late onset hypertension in the offspring. The underlying causes of such programmed hypertension are not known, but may involve alterations in cardiovascular autonomic control. Here, we examined baroreflex and peripheral chemoreflex changes in heart rate (HR) and sympathetic nerve activity (SNA) in the rat following gestational dexamethasone administration (DEX), using the in situ working heart brainstem preparation.

Pregnant dams were administered DEX (200 μg/kg s.c.) at days E15-E16. At birth, litter size was reduced to 8 and pups were left with the dam until weaning. Naïve age-matched rats were used as controls. At 3-5 weeks experiments were performed in the in situ working heart brainstem preparation (Paton, 1996). Under halothane anaesthesia, rats were transected below the diaphragm and decerebrated to the level of the superior colliculus. The rostral portion of the rat was perfused via the descending aorta using oxygenated Ringer's solution. Perfusion pressure, HR, phrenic nerve activity (PNA) and thoracic SNA were recorded. HR and SNA responses to both baroreceptor (a ramp increase in perfusion pressure of 20-30 mmHg) and peripheral chemoreceptor stimulation (injection of 75 μL 0.03% sodium cyanide) were measured. Data are expressed as mean ± SEM and statistical significance determined using unpaired student's t-test.

There was no difference in baseline perfusion pressure (control, 65 ± 3 vs DEX, 66 ± 5 mmHg), HR (control, 327 ± 3 vs DEX, 309 ± 16 bpm) or PNA cycle length (control, 3.3 ± 0.4 vs DEX, 2.8 ± 0.3 s). Baroreflex changes in HR gain (control, -1.3 ± 0.2 vs DEX, -0.8 ± 0.2 bpm/mmHg) and SNA gain (-16 ± 4 vs -6 ± 1 % baseline/mmHg) were both lower in the DEX rat than controls ($P<0.05$, n=9). In contrast, the peripheral chemoreflex changes in HR (control, -161 ± 30 vs DEX, -217 ± 15 bpm) and SNA (control, 146 ± 11 vs DEX, 185 ± 10 % baseline, $P<0.05$, n=5) were augmented, although there was no difference in the change in PNA cycle length (decrease: control, 2.0 ± 0.4 vs DEX, 1.7 ± 0.2 s).

The results indicate that cardiovascular autonomic reflexes are altered in the DEX model of programmed hypertension in such a way as to facilitate an increase in sympathetic drive. This may contribute to the ontogeny of hypertension in this model.

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Supported by British Heart Foundation

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SA1

The feedforward reflex arc for sympathetic thermogenesis in cold defense

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The central nervous system orchestrates the regulation of body temperature within a narrow range to optimize cellular function and facilitate homeostasis. The neural networks mediating thermoregulatory compensations for a cold external environment include the afferent pathway for cutaneous cold, thermoregulatory integrative sites in the preoptic area and hypothalamus and descending pathways to spinal neurons controlling effector function. We have conducted a series of anatomical and *in vivo* electrophysiological studies to elucidate central thermoregulatory networks. Experiments were conducted on rats anesthetized intravenously with urethane (0.8g/kg) and chloralose (70 mg/kg) and under neuromuscular blockade with d-tubocurarine (0.2mg/hr), during which, adequacy of anesthesia was monitored as described(3). Here we describe the feedforward reflex pathway underlying the stimulation of thermogenesis in brown adipose tissue (BAT) in response to skin cooling. The presence of uncoupling protein in the mitochondrial membrane of brown adipocytes allows them to respond to their sympathetic neural input by generating heat through fatty acid oxidation.

Cutaneous cold signals, sensed by TRP channels in thermal receptor membranes, excite cool-responsive neurons in the spinal dorsal horn that provide a glutamatergic excitation to neurons in the lateral parabrachial nucleus(6). Lateral parabrachial neurons excited by skin cooling send their axons primarily to the median preoptic nucleus (MnPO)(6) where they excite GABAergic interneurons that reduce the activity of warm-responsive neurons in the medial preoptic area. The discharge of warm-responsive neurons in the preoptic area is increased as local brain temperature rises(1) and this core temperature information is integrated with inhibitory synaptic input from the cutaneous cold afferent pathway and excitatory synaptic drive from cutaneous warm afferents to produce a preoptic area efferent signal that inhibits cold defense mechanisms, including BAT thermogenesis(2). Thus, stimulation of BAT thermogenesis in response to skin cooling involves disinhibition of the sympathoexcitatory drive to BAT preganglionic neurons. Potential targets of the inhibitory projection neurons in the preoptic area are the dorsomedial hypothalamus (DMH) and the rostral raphe pallidus (rRPa): (a) both sites receive direct projections from the preoptic area, (b) blockade of $GABA_A$ receptors in either the DMH or the rRPa stimulates BAT thermogenesis and (c) blockade of glutamate receptors in either site eliminates skin cooling-evoked increases in BAT sympathetic nerve activity (SNA)(4,5,7). The rRPa and the neighboring parapyramidal area contain BAT sympathetic premotor neurons whose activity is necessary for activation of BAT thermogenesis, including that evoked from the DMH, which sends a direct projection to the rRPa(7). At least some BAT sympathetic premotor neurons in rRPa contain serotonin and spinal serotonin release can augment the level of BAT SNA by potentiating the glutamate receptor-mediated excitation of BAT sympathetic preganglionic neurons(3). In summary, the cutaneous cold afferent pathway involves dorsal horn, lateral parabrachial nucleus and MnPO neurons that act to inhibit warm-responsive inhibitory projection neurons in the medial preoptic area which allows increased activity in an efferent pathway involving DMH neurons, BAT premotor neurons in rRPa and BAT sympathetic preganglionic neurons that drives a feedforward increase in BAT thermogenesis to prevent a fall in brain and core body temperatures.

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Research supported by NIH grants NS40987 (SFM), DK57838 (SFM) and DK65401 (CJM) and by the Japan Society for the Promotion of Science (KN).

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

SA2

Altered brainstem vasculature in neurogenic hypertension

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Essential hypertension is an enigma. It has escalated to 900 million people worldwide and is rising[3]. One-in-three of the UK population are now affected (www.heartstats.org). With the alarming statistic that between 55-60% of essential hypertensive patients on medication remains hypertensive[1,4], there is an urgent need to discover new targets. One relatively unexploited organ is the brain. Indeed, central autonomic nervous mechanisms may contribute to the pathogenesis of essential hypertension (e.g. Grassi 2004; Smith et al. 2004) yet current anti-hypertensive drugs were designed to target peripheral organs. Because of its role in both set-point determination of arterial pressure and control of the gain of the arterial baroreceptor reflex, we have focussed on the nucleus tractus solitarii (NTS) located in the dorsomedial medulla oblongata. Our previous studies on NTS have revealed a novel target that of the microvasculature and related proteins such as endothelial nitric oxide synthase[7] and junctional adhesion molecule-1[6] as major regulators of arterial pressure in hypertensive rats. To further delineate genes associated with the microvasculature that are associated with hypertension, we used an Affymetrix rat gene chip and compared differentially expressed genes from enriched isolated vessels from the brainstem of pre-hypertensive spontaneously hypertensive rat (SHR, n=5) and aged matched Wistar Kyoto (WKY, n=5) rats. We found 210 differentially expressed genes of which 94 were up-regulated in SHR and 116 down-regulated. Following a cluster analysis, differentially expressed genes from both whole NTS and enriched vessels showed similarity in revealing associations with inflammation, hypoxia and angiotensin II mediated intracellular signalling. Validation of some of the differentially regulated genes has commenced using both real time RT-PCR and *in vivo* studies including both pharmacological approaches (see Hendy et al. – this meeting) and gene transfer into NTS using viral vectors to determine any functional role in generating hypertension. We are also making comparisons between SHR/ WKY rats and human (hypertensive *versus* normotensive) brainstem from post-mortem tissue to see whether our rat data translates to the human condition. The presentation will illustrate that the endothelium within the brainstem, including the NTS, of the SHR appears 'sticky' and inductive to leukocyte adhesion, and that when induced can cause high blood pressure. It also is apparent that the SHR brainstem may be borderline hypoxic due to both the leukocyte adhesion and resultant increased vascular resistance as well as the smaller internal diameter of the feeder arteries (basilar and vertebral) to the brainstem. We will propose a hypothesis that an inflamed vasculature, high vascular resistance and relative low oxygen levels in the brainstem may be a pre-requisite to elevated arterial pressure in the SHR, and possibly also in man.

