Arsenijevic *et al.* 2000). The effect of maternal nutrient restriction in early to mid gestation, coincident with the period of maximal placental growth, on UCP2 and GCR mRNA abundance in the ovine fetal placenta and adolescent lung has not been determined.

Fetal placental tissue was sampled at 80 or 140 days (term ≅147 days) gestation after humane euthanasia from nutrient restricted (NR) singleton-bearing ewes, receiving 60% of their metabolisable energy requirements (MER) from 28 to 80 days gestation, or Controls (C) receiving 100-150% MER (n=5 per group). All ewes were fed to meet their MER up to term. Lung tissue was also sampled from humanely killed adolescent offspring at 6 months born to NR singleton-bearing ewes (n=5 per group). Total RNA was isolated and mRNA abundance was measured by RT-PCR using oligonucleotide primers designed specifically to ovine UCP2 and GCR. Results are given as means (±SEM) relative to 18S rRNA. Statistical differences between groups were analysed by Mann-Whitney U test.

Fetal placental weights decreased in the NR group (C 507.8±64; NR 326.2±20.3g, $p<0.05$) at 80 days, but increased at 140 days (C 183.6±9.6; NR 364.4±21.3g, $p<0.05$) compared to C. Lung fresh and dry weights, and total protein concentration were similar between groups. UCP2 and GCR mRNA abundance in fetal placenta increased with gestational age and with nutrient-restriction in mid-gestation. UCP2 mRNA levels were low in the adolescent lung but increased by NR.

Maternal nutrient restriction in early to mid gestation increases UCP2 mRNA abundance in the fetal placenta and adolescent lung. These changes may be important in modulating ROS and apoptosis and may indicate long-term susceptibility to infection in the offspring.

mRNA abundance in Fetal Placenta and Adolescent Lung

	80D C Fetal Placenta	80D NR Fetal Placenta	140D C Fetal Placenta	140D NR Fetal Placenta	6M C Lung	6M NR Lung
UCP2 mRNA Mean	45.1	68.7**	80.2	97.0**	1.3	3.5*
SEM	3.7	3.0	3.5	3.4	0.2	0.3
GCR mRNA Mean	72.5	101.9**	90.7	118.0*	20.3	37.8
SEM	3.1	4.6	6.5	6.5	2.4	2.8

Significantly different from age matched controls:*$p<0.05$, **$p<0.01$

Whorwood CB *et al.* (2001). *Endocrinology* **142**, 2854-2864.

Arsenijevic D *et al.* (2000) *Nature Genetics* **26**, 435-439.

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

PC1

EXTENDED-VOLUME 3D IMAGING OF RENAL VASCULATURE.

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There is evidence that high blood pressure is associated with changes in renal function and structure of renal vasculature (Anderson et al., 2000). Thus knowledge of the architecture of the renal vasculature is likely to be important for our understanding of the relationship between renal function, structure and blood pressure. The renal vasculature is difficult to measure and reconstruct. A current method requires injection of resins and digestion of the kidney tissue to reveal casts of blood vessels that are imaged using scanning electron microscopy (Denton et al., 2000). This approach provides detail of vasculature but does not allow for easy reconstruction of a 3 dimensional view of the whole renal vessel network. Therefore, we have further developed a method first used to produce extended volume 3D images of myocardium whereby immunofluorescence labelling of blood vessel can be visualised using extended confocal microscopy (Young et al., 1998).

Experimental procedures were approved by the University of Auckland Animal Ethical Committee. The animals were anaesthetised with 3% halothane in oxygen and the abdominal aorta was tied above and below the level of the renal arteries. The animals were killed humanely with sodium pentobarbitone (160 mg/kg) followed by perfusion with saline/heparin/sodium nitrite then wheat germ agglutinin conjugated with tetramethylrhodamine isothiocyanate (WGA-TRITC) and finally with 4% paraformaldehyde in phosphate buffered saline (PBS). The kidneys were removed, post fixed then stored in 0.1 M PBS until resin embedded. Confocal fluorescence laser scanning microscopy was used to obtain 3D images in a contiguous mosaic across the surface of the block. The system consisted of a confocal microscope (Leica TCS 4D) with a Kr/Ar laser and a variable speed Ultramill (Leica). Z-stack volume images were acquired for overlapping x-y areas that covered the region of interest. The scanned volume was milled and the imaging process repeated. The acquired images were combined to reconstruct the volume in 3D.

