SA1

Differential effects of behaviour on renal and lumbar sympathetic outflow in conscious rats

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The sympathetic nervous system is widely assumed to respond to physiological challenges in a global and generalized manner. However, a number of observations have highlighted regional variations in the pattern of sympathetic outflow consequent to external stimuli, including electrical stimulation, thermal stimulation, acute exposure to hypoxia and injection of drugs (Futuro-Neto HA & Coote JH. 1982; Morrison SF. 2001). Since the majority of previous studies on the regional differences in sympathetic outflow have been generated in acutely prepared anesthetized animals, little is known as to whether the patterning of sympathetic outflow occurs in a regionally different manner during daily activity in the conscious animal. We have recently developed a method to measure renal and lumbar sympathetic nerve activity simultaneously and continuously in freely moving rats. This has been utilized to examine the regional differences in sympathetic outflow which occur as a result of natural behavioural activity in rats. Further, the functional relationships between the behavioural related changes in sympathetic outflow and cardiovascular function were evaluated. Wistar rats were used for all experiments. All procedures were undertaken in accordance with National and International guidelines. Rats were operated on in two stages. During the first surgical procedure, the electroencephalogram (EEG), electrocardiogram (ECG), and electromyogram (EMG) electrodes were implanted. At least five days after the first surgery, the following were implanted: electrodes for the measurement of renal sympathetic nerve activity (RSNA) and lumbar sympathetic nerve activity (LSNA); catheters for the measurement of systemic arterial pressure (Pa) and venous pressure (Pcv); Doppler flow cuffs for the measurement of iliac, mesenteric and renal blood flows. Three days after the second surgery, recordings were begun in a sound attenuated, temperature (24°C) controlled chamber. Behavioural states were scored by standard criteria based on EEG and EMG as well as behavioral observations noted at the time of data collection. The animal's behaviour was classified as rapid eye movement (REM) sleep, non-REM (NREM) sleep, quiet awake, moving and grooming states.
Interestingly, a key observation was that there was a regional diversity in sympathetic outflow during REM sleep. Thus, RSNA decreased in a step manner during REM sleep that was accompanied by a step increase in LSNA. This indicated that the sympathetic regulation did not consist of a simple unidirectional readjustment, indeed, the renal sympatho-inhibition coexisted with a sympatho-excitation in the nerves to the muscle during REM sleep. This was reinforced at the functional level in that mesenteric and renal blood flows and vascular conductances were increased while iliac blood flow and vascular conductance was decreased in REM sleep. These changes were accompanied by an increase in systemic arterial pressure at a time when heart rate was decreased, which could be partly explained by the regional diversity in sympathetic outflow; thus, the onset of REM sleep may cause an increased resistance in the muscle vascular bed which is able to partly compensate the decreased vascular resistance in the visceral organs and the reduction in cardiac output associated with the decrease in heart rate. A further point of interest was the finding that during the other behavioural states including NREM, quiet awake, moving, and grooming state, both LSNA and RSNA increased in a parallel manner and in proportion to the rise in physical activity level. At the same time, systemic arterial pressure increased and was associated with an elevation in heart rate. The increases in LSNA and RSNA, which occurred together with the rise in heart rate, would cause the vasoconstriction of the visceral organs and the non-active muscle, which would contribute to the elevation in systemic arterial pressure. Together, these findings demonstrate that during REM sleep and other behavioural states, different patterns of activity occurred in the RSNA and LSNA. This differential response in sympathetic outflow can be related to state-related changes in organ and cardiovascular function during natural behaviour in rats.