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Supported by the British Heart Foundation, BBSRC and the NIH.

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SA3

From the beginning: Differential pre- and postsynaptic mechanisms tune afferent processing within the solitary tract nucleus

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The nucleus of the solitary tract (NTS) contains the central terminations of a broad range of cranial primary afferents that engage homeostatic reflex pathways. These afferent-activated pathways both directly regulate visceral organs (e.g. heart) and indirectly affect integrated responses (e.g. stress or satiety). The heterogeneity of NTS – its various cell types, transmitters, receptors and interconnections with other brain regions - complicates experimental work. Thus, understanding is limited about the mechanisms by which afferent information is conveyed, transformed and then transmitted beyond NTS. Recent work suggests that NTS may be organized quite distinctly depending on the central destination of the afferent information and the evidence suggests that selective deployment of molecular effectors result in distinctly different integrative outcomes.

The sources, myelination and modality subtypes of cranial visceral afferents to NTS are diverse across and within organs.

Afferents are viscerotopically distributed in NTS and feature characteristic differences in key receptors and enzymes consistent with physically interspersed cohorts of neurons sharing a common property. Understanding this cellular patterning and how it impacts processing seem key to appreciating the broader contributions of NTS neurons to integrated response behaviors.

Visceral afferent transmission with NTS relies on glutamatergic mechanisms but overall appears to transgress conventional precepts of central integration of excitation. Central glutamatergic excitatory synapses are: 1. often preferentially deployed on distal dendrites, 2. low in their probability to release vesicles, and 3. relatively weak – properties that generally favor excitation of central neurons by highly redundant, convergent inputs such as at Schaeffer collaterals to CA1 pyramidal cells (Allen and Stevens, 1994). EPSCs from solitary tract (ST) afferents at second order NTS neurons uniformly violate each of these transmission features. ST activation evokes EPSCs that largely rely in non-NMDA receptors and these afferent synapses rarely (<1%) fail to release glutamate. The large amplitude of such currents assures a safety factor of generating an action potential nearly every time and even can endure substantial synaptic depression before attenuating spike output of the second order neurons. Quantal analysis of ST transmission reveals an extraordinarily uniform mechanism that produces a high probability of glutamate release across second order NTS neurons. So with respect to basic glutamate release, ST afferent terminals are remarkably homogeneous, much more so than is common in most brain regions.

Another approach in brain slices identifies for study cohorts within NTS by their destination. The approach marries classic neuroanatomical tracers to high resolution electrophysiological recordings (Bailey et al., 2006; Bailey et al., 2007). This work offers a new perspective about homogeneities within NTS sub groups associated with particular central destinations – i.e. NTS projection neurons. While retrograde dyes disclose projection targets, electrophysiology probes functional aspects of circuit organization. Basic properties are probed with pharmacological tools such as capsaicin (CAP) to classify afferents as unmyelinated (CAP-sensitive)(Doyle et al., 2002). Questions include transmitter interactions; voltage-dependent ion channels; and the organization of intra-NTS circuits. Interestingly, the organization and intrinsic cellular differences in myelination subtypes substantially impact information transfer to the central nervous system.

Retrograde fluorescent tracers injected into central target regions identify pools of neurons within NTS that project to that particular region. Dye injected into the hypothalamic paraventricular nucleus (PVN) illuminate cohorts of single NTS neurons in vitro with axons projecting to PVN. Likewise, injections within the caudal ventrolateral medulla (CVLM) identify CVLM-projecting NTS neurons. ST activation indicates that all CVLM-projecting NTS neurons were directly connected to ST afferents with an EPSC latency that varied (jitter=S.D.) <200 μsec. In contrast, similar tests of PVN-projecting, medial NTS neurons revealed weaker, polysynaptic intra-NTS excitatory pathways from ST with higher jitter and such neurons expressed substantially larger expression of the A-type, transient potassium channel (IKA). Together, both the convoluted intra-NTS pathway as well as the elevated IKA expression attenuated the afferent information content sent to PVN compared to that via the CVLM pathway. Overall, projection target information identifies relatively homogeneous populations of neurons within the more general heterogeneity of neurons that dominates views of NTS.

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Work supported by NIH grants HL-41119 and HL-56460.

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SA4

The cortical network associated with baroreflex cardiovascular control in humans

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The baroreflex regulates cardiovascular dynamics by integrating afferent feedback signals from the heart and blood vessels with parasympathetic (PNS) and sympathetic (SNS) efferent activity to cardiovascular end organs. In addition to known brainstem pathways there is growing evidence from neuroanatomical, clinical and surgical neurostimulation studies that forebrain cortical sites have an important role in modulating cardiac rhythms and blood pressure through the autonomic nervous system (Cechetto & Saper, 1990; Critchley et al. 2003; Oppenheimer et al. 1992). These sites include the insular cortices (IC), anterior cingulate cortex (ACC), medial prefrontal (mPFC), amygdala and cerebellum. Recent advances in neuroimaging technologies have allowed exploration of this cortical autonomic network (CAN) in conscious humans. Studies using effortful volitional and cognitive tasks have associated activity in the dorsal ACC and IC regions with autonomic aspects of cardiovascular arousal. However, such tasks produce elevations in heart rate (HR) and mean arterial pressure (MAP) as well as changes in PNS and SNS efferent activity. As afferent signals from the heart and baroreceptive vascular regions are represented in the forebrain, and efferent signals generate changes in HR and MAP, it remains uncertain whether the cortical activity during volitional effort represents viscerosensory or visceromotor components of baroreflex-mediated cardiovascular arousal and/or muscle activation. Our goal has been to study the cortical organization of baroreflex-cardiovascular integration and differentiate the afferent and efferent components. Functional magnetic resonance imaging (fMRI) is used to assess cortical activation patterns during various manoeuvres that differentially affect HR, MAP, sympathetic nerve activity (SNA) and cardiac output. Our findings presented here focus on the most robust responses in the CAN. During a 30-sec isometric handgrip (IHG) contraction of moderate intensity (e.g. 30% maximal), both HR and MAP are increased (Wong et al. 2007b; Wong et al. 2007a). The tachycardia is due to PNS withdrawal and is associated with deactivation in the mPFC and bilateral IC activation. Additional studies were designed to differentiate