The renal vasculature was revealed by the presence of fluorescing WGA-TRITC bound to the endothelium of the blood vessel wall. The resultant image reconstruction provides structural detail of the branching network of renal blood vessels as well the orientation relationships.

We have successfully developed a method that allows 3D volume reconstruction of renal vessel architecture of relatively large tissue blocks. The structural information is important for our further understanding of the role of the kidney in the development of hypertension.

Anderson WP et. al. (2000) Hyp. 36, 648-652.

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Young AA et. al. (1998) J. Micros. 192, 139-150.

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

PC2

NOCTURNAL RESETTING OF THE CAROTID BAROREFLEX - A MECHANISM FOR PROMOTING HYPERTENSION

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Obstructive sleep apnoea (OSA) is associated with an increased risk of hypertension, but the reasons for this are unclear. We hypothesise that in normal sleep, as arterial pressure decreases, baroreceptors may reset towards lower pressures and thus protect against hypertension. In OSA however, obstructive episodes result in large increases in arterial pressure which would prevent the downward resetting and could have the opposite effect of resetting baroreceptors towards higher pressures,thus promoting hypertension.

We studied 7 healthy control subjects and 14 patients with OSA. The patients were divided into 2 groups, based on 5 separate blood pressure readings, into normotensive (NT, n=7) and hypertensive (HT, n=7). Baroreceptor testing was performed at 9am and 12pm. We used the neck chamber technique to increase and decrease carotid sinus transmural pressure between -40 and +60mmHg. The responses of mean arterial pressure were assessed. Stimulus-response curves were constructed by fitting a sigmoid function to the data. The maximal differential of this curve (equivalent to sensitivity) and corresponding carotid sinus pressure (equivalent to "set point") were calculated.

Baroreflex sensitivity was not different between the 3 groups and did not change significantly with time. In the control group "set point" was significantly lower at 9am compared to 12pm. However, in both patient groups "set point" was significantly higher at 9am (Table 1.). At both 9am and 12pm "setpoint" was significantly higher in HT compared to both controls ($P<0.001$) and NT ($P<0.05$). Controls and NT were not different at either timepoint.

The results of this study do suggest that in controls there is a downward resetting of the baroreflex in the morning, which may aid in preventing hypertension. The morning upward resetting of the baroreflex in both patient groups may partly explain the development of hypertension in the HT group and may predispose to hypertension in the NT group.

Table 1.

	controls	NT-OSA	HT-OSA
09:00	83.3 ±2.9	92.4 ±5.5	108.5 ±1.6
12:00	88.9 ±2.8	78.1 ±4.2	103.3 ± 2.1
P	<0.05	<0.05	<0.05

Baroreflex "set point" (mmHg)

This research was funded by a grant from the British Heart Foundation.

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

PC3

HCN1 ION CHANNEL SUBUNIT IMMUNOREACTIVITY IN SUPERIOR LARYNGEAL MOTOR NEURONES

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) non-selective cation channels in neurones carry cationic currents proposed to perform diverse functions, such as, generation of rhythmic activity and may underlie the hyperpolarisation activated I_h current. (Pape, 1996). The 4 HCN subunits are differentially expressed in the CNS. Here we examined the distribution of HCN1 channel subunits in the rat brainstem, using immunohistochemistry.