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SA2

Angiotensin in the brain and the autonomic control of the kidney

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It is now recognised that all components of the renin-angiotensin system exists within the brain, renin, angiotensinogen and converting enzyme, indicating that it is possible to generate angiotensin locally within particular neural regions (Wright & Harding, 1997). A high density of angiotensin receptors have been found to exist in many areas involved with cardiovascular control, the nucleus tractus solitarius, caudal and rostral venterolateral medulla, paraventricular nucleus and the concept has developed that angiotensin II may play a role as a neuromodulator or neurotransmitter at these sites. What is unclear at present are the factors that may alter the level of activity of brain angiotensin II and the impact this may have on the reflex regulation of sympathetic outflow.
One area of focus has been the importance of the level of dietary sodium intake which has a major impact on the renal renin-angiotensin system. It has been demonstrated in acute studies in anaesthetised rats that administration of angiotensin II or drugs blocking its action into the cerebroventricles or directly into more restricted brain areas has a larger impact in adult rats on a low salt diet for two weeks than in rats fed a high salt diet (DiBona, 2003). Interestingly, other reports have demonstrated that exposure of young animals to a high dietary sodium intake through the period of growth and development caused a small rise in basal blood pressure, but the blood pressure elevation caused by a slow low-dose infusion of angiotensin II icv elicited a marked rise in blood pressure and a renal nerve-dependent antinatriuresis (Camara & Osborn, 1998). This suggested that under these particular circumstances that exposure to a life-long high salt diet enhanced the importance of the brain renin-angiotensin system in the regulation of sympathetic activity, at least to the kidney.
Our own studies have been aimed to develop these observations further by determining whether this long term exposure to a high salt diet through the period of growth and maturation altered the impact of brain angiotensin II on the baroreflex control of

renal sympathetic nerve activity (RSNA) and heart rate (HR). To do this, rats were fed a 3.1% sodium diet from 4 weeks of age for 7 weeks. At this time they were chronically implanted with arterial and venous cannulae, renal nerve recording electrodes and an icv cannula. Following a 3-day recovery from the surgical procedures, baroreflex gain curves were generated for RSNA and HR before and after an icv injection of losartan which was sufficient to block the drinking response to bolus dose of angiotensin II icv. It was apparent that basal blood pressure, RSNA and gain curve parameters for RSNA and HR were very similar in the rats fed on a regular sodium diet and those fed a high salt diet. Following the icv losartan, the baroreflex gain curves for RSNA and HR were unchanged but in the rats fed a high salt diet, the sensitivity of the baroreflex gain curve for RSNA was elevated by over 30%.

These data, generated in conscious unstressed rats show that the chronic elevation of dietary sodium intake over the growth and maturation period results in a relatively normal baroreflex control of RSNA. To a degree, this is achieved by a greater role for the brain renin-angiotensin system in that following blockade of angiotensin receptors, the sensitivity of the baroreflex control of RSNA was enhanced. Interestingly, these observations in the conscious unstressed rat provided with lifelong high dietary salt intake seem to be somewhat different from those obtained in adult rats subjected to a relatively short period of high salt intake. This raises the question as to how the prolonged exposure to high salt during a developing phase alters the role of angiotensin within the brain. Exactly at which specific sites this action of the brain renin-angiotensin system is exerted remains to be evaluated.

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SA3

Dynamic Analysis of Renal Sympathetic Nerve Activity: Implications for Renal Function

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I. Patterns of Innervation of Renal Neuroeffectors

One pattern is a single renal sympathetic nerve fiber making contact with each of the 3 neuroeffectors. This suggests that each effector possesses response characteristics enabling it to respond to effector-specific information encoded in the RSNA signal. The other pattern is a selective and specific single renal sympathetic nerve fibers making contact with 1 neuroeffector but not the other 2, functionally specific renal sympathetic nerve fibers. This suggests that unique fibers are coupled to separate central sites with specific and selective afferent inputs.

Renal sympathetic nerve fibers show a bimodal distribution of diameters. The strength duration curves for the antidiuretic and renal vasoconstrictor responses have different rheobase and chronaxie values, indicating they are different fiber populations. Single fiber analysis discloses a heterogeneous response to many afferent inputs supporting the existence of functionally specific renal sympathetic nerve fibers.