the baroreceptor "loading" phase from concurrent bottom-up changes in MAP and HR from top-down volitional effort during IHG. HR rises rapidly during a 2-sec isometric contraction whereas the MAP response is delayed, cresting 2-5 sec after the contraction. During a 30% maximal effort IHG lasting 2-sec, where HR increases but SNA is unchanged, deactivation in vMPFC is observed along with bilateral IC activation. SNA does increase during a strong (e.g. 70% maximal) 2-sec contraction, along with HR, and is associated with additional activation in the dACC. However, the post-contraction rise in MAP was associated with activation only in the left posterior IC. More direct and physiologic assessment of baroreflex control can be accomplished using low to moderate levels of lower body negative pressure (LBNP) that unloads the baroreceptors through reductions in central filling pressure and pulse pressure. In this model MAP remains constant and the HR and SNA responses can be differentiated with varying levels of suction. When only SNA rises (-15 mmHg suction) dACC and/or genual ACC activation is observed along with right posterior superior IC activation. When both HR and SNA rise (-35 mmHg), additional deactivation in the mPFC, amygdala, and bilateral central IC (emphasizing posterior inferior right IC) is observed (Kimmerly et al. 2005; Kimmerly et al. 2007a; Kimmerly et al. 2007b). Passive rises in MAP and reductions in HR were elicited with phenylephrine infusions producing a direct arterial baroreflex effect. These events are associated with mPFC activation (i.e. mPFC is still negatively correlated with HR), bilateral inferior IC activation and dACC activation. In summary, the studies suggest that: 1) baroreceptor loading through increased MAP (passive and active) produces bilateral (and predominantly left) anterior IC activation; this suggests a viscerosensory role of these regions. 2) During baroreceptor unloading with stable MAP (LBNP), increased SNA alone is associated with right superior posterior IC activation; if both SNA and HR increase there is additional activation in the dACC and deactivation in the mPFC and inferior IC regions. 3) Changes in SNA, be they an increase or decrease, were always associated with activation of the dACC or genual ACC. 4) Changes in HR were always negatively correlated with mPFC. Our studies emphasize the CAN regions that are involved in reflex cardiovascular control, independent of effort.

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Supported by Heart and Stroke Foundation of Canada, Canadian Space Agency.

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SA5

NOS activity as a marker for cardiac neural regulation in health and disease

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Abnormal neurohumoral activation (as seen in hypertension) is a negative prognostic indicator for sudden cardiac death and a strong independent predictor of mortality. Our work and that of others has recently established that nitric oxide (NO) inhibits cardiac sympathetic activity, decreases adrenergic regulation of ICaL in sino atrial node cells, and facilitates cardiac parasympathetic transmission. This talk will review the emerging evidence that supports the idea that upregulation of neuronal NOS by either exercise training or gene transfer is beneficial in restoring the normal cardiac neural phenoptype. However, a reduction in NO bioavailability in hypertension caused by oxidative stress impairs cyclic nucleotide signalling and contributes to sympathetic hyperresponsiveness and vagal impairment. Overexpression of nNOS into cardiac sympathetic nerves induced by cell specific adenoviral gene transfer can rescue this effect by increasing cGMP dependent modulation of intracellular calcium leading to normal neurotransmission. The significance of these observations in the wider context of cardiac neural control will be discussed.

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SA6

Factors influencing orthostatic tolerance in humans

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Orthostatic tolerance (OT) refers to a person's ability to maintain normal blood pressure and consciousness when subjected to gravitational stress. It varies widely between healthy individuals. Gravity imposes a pressure gradient along the body resulting, when upright, in the loss of effective blood volume through distension of dependent veins and transudation through capillaries. This reduces venous return and cardiac output but, due to reflexes, blood pressure does not normally fall. However, if the stress is sufficient, for example by increasing gravity using a centrifuge or applying suction to the lower body, fainting occurs in all subjects. My research, undertaken over several years, has been concerned with ways of assessing orthostatic tolerance, factors which influence it, and methods to increase it.
Orthostatic tolerance is usually assessed by determining whether a subject faints when upright for varying periods. However, this is insensitive and unreliable and we have developed a method which combines head-up tilting with graded lower body suction and measures orthostatic tolerance as the time required to induce presyncope. Using this we showed that an

important determinant of OT is plasma volume (El-Sayed & Hainsworth, 1995). Interestingly, we have recently shown that Andean high altitude dwellers, who have very large packed cell volumes, also have an exceptionally high OT (Claydon et al. 2004).

Blood pressure is regulated mainly through the control of vascular resistance and subjects with good OT show greater increases in vascular resistance. This is partly due to increased sensitivity of the baroreceptor reflex during the orthostatic stress (Cooper & Hainsworth, 2001). The factor that is critical for normal consciousness is actually cerebral blood flow, and autoregulation of this is more effective in subjects with good OT. During orthostatic stress people usually hyperventilate to some extent and the resulting hypocapnia dilates peripheral vessels and constricts those in the brain. Both effects reduce cerebral flow. Norcliffe et al. (2008) showed that both these responses were greater in fainting subjects and this is likely to contribute to their attacks.

Strategies for increasing OT are based on manipulating the above factors, particularly plasma volume. Salt loading had been shown to expand plasma volume in dogs and we conducted a double-blind placebo controlled study of the effects of salt supplement in patients with poor OT and fainting attacks (El-Sayed & Hainsworth, 1996). This showed improvement in those taking salt and that all those in whom plasma volume increased OT also increased. The effects of salt, however, are complex as it also results in increased baroreceptor sensitivity and improved autoregulation of cerebral flow (Claydon & Hainsworth, 2004). Interestingly, drinking just water also improves OT although the mechanism is uncertain (Claydon et al. 2006). Of particular relevance to this symposium is the effect of exercise training. Training is known to increase blood volume and so should be of benefit to fainting patients. Mtinangi & Hainsworth (1999) put untrained subjects on a training schedule (5BX/XBX, Royal Canadian Air Force) to reach a "target" level of activity. Fitness increased in all subjects, as assessed by the heart rate-oxygen uptake relationship, and all showed increases in plasma and blood volumes. However, OT increased only in those with a relatively low initial OT and this may be related to earlier observations of fainting in some very highly trained subjects. The same study was then carried out on 14 patients with fainting attacks (Mtinangi & Hainsworth, 1998). Twelve of these trained successfully and all 12 had increases in plasma volume and OT, and were symptomatically improved. Since then we have advocated exercise training as an option for managing such patients.

Although increasing plasma volume is usually effecting in increasing OT, another approach is to reduce the actual stress. Venous pooling and capillary fluid loss are minimised by leg movement and the "muscle pump". We have identified asymptomatic subjects who, despite never normally fainting, have poor orthostatic test results. They apparently compensate by increased postural sway movements during normal standing. Many patients, on the other hand, who have similar poor test results, have smaller postural movements, possibly explaining why they faint and the volunteers don't (Claydon & Hainsworth, 2006).

In conclusion, OT is influenced by several factors including plasma or blood volume, reflex responses and cerebral autoregulation, and any interventions that change these are likely to change OT. However, the stress may be minimised by avoiding standing still and encouraging exaggerated postural movements.

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SA7

Amelioration of autonomic nervous system activity in chronic heart failure patients: Exercise better than cardiac resynchronisation therapy?

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Exercise training represents an efficient therapy in stable, chronic heart failure (CHF). The impact of cardiac rehabilitation programmes on autonomic nervous system (ANS) equilibrium is now well established. Furthermore, biventricular pacing (BiP) is emerging as an important long-term therapy for symptomatic CHF patients. Such resynchronisation therapy may achieve several of the treatment goals in CHF including slowing of disease progression and survival.

Analysis of heart rate variability (HRV) has become an important method for assessing cardiac autonomic regulation and has been shown to predict clinical outcome in CHF (arrhythmic as well as non-arrhythmic mortality). Cardiac resynchronisation therapy improves autonomic function by increasing HRV as well as exercise training do. The interpretation is a shift of cardiac autonomic balance toward a more favorable profile that is less dependent on sympathetic activation. The effect is sustained in advanced CHF. Lack of HRV improvement four weeks after BiP could identify patients at higher risk for major cardiovascular events. Lack of baroreflex response improvement after cardiac rehabilitation could also identify subjects at high risk for arrhythmic event.

Cardiac resynchronisation therapy is also associated with long-term improvement in cardiac sympathetic nerve activity as reflected by improvements in cardiac 123I-MIBG uptake.