Male rats (150-250g) were humanely killed by intraperitoneal injection of Sagatal (60mg/kg i.p.) followed by transcardial perfusion with 0-4% paraformaldehyde in 0.1M phosphate buffer. Autonomic preganglionic and motor neurones were pre-labelled by intraperitoneal injection of 0.1ml of 0.1% of Fluorogold (Fluorochrome Int.) or application of tracers to the superior laryngeal nerve 3-7 days prior to perfusion. Brainstem sections (50μm) were incubated in either rabbit anti-HCN1 (1:1000 Alomone) with goat anti-choline acetyltransferase (ChAT, 1:500, Chemicon) or mouse anti-non-phosphorylated neurofilament 200 (NF200, clone N52, Sigma, UK) antisera.

All motor neurone pools in the medulla oblongata contained HCN1-immunoreactivity (IR). In the nucleus ambiguus (NA) HCN1-IR neurones contained Fluorogold and ChAT-IR indicating that they have peripherally projecting axons and synthesize acetylcholine, but not all ChAT or FG cells contained HCN1-IR. HCN1-IR in the NA was present only in NF200-IR cells, suggesting that it is expressed in motor but not autonomic preganglionic neurones. The localisation of these HCN1-IR cells was also consistent with motor neurones – they were present in the semi-compact formation of the NA that contains neurones innervating the striated muscle of the larynx and pharynx. Curiously, the compact formation of the NA was unlabelled with HCN1-IR even though it also contains motor neurones, whose target is the oesophagus. However, these cells were NF200 negative, suggesting that they are not α motor neurones. Preliminary experiments indicate that HCN1-IR is present in neurones specifically labelled retrogradely from the superior laryngeal nerve. HCN1-IR may therefore be a useful immunohistochemical marker to specifically identify motor neurones in the NA. Since cells projecting to the larynx display patterns of activity in vivo related to the respiratory cycle it will be of interest to determine if these motor neurones are the prime source of respiratory neurones with I_h recorded in the pre-Botzinger complex (Mironov et al. 2000).

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Supported by the Wellcome Trust and the British Heart Foundation

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

PC4

ACUTE INTRACEREBROVENTRICULAR (ICV) LEPTIN ADMINISTRATION AND THE BAROREFLEX CONTROL OF RENAL SYMPATHETIC NERVE ACTIVITY (RSNA) IN THE ANAESTHETIZED RAT.

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Leptin is a hormone secreted by adipose tissue which may have a role in the generation of obesity-induced hypertension. Previous studies have demonstrated that leptin given icv caused a slow increase in sympathetic outflow to a number of organs including the kidney. Moreover, after a period of 3h, Hausberg et al (2002) reported that baroreflex control of RSNA was enhanced. The aim of this study was to evaluate whether an acute increase in brain leptin, that may occur in the post-prandial state, could influence RSNA and the baroreflex.

Male Wistar rats, 275-375g, were anaesthetized with ip injection of 1ml of a chloralose/urethane (16.5/250mg/ml) mixture. The right femoral artery and vein were cannulated for recording blood pressure (BP) and heart rate (HR), or giving drugs. The left kidney was exposed and an electrode was sealed onto the renal nerves. Rats were placed in a stereotaxic frame and a guide cannula was implanted into the right lateral cerebral ventricle. The BP and RSNA were measured while BP was manipulated by administration of phenylephrine hydrochloride and sodium nitroprusside (both at 10μg in 0.2ml 154mMol NaCl) before and 20min following leptin infusion (5μg bolus followed by infusion at rate 20μg/h). Animals were killed humanely at end of the experiment. Baroreflex curves for RSNA were analysed using a 4-parameter algorithm as described by Miki et al (2003). Data (means±SEM) were subjected to Student's t-test and significance taken as $p<0.05$.

Before the leptin infusion, BP was 97± 4 mmHg, HR was 414±15beat/min. The absolute RSNA values varied between animals, therefore to quantify the RSNA response, percentage changes were calculated by taking the mean of the values before the first baroreflex as 100%RSNA. The logistic parameters describing the baroreflex curves for RSNA ie, the range (A1), slope (A2), midpoint BP (A3), and minimum point (A4) were 111.2±6.8%, 0.101±0.008%/mmHg, 114±3mmHg and 13.5±3.3% respectively. Following 20min leptin infusion, while the BP and HR were unchanged, at 97±4mmHg and 409±15beat/min respectively, the RSNA at 149.0±16.5% was significantly increased. Also, while A1 at 134.3±24%, A2 at 0.108±0.01%/mmHg, A3 at 113±4mmHg were stable, A4 at 47.7±14% was significantly increased.