II. Importance of Dynamic Information Encoded in RSNA

Renal nerves were stimulated with a square wave or diamond wave stimulation pattern, matched for integrated voltage and peak amplitude. Diamond wave pattern produced greater renal vasoconstrictor response. When 2 stimuli were matched for intensity that was subthreshold for renal vasoconstriction, only diamond wave pattern decreased UNaV.

Two reflex stimuli, peripheral thermal stimulation (heat) and tail pinch, while eliciting same increase in total integrated RSNA voltage, produced different effects on RBF. Heat produced greater decrease in RBF and greater increase in renal vascular resistance. Power spectral and transfer function analysis disclosed additional oscillations in RSNA signal during heat (not present in tail pinch) that were transferred into RBF signal; they were absent following renal denervation indicating their derivation from RSNA.

Renal vasculature acts as a low pass filter (i.e. renal vascular frequency response), passing oscillations at frequencies $\langle$ 0.4 Hz without significant attenuation but progressively attenuating (filtering out) oscillations at frequencies $\rangle$ 0.4 Hz.

III. Dynamic Role of Subvasoconstrictor versus Vasoconstrictor Intensities of RSNA

Using rat models with subvasoconstrictor (control, WKY) and vasoconstrictor (congestive heart failure, CHF; SHR) intensities of RSNA, effect of renal denervation (DNX) on dynamic autoregulation of RBF and spontaneous variability of RBF was examined. DNX did not affect basal RBF, dynamic autoregulation of RBF and spontaneous variability of RBF in control and WKY. In CHF and SHR, DNX significantly increased basal RBF, significantly improved abnormal dynamic autoregulation of RBF toward normal and significantly increased spontaneous variability of RBF. Vasoconstrictor intensities of RSNA: (a) impair the dynamic coupling (coherence) between AP and RBF; (b) minimize spontaneous variability of RBF (i.e. improve stability) which may contribute to stability of GFR.

IV. Dynamic Effects of Angiotensin II

This was studied in CHF rats who have increased activity of the renin-angiotensin system (and increased RSNA) with increased circulating plasma angiotensin II concentration (ang II) and in normal rats fed low, normal and high dietary sodium to produce physiological changes in ang II. Losartan was used to assess role of ang II acting on AT1 receptors.

In low dietary sodium rats (not normal or high), physiological elevations in circulating ang II impaired renal vascular frequency response but had a selective effect to enhance slower tubuloglomerular feedback component (but not myogenic component) of dynamic RBF autoregulation.

In CHF rats, the pathophysiological elevations in ang II impaired both renal vascular frequency response and dynamic RBF autoregulation.

These changes are functional (losartan reversible) occurring at interface between renal sympathetic nerve terminals and renal resistance vasculature.

V. Dynamic Models of Renal Blood Flow

RBF can be modeled as single output of a system with 2 inputs, arterial pressure (AP) and RSNA, considered separately or combined. To assess role of RSNA in modeling process, RBF was modeled as single output of a system using single input of AP before and after DNX. DNX did not affect ability to model RBF from AP in rats with subvasoconstrictor intensities of RSNA (WKY) and significantly improved it in rats with vasoconstrictor inten-

sities of RSNA (SHR). In comparing modeling efficacy using 2 inputs separately or combined, RSNA or AP&RNSA was no better than AP alone in WKY. In SHR, RSNA was worse than AP and AP&RSNA was still less efficacious than AP alone.

SA4

ROLE OF THE PVN IN THE AUTONOMIC CONTROL OF HEART AND KIDNEY

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Little more than twenty years ago studies on the paraventricular nucleus (PVN) in the hypothalamus were confined to its relationship with the pituitary. Then some elegant studies by several neuroanatomists alerted us to the numerous extrahypothalamic projections and especially those to the spinal cord. Subsequently studies have established that PVN neurones project to key cardiovascular control regions, notably the rostral ventrolateral medulla (RVLM) where they synapse with spinally projecting vasomotor neurones. A separate population of PVN neurones projects directly to innervate spinal sympathetic neurones involved in cardiovascular control, whilst a third group branch to innervate both RVLM and spinal cord neurones. These PVN neurones are topographically arranged and separate populations contain arginine vasopressin, oxytocin and glutamate although the latter is possibly also a co-transmitter with the peptides. Perhaps the most clearly documented function of the PVN is its role in blood volume regulation and unsurprisingly this seems to be a pivotol influence of the extrahypothalamic projecting neurones. Two key organs the heart and kidney are involved in blood volume regulation and sympathetic control of these allows fast and moment by moment adjustments.