Two months BiP, as well as 8 weeks of exercise training, decreases muscle sympathetic nerve activity (MSNA) in patients with severe CHF. Such reversible sympathoinhibition is a marker of the clinical response to cardiac resynchronisation therapy.

In conclusion: exercise training, biventricular pacing have significant favourable effects on ANS equilibrium in CHF. Modifications of autonomic balance following BiP or exercise training could be related to a possible decrease in mortality in CHF patients.

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SA8

5-Hydroxytryptamine – a critical neurotransmitter in cardiovascular regulation - a scientific remembrance of David Jordan

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Considering the relatively small number (thousands cf. billions) of 5 HT (serotonin) containing neurones that are found in the brain it is surprising that 5 HT is involved in so many functions. The function of 5-HT in central cardiovascular respiratory regulation in a sense has been overlooked and even here 5-HT may be considered to play "third fiddle" to amino acid and adrenergic transmission. This is not surprising as when David Jordan and I started our collaboration in the mid 80s the view was that "alteration in ... cerebral 5-HT systems can alter BP but unfortunately ... the role of 5-HT in BP regulation can only be made with caution"(Kuhn *et al.,* 1980 Hypertension 2:243). The system needed a drug similar to clonidine in the adrenergic system. For the 5-HT system this turned out to be 8-OH-DPAT, a simplified ergot congener, which was selective for 5 HT_{1A} receptors (there are 14 receptor subtypes). The observations that 8-OH-DPAT caused a fall BP and a very large increase in vagal drive to the heart quickly triggered our collaboration.

Initially, this led to the demonstration that 5-HT_{1A} receptors, at the level of the nucleus ambiguus, are involved in the genesis of cardiopulmonary afferent evoked vagal bradycardia. The role of 5-HT_{1A} receptors in other reflex bradycardias shows a degree of species variation (see Jordan, 2005). In contrast there is no evidence for a role for 5 HT_{1A} (sympathoinhibitory) or 5-HT_2 (sympathoexcitatory) receptors, in the sympathetic regulation of the heart (see Ramage 2001). Interestingly, activation of central 5-HT_2 receptors causes vasopressin release and this has been suggested to play an important role in blood volume regulation. In this respect chronic treatment with a 5-HT_2 receptor antagonist prevents the development of DOCA-salt hypertension, which is consistent with the view that this form of hypertension requires the release of vasopressin. This system may also be involved in stress hypertension.

The involvement of 5-HT_3 receptors in cardiovascular regulation was also studied. This receptor is found in a very high density in the nucleus tractus solitarius (NTS), the site of termination of baroreceptor and other visceral afferents (Jordan & Spyer, 1986), and the dorsal vagal nucleus (DVN). Within the DVN and NTS ionophoretic application of the highly selective 5-HT_3 receptor agonist phenylbiguanide (PBG) excited most neurones tested, and this excitation was blocked by the antagonist granisetron. Intracellular recording (*in vivo*) showed that PBG application caused little change in the membrane potential although firing rate increased, while the glutamate receptor agonist DLH caused membrane depolarization with increased firing. This indicates that neuronal excitation by 5-HT_3 receptors is indirect and is consistent with the view that these receptors are mainly on afferent terminals. Further experiments demonstrated that both in the DVN and NTS, activation of 5-HT_3 receptors causes the release of glutamate which acts on NMDA and/or kainate receptors to activate these neurones (Jeggo *et al.,* 2005). It was suggested that the source of glutamate could also be glial cells in the NTS (Llewellyn-Smith *et al.,* 2004).

More recently 5-HT_7 receptor blockade was shown to abolish all reflex (cardiopulmonary, baroreceptor and chemoreceptor) evoked increases in vagal drive to the heart in both anaesthetized and conscious rats (Kellett *et al.,* 2005). This is believed to occur at the level of the NTS (Oskutyte *et al.,* 2008). In addition, depletion of 5-HT with p-CPA causes an increase in BP in awake rats and attenuates the baroreflex gain in both awake and anaesthetized rats.

In conclusion it can be stated that 5-HT is released in the reflex activation of cardiac vagal pathways and that activation of 5-HT_7 receptors is essential for the mediation of such effects. However the role for 5 HT_{1A} and 5 HT_3 receptors is far less clear. In blood pressure regulation there is an indication that 5-HT pathways are involved, at least in hypertension, especially that which involves vasopressin release.

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I wish to acknowledge the late David Jordan's essential contribution to these studies.

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SA9

Sympathetic Rhythms: some underlying mechanisms and speculations on function

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In a seminal paper "Discharges in mammalian sympathetic nerves", Adrian et al.(1932) described the rhythmic nature of sympathetic nerve discharges. Since then some of the mechanisms underlying sympathetic rhythm generation have been unravelled, yet many aspects of their functional significance remain elusive.

One of the rhythms documented by Adrian et al. was that related to central respiratory drive, which may be relevant to

the coordination of cardiovascular and respiratory functions. Work from Polosa's laboratory (e.g., Priess & Polosa, 1975) documented patterns of respiratory-related activity of single sympathetic preganglionic neurones (SPN). Such activity may arise from the interaction of rhythmic and tonic components at the level of the SPN. This contention is supported by the observation of Gilbey et al. (1986) that quiescent SPNs brought to threshold by the ionophoretic application of glutamate could have firing patterns related to central respiratory drive.

Probably the first indication that rhythmic activity might arise within sympathetic networks themselves, rather than from rhythmic inputs to tonic sympathetic-tone generating networks, came from the work of Green & Heffron (1967; see Barman & Gebber, 2000). Subsequently, Gebber became a main proponent of the sympathetic rhythm generator hypothesis and identified sympathetic rhythms within medullary networks (see Barman & Gebber, 2000). However, is now becoming established that some sympathetic rhythm generating substrates are located within the spinal cord.

Yoshimura et al. (1987) observed noradrenaline-induced membrane potential oscillations in SPNs recorded in slice preparations. These remained in the presence of tetrodotoxin. Chitz et al. (1997) reported somatic motor coupled postganglionic rhythmic sympathetic discharges in an in situ perfused spinalised rat. Recent work from Sue Deuchars laboratory has demonstrated, in sympathetic regions of rat spinal cord slices, 5-HT-driven rhythmic population activity . Marina et al. (2006) noted that intrathecal application of 5-HT to the thoracolumbar spinal cord in lower thoracic spinalised anaesthetised rats generated a ~ 1 Hz rhythm in sympathetic activity supplying the rat tail circulation. The rhythm was similar to the spontaneous rhythm observed in an intact anaesthetised animal (T-rhythm, see Gilbey, 2007). Entrainment of such spinal rhythms may be a mechanism for the coupling of networks involved in controlling various motor outflows; e.g., sympathetic and respiratory. Indeed studies from my laboratory have shown that whereas in the absence of an entraining input the T rhythm discharges recorded from pairs of single postganglionic neurones innervating the rat tail circulation can be dissociated, they can be entrained by various input(s); e.g., those related to central respiratory drive (see Gilbey, 2007).

It can be hypothesised that sympathetic rhythm generation within the spinal cord allows for the flexible coupling of sympathetic activity with other networks; e.g., respiratory and locomotor. The degree of synchronisation of rhythmic sympathetic activity can depend on the strength of the entraining input and its timing with respect to the phase of the rhythm generator. Regulating the degree of synchronisation of sympathetic discharges in this manner may increase the efficacy of transmission along the efferent pathway from spinal cord to effector. Furthermore synchronisation of activity of SPNs may lead to the recruitment of quiescent SPNs (see Gilbey 2007). These hypotheses remain to be tested.