The data show that leptin acutely raises the baseline RSNA, which was associated with an increase in A4, but without a significant increase in A1. This suggests that brain leptin can acutely increase baroreflex control of RSNA but does not affect the sensitivity of the baroreflex significantly.

Hausberg M, et al (2002). J Hypertens 20, 1633-1641.

Miki K, Yoshimoto M & Tanimizu M (2003). J Physiol 548.1, 313-322.

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

PC5

HYPOGLYCAEMIA-EVOKED HYPERMETABOLISM INCREASES PERIPHERAL CHEMORECEPTOR GAIN IN ANAESTHETIZED RATS

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Insulin-induced hypoglycaemia increases ventilation in a carotid body-dependent manner due to the associated increase in metabolic rate (Bin-Jaliah *et al.* 2004). As an augmentation of peripheral chemoreceptor gain may underlie exercise hyperpnoea (e.g. Weil *et al.* 1972), in this study we evaluated chemoreceptor sensitivity to $P_{a,CO2}$ during elevated metabolism in anaesthetized rats.

Phrenic nerve activity was recorded in adult Wistar rats (300-350g) which were vagotomized, neuromuscularly blocked (pancuronium bromide; 3 mg kg^{-1}, I.V.) and artificially ventilated (O_2-enriched air); adequacy of anaesthesia was continuously monitored (Bin-Jaliah *et al.* 2004).

The level of pulmonary ventilation (V_E) was measured from integrated tracheal airflow which was varied to alter the $P_{a,CO2}$. Thus, both metabolic hyperbolae (the effect of ΔV_E upon $P_{a,CO2}$) and CO_2 chemosensitivity (the effect of $P_{a,CO2}$ upon phrenic nerve activity) were assessed simultaneously. All animals were humanely killed at the end of the experiment. Data are expressed as means ± S.E.M.

Insulin (0.4 U min^{-1} kg^{-1}) lowered blood glucose from 8.96 ± 0.34 mmol L^{-1} to 3.37 ± 0.12 mmol L^{-1} ($P < 0.0001$, ANOVA; $n = 8$). Hypoglycaemia induced an increase in metabolism, as demonstrated by a significant elevation of the $P_{a,CO2}$ by 4.6 ± 1.2 mmHg ($P < 0.01$, paired t test) at a fixed (euglycaemic, eucapnic) level of V_E. The CO_2 chemosensitivity, measured by linear regression, was increased by more than two-fold (from 1.33 ± 0.13 V min^{-1} kg^{-1} $mmHg^{-1}$ to 3.31 ± 0.28 V min^{-1} kg^{-1} $mmHg^{-1}$; $P < 0.0001$, paired t test) by hypoglycaemia. This elevation was mediated via an increase in the inspiratory drive (V_T / T_I) component of each breath (from 0.030 ± 0.009 V s^{-1} $mmHg^{-1}$ to 0.073 ± 0.021 V s^{-1} $mmHg^{-1}$; $P < 0.02$, paired t test). The normalized mean metabolic hyperbolae and linear CO_2 sensitivities showed that $P_{a,CO2}$ remained unchanged (37.3 ± 2.6 mmHg) during hypoglycaemia from the basal setting of 40 mmHg ($P > 0.21$, paired t test).

Thus hypermetabolism during insulin-induced hypoglycaemia is associated with an increase of CO_2 chemosensitivity which is important for the maintenance of eucapnia during hypermetabolism.

Bin-Jaliah I *et al.* (2004). *J Physiol* **556,** 255-266.

Weil JV *et al.* (1972). *J Appl Physiol* **33,** 813-819.

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

PC6

GENE EXPRESSION PROFILES IN THE NUCLEUS TRACTUS SOLITARII OF THE SPONTANEOUSLY HYPERTENSIVE RAT

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Human essential hypertension is a complex polygenic trait with underlying genetic components that remain unknown. Since the nucleus tractus solitarii (NTS) is a pivotal region regulating both baroreceptor reflex function and set-point control of arterial pressure, it may be a participating central nervous site for causing primary hypertension. In this study, we performed cDNA microarray analysis to screen for differentially expressed genes in the NTS between spontaneously hypertensive rats (SHR), which is a well-known animal model for hypertension, and their progenitor, Wistar-Kyoto rats (WKY).

Three week old and 18-week-old male rats were humanely killed by cervical dislocation and the caudal extent of the NTS was micro-dissected out from each animal. Total RNA was extracted and fluorescently labeled cDNA array probes were synthesized using CY-3 as a marker for SHR and CY-5 for WKY. The SHR and WKY probes were mixed and hybridized to a rat cDNA array (Agilent Technologies), representing 14,815 genes. The level of signal in each array was compared between SHR and WKY. Signals exhibiting a >2 difference between these strains were validated using real-time RT-PCR.

22 genes showed a greater expression in young (pre-hypertensive) and adult (hypertensive) SHR relative to WKY, whereas two other genes were down-regulated. So far our validation using real-time PCR has confirmed that junctional adhesion molecule-1 (JAM-1) is highly expressed in both pre-hypertensive (n=4) and hypertensive SHRs (n=6) compared to WKY (young, n=4; adult, n=6). These data suggest that some endothelium-related genes within NTS are differentially expressed between SHR and WKY and that these differences are not secondary to the hypertension. Functional contribution of JAM-1 to blood pressure phenotypes of the SHR and WKY is currently under investigation.

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

PC7

REVERSAL OF DIGOXIN CARDIAC TOXICITY BY AN ANTICALIN (DIGICAL II) IN THE ANAESTHETIZED GUINEA PIG

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Anticalins are a novel class of engineered receptor proteins with prescribed ligand specificity. Digical II is an Anticalin with high affinity (KD = 1.2± 0.2 nM) for digoxin and as such has the potential to reverse digoxin toxicity in man (Schlehuber et al., 2000).

We have tested the ability of Digical II to reverse digoxin toxicity in the guinea pig and described the pharmacokinetics of the interaction between digoxin and Digical II in the pig.

Eleven guinea pigs (average wt 500g) were anaesthetized (ketamine 60mg/kg i.p. and xylazine 8mg/kg i.p., maintenance 1/10 induction dose every 30min), artificially ventilated, a jugular vein cannulated and the ECG lead II recorded. Atropine (0.8 mg/kg i.v.) and digoxin (500ug/kg i.v.) were given followed by an infusion of digoxin (1ug/min i.v.) for 20min. All guinea pigs showed ECG signs of digoxin toxicity by 13.9 min (mean: range 6-35min). In 6 guinea pigs, given saline placebo no reversal of toxicity took place and all animals were dead within 40.8±3.6 min (mean±S.E.M.). In 5 guinea pigs given Digical II (mean dose 12.8 mg i.v. range 9.5—16.4 mg) the mean survival time was significantly increased (p=0.006 unpaired t test) to 112.8±16.3 min (mean±S.E.M.). In these guinea pigs reversal of digoxin toxicity was observed within 29.2 min (mean: range 15—43 min) and all were alive at 60 min or longer and were then humanely killed.

Preliminary experiments in anaesthetized pigs (induction pentobarbitone 30mg/kg i.v. maintenance 6mg/kg/h) were carried out to define the kinetics of the distribution (a) and elimination (b) phases of free digoxin, Digical II and protein bound digoxin. A jugular vein was cannulated for injection of drugs and withdrawal of blood samples. In the absence of Digical II, the t1/2(a) values of free digoxin were 12 and 18min and protein bound digoxin 10 and 20min (digoxin 12.5 ug/kg i.v.). When Digical II (450 ug/kg i.v.) was administered 90min after the initial dose of digoxin, the free plasma digoxin concentration fell below detection limit within 10 min and the elimination phase could not be measured. In contrast, protein bound digoxin increased and then declined with t1/2(a) values of 30 and 35min. These results show that Digical II is capable of rapidly clearing free digoxin from plasma and reversing digoxin cardiac toxicity. Experiments were carried out under licence from the Irish Government.