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The British Heart Foundation & The Wellcome Trust

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SA10

Sympathetic preganglionic neurones *in situ*: Respiratory drives

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The sympathetic nervous system regulates the activity of a diverse range of target tissues through functionally specified efferent pathways. Since the earliest recordings of sympathetic nerves (Adrian et al. 1932) it has been known that many sympathetic outflows exhibit a pronounced, centrally generated, respiratory modulation of their activity (reviewed by (Habler et al. 1994)). In the specific case of the sympathetic control of the vasculature this respiratory modulation produces Traube-Hering waves, fluctuations in arterial pressure in phase with respiration. We have hypothesised that alterations in the strength of this respiratory-sympathetic coupling may be a causative factor in the development of hypertension.

Previous work in the adult spontaneously hypertensive rat has reported changes in the phase relationship between respiration and sympathetic nerve activity (Czyzyk-Krzeska & Trzebski, 1990). We have extended these observations and find an enhancement of respiratory-sympathetic coupling in neonatal and juvenile spontaneously hypertensive rats, during the "pre-hypertensive phase" (see Simms et al. this meeting). This change in respiratory-sympathetic coupling occurs early in the development of elevated blood pressure, suggesting a potential causative link.

At present, we have limited knowledge of the cellular and network interactions that produce the respiratory modulation of sympathetic activity at the level of the sympathetic preganglionic neurone (SPN). We have developed an approach allowing whole-cell patch clamp recordings from SPN in the neonatal rat working heart-brainstem preparation (Paton, 1996). This preparation generates robust central respiratory activity and shows characteristic respiratory modulated patterns of sympathetic output to that reported in vivo. Recordings from over 80 SPN in the upper thoracic spinal cord have shown several different patterns of respiratory modulation with either excitatory and/or inhibitory drives associated with the phases of the respiratory cycle. We have also functionally identified SPN on the basis of their responses to cardiorespiratory afferent activation e.g. peripheral chemoreflex, diving response and baroreceptor reflex. This

has shown that some of these patterns of respiratory modulation are associated with different functional classes of SPN. For example putative muscle vascoconstrictor SPN, which play a key role in determining blood pressure, are predominantly excited in late inspiration/early expiration and inhibited in late expiration/early inspiration (Stalbovskiy et al. this meeting).
From these data, we conclude that alterations in respiratory-sympathetic coupling appear to have a role in the development of hypertension and using in situ recordings we are able to examine the alterations in both inhibitory and/or excitatory respiratory drives to the muscle vasoconstrictor sympathetic preganglionic neurones, which are key determinants of arterial pressure.

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Funded by the British Heart Foundation and NHMRC.

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SA11

Local influences on sympathetic activity in the spinal cord – role of interneurones and tonic inhibition

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Many CNS regions and neuronal types shape the level of sympathetic activity by ultimately influencing spinal sympathetic preganglionic neurones (SPNs), the sole sympathetic output from the CNS. The spinal cord itself provides a high degree of complexity and co-ordination of sympathetic control through the many interneurones that are crucial components of CNS circuits. Surprisingly, there is little information regarding the characteristics of these interneurones involved in sympathetic control, partly due to problems associated with their identification. We have identified novel groups of local sympathetic interneurones and here we discuss how these interneurones may fit into circuits involved in sympathetic control and how activation of specific receptors, both on these interneurones and on SPNs may contribute to control of SPN activity.
These data are obtained using recording and filling of neurones in thoracic spinal cord slices of rats (11-14 days) that were terminally anaesthetised with urethane (2g/kg, i.p.) and transcardially perfused with ice cold 215 mM sucrose aCSF (Deuchars et al. 2005). One group of interneurones in particular will be considered here, within the central autonomic area (CAA). We have shown that GABAergic neurones in the CAA form direct monosynaptic, inhibitory connections with SPNs (Deuchars et al., 2005). Recording and filling interneurones within the CAA has revealed a complex degree of local axonal projection. The local axon ramifies extensively in the intermediolateral cell column and intercalated nucleus where it forms direct synaptic contacts (verified at the electron microscope level) onto immunohistochemically identified cholinergic SPN dendrites. Furthermore, axons also extend to the ventral horn where they also directly synapse onto cholinergic motoneurones. This exciting finding has implications for a possible spinal cord site of co-ordination of sympathetic and motor outflow, as suggested by (Chizh et al., 1998).
Since serotonin is known to profoundly influence sympathetic outflow at the spinal level (Madden & Morrison, 2006;Marina et al., 2006) and also shapes the rhythmic activity of motor outflow (e.g. (Schmidt & Jordan, 2000), the effects of serotonin on CAA interneurones and on rhythmic oscillations recorded from populations of neurones in the IML has been investigated. Depolarisations and hyperpolarisations of CAA neurones were elicited with both 5-HT and, somewhat surprisingly, the 5-HT 2 receptor agonist α-methyl5-HT. The unusual pharmacological profile of the hyperpolarisations is currently under investigation. 5-HT also induced or increased the power of ongoing oscillations recorded in the IML, using "field" recording techniques. We have shown that this 5-HT driven oscillatory activity relies on the presence of gap junctions in the network and on ongoing GABAergic activity.
Since these GABAergic interneurones provide the first evidence of a local GABAergic inhibitory influence on SPNs, we have also re-examined how GABA may affect SPN activity and have uncovered a novel tonic inhibitory GABAergic role, mediated by receptors containing α5, rather than delta subunits. This tonic GABAergic inhibition is maintained in low Ca2+/high Mg2+ and also in tetrodotoxin and acts to reduce the excitability of SPNs by holding them at a more hyperpolarised potential and reducing the numbers of action potentials elicited during a depolarising pulse. Elevation of such a tonic inhibitory influence may be a potential target for treatment of conditions where there is a chronic elevation in sympathetic activity.
To summarise, these data provide evidence for a complex level of control of sympathetic outflow within the spinal cord. As we unravel the circuits involved in influencing sympathetic activity, we gain insight into ways in which precise control of specific pathways may be achieved.

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The generous support of the British Heart Foundation is gratefully acknowledged

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SA12

Interactions between supraspinal, propriospinal, and segmental inputs to sympathetic preganglionic neurons: challenges in spinal cord injury and repair

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Sympathetic preganglionic neurons (SPN) receive direct input from many levels of the neuroaxis, including the cervical, thoracic, and lumbosacral spinal cord, brainstem, hypothalamus, and even the cerebral cortex. In animals with intact spinal cords, nearly all tonic excitatory drive to SPNs descends from supraspinal systems. In these animals, tonic inhibitory input to SPN from segmental and propriospinal neurons is more important than excitatory input from these sources. Indeed, spinal excitation of SPN is inhibited both by descending pathways originating from supraspinal systems and from intraspinal systems. Thus, tonic and reflex-elicited sympathetic activity after spinal cord injury (SCI) is thought to result from disinhibition of excitatory spinal interneurons (IN) and from loss of descending excitation of spinal inhibitory IN. Where are the segmental and propriospinal IN that affect SPN located? Both retrograde transynaptic tracing and neurophysiological recordings indicate that the longitudinal distribution of excitatory neurons affecting renal sympathetic nerve activity (RSNA) is similar to the distribution of the SPN that generate that activity; the maximum concentration of both SPN and their associated IN is between T10 and T12 (1,2,3). On the other hand, propriospinal neurons projecting from rostral cervical spinal cord to caudal thoracic cord appear to be exclusively inhibitory (4). Both sympathoexcitatory and sympathoinhibitory propriospinal projections from lumbosacral spinal cord to caudal thoracic spinal cord have been reported. Surprisingly, these ascending propriospinal pathways, important as they are in autonomic dysfunction after SCI, are only now being examined in detail. Although previous studies have confirmed direct projections from the major sympathoexcitatory site in the rostral ventrolateral medulla (RVLM) to SPN, the degree to which the RVLM's excitatory effects are mediated by these direct projections, or by projections to excitatory IN, has only been determined recently. We found that the incidence of retrogradely-identified renal sympathetic IN closely apposed by RVLM projections was only one-fifth the incidence of SPN closely apposed by these projections (5). These data confirm our electrophysiological observations that in rats with intact spinal cords, many SPN but few spinal IN exhibit activity correlated with RSNA (6).

In recent years, a major concern of this laboratory has been the possibility that treatments currently under development for spinal cord injury may disrupt the function of the complex (and still incompletely-characterized) interactions between segmental, propriospinal, and supraspinal sympathetic systems. Yet in developing treatments, few laboratories assess autonomic function and dysfunction other than bladder control and the severity of autonomic dysreflexia. In a recent study, we used as our model the abundant sprouting of the corticospinal tract (CST) rostral to a chronic spinal cord injury (7). This model is relevant because encouragement of sprouting of lesioned descending pathways is being investigated in experimental animals as a promising treatment for SCI. We hypothesized that the synapses of sprouting axons, if they impinged on SPN, might amplify the modest increase in RSNA elicited when we microstimulated the thoracic CST. We identified "renal" SPN and IN by retrograde transport of pseudorabies virus from the kidney. We identified CST axons by anterograde transport from the cortex. Six times as many labeled SPN and three times as many labeled IN were closely apposed by CST axons one month after a chronic lumbar lesion than in unlesioned rats. Nevertheless, responses in RSNA to stimulation of the thoracic CST just caudal to an acute spinal transection were unaffected in the lesioned, sprouting rats. Apparently, the new synapses on sympathetic neurons were not sufficiently powerful, dense, or numerous enough to affect CST control of RSNA. These results are encouraging. They show that an increase in contact between a sprouting descending pathway and spinal sympathetic neurons need not lead to dysfunction. Nevertheless, we suggest that it would be prudent to conduct similar experiments routinely to assess the autonomic effects of treatment-induced sprouting and regeneration after SCI.

In summary, appropriate levels of sympathetic activity are achieved through a balance of excitatory and inhibitory input from both spinal and supraspinal systems. Spinal cord injury disrupts that balance, resulting in dysfunction. Treatments for SCI may result in similar, or even more serious, imbalance. Therefore, assessment of autonomic function should be included in the development of treatments for SCI.

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Supported by NIH grant HL16315.

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SA13

Autonomic dysfunction in spinal cord injury

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The autonomic nervous system has a cranio sacral parasympathetic and a thoraco-columbar sympathetic outflow, each of which supply every organ in the body. Autonomic dysfunction is common after spinal injuries, especially in high lesions where there is substantial disruption to descending spinal sympathetic and sacral parasympathetic pathways, which are separated from cerebral control. This can result in cardiovascular, sudomotor and pelvic dysfunction (the latter involving bladder, bowel and sexual organs), and impair key integrative control mechanisms that maintain blood pressure, heart rate and body temperature.

This overview will focus on cardiovascular and sudomotor autonomic dysfunction that results from spinal cord injury; dysfunction which can result in considerable morbidity, and sometimes in death. Autonomic abnormalities will also be considered on a temporal basis, in the stage of spinal shock and later in the chronic stage. The pathophysiological mechanisms that form the basis of therapeutic strategies to overcome the key features of autonomic dysfunction will be described. Newer approaches which quantify autonomic dysfunction, especially in relation to a more complete classification of spinal cord injuries will be discussed, as this has major implications in relation to newer repair interventions that reverse spinal injury.

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SA14

Effects of spinal cord injury on spinal autonomic neurons

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Understanding central circuits that control the cardiovascular system and how these circuits change with disease or injury is a major challenge. Sympathetic preganglionic neurons (SPN) in the intermediolateral cell column (IML) of thoracic and upper lumbar cord are critical neurons for blood pressure control because they provide central drive that regulates blood vessel diameter. SPN are topographically organized. Rostrally, they regulate targets in the upper body, such as the heart, whereas caudally, they control abdominal and pelvic viscera. Spinal cord injuries disrupt the connections between SPN and neurons above the lesion. The rostrocaudal topography of SPN means that injury location determines its autonomic sequelae. If damage occurs in the upper thoracic or cervical cord, blood pressure control can be profoundly disturbed. People and animals with such injuries experience hypotension and autonomic dysreflexia, a condition characterized by hypertensive episodes that are triggered by noxious or innocuous sensory input entering the cord below injury level. Dysreflexia is thought to occur because of loss of baroreflex input to SPN controlling the splanchnic vasculature, an important bed for reflex control of arterial pressure. My collaborators and I have revealed some of the major effects of spinal cord injury on SPN and their synaptic inputs. After a complete transection (under anaesthesia) at segments T4/5, SPN retract and then regrow their dendrites. Their cell bodies shrink and return to normal size. These changes in SPN correlate with the time required for clearance from the IML of axons severed by the transection and are completed by two weeks after injury. Significant changes to the synaptic input of mid-thoracic SPN also occur acutely. We compared the density of synaptic input to choline acetyltransferase-immunoreactive SPN in T8 from rats with intact cords and rats with 3- or 14-day transections and determined the amino acid content of inputs using immunogold labelling. At 3 days after injury, the number of synapses/10 μm of SPN membrane had decreased by 34% on somata but increased by 66% on dendrites. Almost half the inputs lacked amino acids. By 14 days, the density of inputs to dendrites and somata decreased by 50% and 70%, respectively. The proportion of input that contained glutamate was less in rats with 14-day injuries than in rats with intact cords whereas the proportion of input that contained GABA increased. Thus, in acute injuries, SPN participate in vasomotor control despite profound denervation. Furthermore, an altered balance of excitatory and inhibitory input may explain injury-induced hypotension. Innervation of the IML by other populations of neurochemically identified axons also changes caudal to a complete transection. In intact cord, supraspinal axons containing tyrosine hydroxylase (TH) or phenylethanolamine-N-methytransferase densely supply the IML and synapse on SPN. At 14 days post-transection, the density of these axons is substantially decreased although some TH synapses persist on SPN. By 11 weeks after injury, all of the catecholamine axons have disappeared from the IML. Serotonergic axons disappear more quickly. These supraspinal axons abundantly supply the IML of intact cord but are absent by 2 weeks after injury. Caudal to both acute and chronic (10-12 week) transections, axons containing substance P, enkephalin and neuropeptide Y are present in the IML. Synapses containing each of these neuropeptides occur on SPN from acutely transected cord. These observations indicate that, in injured cord, the IML and SPN receive synaptic input from neuropeptide-containing spinal interneurons. Interestingly, the anatomical consequences of spinal cord injury appear to be quite different for caudal SPN retrogradely labelled from the major pelvic ganglion, which contains post-ganglionic neurons innervating the bladder, lower bowel and reproductive organs. Enkephalin- or galanin-containing axons each supply more than half of the innervation of these SPN and a transection does not appear to produce a significant change in their proportions. Thus, more rostrally located SPN are predominantly controlled by supraspinal neurons whereas SPN that control the pelvic viscera are mainly regulated by spinal interneurons. The dominance of intraspinal control for pelvic visceral SPN has important implications for people with spinal cord injuries. Drugs that target persisting interneuronal pathways may be effective treatments for sympathetically-mediated pelvic dysfunction. Moreover, since circuitry controlling pelvic visceral SPN is not significantly affect by injury, restoring sympathetic control of pelvic organs may be less difficult because re-establishment of direct synaptic input from regrowing supraspinal axons may not be so critical.

Grants from NHMRC (Australia). Technical support from Carolyn Martin, Natalie Fenwick and Lee Travis.