Schlehuber S, Beste G and Skerra A. J. Mol. Biol. (2000) 297, 1105-1120.

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

PC8

SINO-AORTIC AFFERENTS (SAAS) AND POSITIVE PRESSURE VENTILATION (PPV) RELATED SYMPATHETIC MUSCLE AND THERMOREGULATORY VASOCONSTRICTOR RHYTHMS IN ANAESTHETISED RATS

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Häbler et al. (1993; 1996) frequently observed PPV-related (PPVR) modulation of preganglionic cervical sympathetic activity that was abolished by sino-aortic denervation (SAD), whereas few postganglionic vasoconstrictor neurones supplying hairy skin or muscle (MVC) had similar characteristics under comparable conditions. In contrast, rhythmic discharges at PPV frequency (fPPV) were observed in sympathetic vasoconstrictor fibres regulating thermoregulatory circulations (CVCs: Chang et al. 2000 & Häbler et al. 1999). Here we analyse the simultaneously recorded activity of MVCs and CVCs for such rhythmic discharges and the involvement of SAAs.

Sprague-Dawley rats (male, 260-340g; n=6) were anaesthetised (sodium pentobarbitone 60 mg kg^{-1} I.P.; supplemented with α-chloralose (5-10 mg I.V.) as required until humane killing with anaesthetic I.V.) and vagotomised. Three rats were additionally SAD. Simultaneous population recordings (glass suction electrodes) were made from a gastrocnemius nerve (GN, MVC activity) and an ipsilateral tibial nerve at a point distal to the main muscle branches 'plantar' branch (TNp activity typical of CVCs) (Huang & Gilbey, 2003). GN and TNp activities were abolished following ganglionic blockade (trimetaphan, 12mg.kg^{-1}). Rats were in central apnoea (hypocapnic) and fPPV was manipulated (0.8-2.0 Hz; stroke volume, 2 ml). Autospectra and coherence spectra were computed from rectified and smoothed nerve activities (τ=20ms), arterial blood pressure (BP) and tracheal pressure (TP) (see Chang et al. 2000).

In SAA intact group TP-GN and TP-TNp coherences increased as fPPV decreased: a linear relationship was indicated (regression analysis, TNp data: 16 pts, slope -0.55±0.08 (S.E., $p<0.001$) $r^2 = 0.76$; GN data: 16 pts, slope -0.39±0.10 ($p=0.02$), $r^2 = 0.52$. Following SAD this relationship ceased (32 pts,GN & TNp data pooled, slope -0.008±0.10 ($p>0.09$), $r^2 = 0.11$). In SAA intact group, as fPPV was decreased the power in BP autospectrum at fPPV increased (non-linear regression analysis, 16 pts, Y=A*XB+C*XD; Runs test, P=0.90; best fit, A, B, C and D values = 0.64, -2.63, -0.15 and -0.40). Linear correlation analysis (Spearson r) indicated that BP wave and nerve activity power at fPPV were correlated (BP_TNp, 14 pts, r=0.89, $p<0.0001$; BP_GN, 14 pts, r=0.67, $p<0.01$). In SAA intact group there was a peak at heart rate frequency (fHR) in GN, but not TNp autospectra.

The data support the hypothesis that PPVR variations in SAA inputs, secondary to BP oscillations, contribute to MVC and CVC PPVR rhythms. Such modulation of CVC activity occurs in the absence of a peak in autospectra at fHR.

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Häbler et al (1993) J Neurophysiol 70 920-930

Häbler et al (1996) JANS 61 116-122

Häbler et al (1999) J Neurophysiol 81 2026-2036

Huang & Gilbey (2003) J Physiol 551P PC36

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