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SA15

Modifications of sympathetic vasoconstrictor pathways after lesions to the spinal outflow in guinea pigs and rats

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Spinal cord injuries have direct and indirect effects on sympathetic outflow below the lesion. Loss of descending drive markedly reduces sympathetic discharge leading to hypotension unless the lesion is caudal to T6 when the intact supply above the lesion helps stabilize blood pressure. Injuries to the thoracic cord that directly damage preganglionic neurones may denervate postganglionic vasoconstrictor neurones. Although these neurones receive multiple inputs arising from several spinal segments, ganglionic transmission depends mainly on only one "strong" input that produces a very large suprathreshold postsynaptic potential, as at the neuromuscular junction. The loss of preganglionic inputs was investigated after resecting the caudal lumbar paravertebral chain of guinea pigs above L4 white ramus (under anaesthesia with 60 mg/kg ketamine and 10 mg/kg xylazine i.p.) (Ireland, 1999). Recordings of synaptic responses to graded stimulation in the isolated chain distal to the lesion site showed that only one in 10 neurones in L5 ganglion normally receive a strong input via L4 white ramus but, a few weeks after the lesion, nearly 60% of neurones had a strong input as well as some weak ones. These new inputs arose by sprouting of the few remaining L4 preganglionic axons and enabled many postganglionic neurones again to relay impulses to their peripheral targets. These novel connections, if inappropriate for the targets, might account for undifferentiated sympathetic activation during autonomic dysreflexia, when not only serious hypertensive episodes but also excessive sweating can be triggered in people with spinal cord injury. The hypertensive episodes are initiated by stimuli below the lesion, such as a distended bladder or bowel, and have been suggested to result from exaggerated spinal reflexes following the expansion of afferent inputs within the damaged spinal cord (Weaver et al., 2006), exacerbated by the loss of baroreflex compensation. However, recordings from sympathetic axons in people with spinal cord injury (Stjernberg et al., 1986) show only transient activation of vasoconstrictor neurones from bladder afferents but prolonged vasoconstriction, implying enhanced vascular responses to sympathetic activation. This was investigated in isolated segments of rat arteries taken from above and below a spinal transection (conducted under anaesthesia with 60 mg/kg ketamine and 10 mg/kg xylazine i.p.). In the tail artery (Yeoh et al., 2004a), responses to 1 Hz stimulation were enhanced 2.5x and those to lower frequencies >20x. The effects were similar with T4 or T8 lesions, neither of which damages the sympathetic outflow. Responses to applied agonists and antagonists revealed unchanged postjunctional α1- and slightly increased α2-sensitivity, reduced prejunctional α2-sensitivity and unchanged activity of the prejunctional noradrenaline transporter (NAT) up to 8 weeks after the lesion. Contractions to raised [K+]o were larger and prolonged. When ongoing sympathetic activity was abolished by decentralizing the neurones supplying the tail artery, the changes in neurovascular transmission were almost identical to those following spinal transection (Yeoh et al., 2004b). The potentiation of neurally-evoked vasoconstriction in the tail artery was not unique. Neurovascular transmission was similarly enhanced after T4 transection in the saphenous artery but, in this case, postjunctional α-sensitivity was reduced and prejunctional α2-sensitivity, NAT activity and muscle reactivity were unchanged. Potentiation of NA release is suspected in both the saphenous and tail artery but this needs to be tested. After inactivating the perivascular peptidergic afferents with capsaicin, the responses of mesenteric arteries to 1 Hz were enhanced 8x after spinal transection (Brock et al., 2006). The underlying mechanisms were unlike those in the cutaneous arteries, with unchanged postjunctional sensitivity or reactivity but a reduction in NAT activity leading to higher junctional concentrations of noradrenaline. In contrast to these changes below a spinal transection, nerve-evoked contractions in the median artery were very similar to control, consistent with the idea that the changes below a spinal transection follow the drop in ongoing sympathetic discharge. Although the adjustments of different vascular beds to reduced nerve impulses are distinct, the widespread enhancement of vascular responses is likely to contribute to autonomic dysreflexia.

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Supported by the Christopher Reeve Paralysis Foundation, the National Health & Medical Research Council of Australia and the NSW Government Spinal Cord Injury and other Neurological Conditions Research Grants Program.

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SA16

Local modulation of sympathetic vasoconstriction in skeletal muscle during systemic hypoxia

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It is widely accepted that sympathetic vasoconstriction is blunted in skeletal muscle during muscle contraction. This phenomenon is known as functional sympatholysis. It has been attributed to a particular vulnerability of the α_2 adrenoreceptor component of sympathetic stimulation that arises because local hypoxia can open the KATP channels on vascular smooth muscle that are closed by α_2-adrenoceptor stimulation, and to the action of nitric oxide (NO) that is generated by neuronal NO synthase (nNOS) expressed on the skeletal muscle fibres (Thomas & Segal, 2004).

An apparently similar phenomenon occurs in skeletal muscle during systemic hypoxia. Thus, systemic hypoxia induces an increase in muscle sympathetic nerve activity (MSNA), attributable to peripheral chemoreceptor stimulation, but the predominant response is muscle vasodilatation (see Ray et al, 2004). We have attempted to elucidate these local-sympathetic interactions in experiments that were approved under current UK Home Office Legislation.

By applying intravital microscopy to the spinotrapezius muscle of anaesthetised rats, we showed that although many arterioles dilate during systemic hypoxia, others constrict and local α-adrenoceptor blockade accentuates the dilatator, and reverses the constrictor responses. Thus, sympathetic vasoconstriction is not completely blunted during systemic hypoxia. We have since developed the spinotrapezius muscle preparation to allow focal recordings of MSNA from the surface of identified muscle arterial vessels. These recordings show the typical cardiac- and respiratory-related rhymicity of MSNA. During graded levels of systemic hypoxia these rhythmicities persist, concomitant with hypoxia-evoked increase in respiration and fall in arterial pressure. Moreover, the frequency of MSNA increased in a graded manner such that instantaneous frequencies in discriminated single fibres reached as high as 20-40Hz. And yet, blood flow recorded from the main artery that supplies the spinotrapezius, showed a graded increase in muscle vascular conductance indicating progressive vasodilatation (Hudson, 2008) as occurs in hindlimb muscle (Ray et al, 2004).

In other experiments we used activity recorded from sympathetic fibres on arterial vessels, or patterns of impulses modelled on specific components of this activity, to stimulate the lumbar sympathetic chain (LSC). By using appropriate pharmacological antagonists, we showed that in normoxia, the vasoconstriction evoked in hindlimb muscle by low and high frequencies and by short and longer trains of impulses is mediated by the actions of noradrenaline and ATP which act synergistically and that at frequencies >20Hz, NPY acting on Y1 receptors contributes (Johnson et al, 2001). However when the LSC was stimulated continuously at 2Hz or with bursts of impulses at 20 or 40 Hz so as to deliver the same number of pulses in 1 minute, the evoked vasoconstrictor response was blunted in a graded manner by graded systemic hypoxia, the vasoconstriction evoked by constant, low frequency stimulation being most vulnerable (Coney & Marshall, 2003).

Although ~50% of the muscle vasodilatation induced by systemic hypoxia is mediated by adenosine acting on A1 receptors via a pathway that involves PGI_2 and depends on the new synthesis of NO (Ray et al, 2004), blockade of A_1 or A_{2A} receptors did not reverse the hypoxia-induced blunting of sympathetic vasoconstriction (Coney & Marshall, 2003). Moreover the blunting was unchanged after NOS blockade, providing the tonic dilator influence of NO was restored by infusion of NO donor. Notably, there was no evidence that NO generated by nNOS contributed to the blunting of sympathetic vasoconstriction associated with systemic hypoxia (Coney et al, 2004). However, we recently found that the component of sympathetic vasoconstriction that approximately 50% of the blunting of sympathetic vasoconstriction that occurs during systemic hypoxia is attributable to loss of the α_2-adrenoceptor component, whereas the component mediated by NPY on Y1 receptors during burst at 20 or 40 Hz, persists, more or less unchanged (Coney & Marshall, 2007).

Thus, it seems that the blunting of sympathetic vasoconstriction that occurs in muscle during systemic hypoxia does not involve the actions of the major dilators that are released during hypoxia; adenosine or NO, but does involve a particular vulnerability of the α_2-adrenoceptor component of the actions of noradrenaline. On the other hand, the component of sympathetic vasoconstriction that is attributable to NPY is resistant to systemic hypoxia, so ensuring that muscle vascular resistance can contribute to the regulation of arterial pressure when MSNA reaches high frequencies.

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This work was supported by BHF

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SA17

Autonomic nervous system-dependent and -independent cardiovascular effects of exendin-4 infusion in conscious rats

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Glucagon-like peptide-1 receptor agonists are promising therapeutic agents for the treatment of type 2 diabetes (Knudsen, 2004), but effects other than those on glucoregulation need assessing. The aim of this study was to determine the effects, and possible underlying mechanisms, of 6h infusions of the long-acting glucagon-like peptide-1 receptor agonist, exendin-4 (Nielsen et al., 2004), in conscious rats chronically instrumented for recording regional haemodynamics. Male, Sprague-Dawley rats (400-500g) were implanted with pulsed Doppler flow probes to measure renal (R), mesenteric (M) and hindquarters (H) blood flows. At least 10 days later, catheters were implanted in the caudal artery and jugular vein. All surgery was carried out under general anaesthesia (fentanyl and medetomidine 300μg/kg of each i.p.). Experiments began at least 24h after catheter implantation, in unrestrained, conscious animals. A 6h infusion of exendin-4 (up to 6 pmol/kg/min) had only modest effects on blood pressure, but caused substantial opposing, regionally-selective, vascular effects, and tachycardia (Table 1). Using propranolol (1 mg/kg; 0.5 mg/kg/h) and phentolamine (1 mg/kg; 1 mg/kg/h), a major involvement of beta-adrenoceptors in the vasodilator and cardiac effects was identified, with little or no contribution from alpha-adrenoceptors to the vasoconstriction seen, since the effects of propranolol were not influenced by the additional presence of phentolamine (Table 1). Under conditions where beta-adrenoceptors were antagonised, alone or in combination with alpha-adrenoceptors, or

when ganglionic transmission was blocked (pentolinium, 5 mg/kg; 5 mg/kg/h), exendin-4 caused widespread vasoconstriction (Table 1). No role for endogenous angiotensin II, vasopressin, endothelin, neuropeptide Y or prostanoids could be shown in the vasoconstrictor actions of exendin-4 (data not shown).

In conclusion, the results show, not only an important beta-adrenoceptor-mediated involvement in the cardiovascular actions of exendin-4 infusion, but also an underlying non-autonomically-mediated vasoconstrictor action, the mechanism of which has not been identified.

	HR(bpm)	BP(mmHg)	RVC(%)	MVC(%)	HVC(%)
Exendin	+98±12*	+4±2	+21±5*	-26±4*	+89±14*
+ prop	-19±13	+30±2*	-15±4*	-66±4*	-55±6*
+ phent +prop	-14±14	+31±3*	-19±3*	-55±2*	-35±3*
+pent	-12±6	+28±2*	-29±4*	-41±4*	-31±4*

Table 1. Changes in heart rate (HR), mean blood pressure (BP) and renal (R), mesenteric (M) and hindquarters (H) vascular conductances (VC) (mean ± se mean, n=7-9 per group), 90 min after the onset of infusion of exendin-4 (6 pmol/kg/ min) alone or in the presence of adrenoceptor antagonists ((propranolol (prop); phentolamine (phent)) or ganglion blockade (pentolinium (pent)). *P<0.05 vs baseline (Friedman's test)

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SA18

High resolution studies of neurotransmitter release from sympathetic nerve terminals

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The autonomic nervous system is fundamental for maintaining homeostasis in health and disease. Although we know substantially how central, sensory and motor nerves signal, there is a paucity of data surrounding autonomic nerve signalling at identified individual neuroeffector junctions. Here, we suggest that noradrenaline and ATP, the major neurotransmitters of the sympathetic nervous system, are focally and intermittently ($P \sim 0.01$) released to act, at points of close contact, at junctions that are functionally analogous to directed synapses. Various techniques will be discussed to show how the release of these neurotransmitters has been advanced. In particular, a unique confocal microscopy Ca^{2+}-imaging method to study Ca^{2+} dynamics simultaneously in nerve terminals and the adjacent smooth muscle cells at individual neuroeffector junctions at a resolution previously unachievable will be demonstrated. The development of a high resolution optical recording technique to detect ATP release from single varicosities on the same terminal branch on an impulse-to-impulse basis will also be described. ATP released from varicose nerve terminals, triggers Ca^{2+} influx through $P2X_1$ receptors, a neuroeffector Ca^{2+} transient, and the frequency of occurrence of these events can be used to measure neurotransmitter release probability from identified varicosities on the same nerve terminal branch[1]. The results confirm the intermittent nature of neurotransmitter release in postganglionic sympathetic nerves innervating rodent vas deferens[2] with action potential evoked probabilities ranging between 0.01 – 0.05 at 0.1 – 2 Hz. Interestingly however, little postjunctional neuronal noradrenergic Ca^{2+} signalling has been detected to date. This new approach complements well-established techniques to study neurotransmitter release and has the additional advantage of providing greater spatial resolution. These studies will permit further important new insights into the mechanisms controlling sympathetic neurotransmitter release and its effects on smooth muscle cells.

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SA19

Sympathetic vasoconstrictor activity in human hypertensive heart disease

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Microneurography is a unique technique for directly recording muscle sympathetic nerve activity (MSNA) in man. The sympathetic nervous system (SNS) is fundamental to cardiovascular reflex control constantly regulating heart rate, blood pressure and peripheral vascular resistance. Many of the common cardiovascular diseases are associated with abnormalities of the SNS, including hypertension, heart failure, myocardial infarction, diabetes mellitus and insulin resistance.

The microneurographic technique was set up in Leeds in 1996 (Drs Mary, Stoker & Greenwood) and was refined to include the recording of single-unit nerve activity to the peripheral vasculature. Using this technique, confirmation of the pathophysiological role of sympathetic hyperactivity in essential hypertension was established[1]. Further work defined the relationship between left ventricular hypertrophy, blood pressure and MSNA in essential hypertension, as determined by echocardiography[2] and cardiac MRI[3]. One hypothesis as to the cause of essential hypertension has been neurovascular compression (NVC) of the rostral ventrolateral medulla. Using MRI to determine the presence of NVC in a group of subjects with a range of arterial pressures, greater sympathetic activity was found in those with NVC compared to those without, supporting the hypothesis that that NVC of the RVLM may cause sympathetic activation and hence be implicated in the pathogenesis of hypertension[4]. Both pregnancy-induced hypertension (PIH) and pre-eclampsia (PE) are poorly understood conditions, but remain a major

cause of maternal death in the UK. Whilst many mechanisms have been proposed, abnormalities of the autonomic nervous system have been implicated. Using the microneurographic technique MSNA was found to be increased in normal pregnancy, PIH and PE, and to return to normal in the post-partum period[5,6].

To summarise, the microneurographic technique for directly recording sympathetic nerve activity to the periphery has provided a unique insight into the mechanisms of human hypertension. Further studies will need to confirm whether therapeutic modulation of neurohormonal activation in hypertension will have a prognostic effect.

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This work was supported in part by the British Heart Foundation.

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