Haemorrhage reflexly leads to increases in sympathetic activity particularly to the kidney. This response depends on the activation of RVLM-spinal vasomotor neurones but the effect is lessened by pharmacological block of PVN-vasopressin synapses both in the RVLM and in the spinal cord. It has also been recently shown that the PVN-enhancement of renal sympathetic activity is negatively regulated by sympathetic neurone releasing nitric oxide which excites nearby glycine inhibitory interneurones which depress activity.

An expansion of blood volume has been shown in a number of species to reflexly increase heart rate via sympathetic nerves and this effect is primarily an action of volume receptors at the vein atrial junctions in the heart. Stimulation of these volume receptors also leads to an inhibition of renal sympathetic nerve activity. Thus the reflex response to an increase in plasma volume consists of a distinctive unique pattern of sympathetic activity to maintain fluid balance. This reflex appears to be dependent on neurones in the PVN. Thus neurones in the PVN show early gene activation on stimulation of the cardiac receptors and a similar differential pattern of cardiac excitation and renal inhibition can be elicited by activating PVN neurones. Cardiac afferents selectively cause a PVN-GABA neurone induced inhibition within the PVN of PVN-spinal neurones projecting to renal sympathetic neurones. Furthermore there is a population of PVN-spinal neurones that selectively increase heart rate by the release of oxytocin a peptide pathway that has no action on the renal sympathetic outflow. Therefore we can envisage a reflex inhibition of renal sympathetic/glutamate neurones in PVN and an excitation of cardiac sympathetic oxytocin neurones to elicit the atrial receptor response.

This role in volume regulation is a good example of how the CNS generates unique non-uniform patterns of sympathetic activity. Furthermore it suggests that such responses depend on specific functional and anatomical connections of the afferent input.

SA5

Vagal Control of the Heart: Central Serotonergic Mechanisms

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Vagal preganglionic neurones innervating the heart are located within the dorsal vagal nucleus (DVN) and the nucleus ambiguus (NA) but the relative importance of these two sites varies between species with increasing importance of the nA compared to the DVN in the move from fish to mammal. In mammals, preganglionic neurones in the nA have small myelinated axons and are responsible for the major changes in heart rate whilst those in the DVN have non-myelinated axons and mediate some chronotropic, dromotropic and inotropic effects.

Numerous studies over the last decade have demonstrated that serotonin (5-HT) has important influences at multiple sites responsible for controlling autonomic outflows. These include the nucleus tractus solitarius (NTS) the site of termination of cardiorespiratory sensory afferent fibres, the cardiac preganglionic neurones within the nA and DVN and within the rostral ventrolateral medulla (RVLM) where sympathetic premotor neurones are located. A series of studies in our Lab have demonstrated the roles of some of the numerous 5-HT receptor subtypes in these brainstem regions involved in control of the heart. Intracisternal application of selective ligands has been used to study the effect of 5-HT receptor subtypes on heart rate and its reflex control, whilst electrophysiological studies have delineated their location and cellular mechanisms of action. Activation of 5-HT_{1A} receptors potentiated the bradycardias evoked by aortic baroreceptors, cardiopulmonary and upper airway receptors but not arterial chemoreceptors, whereas 5-$HT_{1B/D}$ receptors had opposing actions. Within the DVN nA and NTS activation of 5-HT_{1A} receptors could excite or inhibit neurones but, surprisingly, only the excitations were antagonised by 5-HT_{1A} receptor antagonists (Wang et al, 1997). 5-HT_2 receptors also have differential effects and this relates to the class of NTS neurone, excitatory effects are mediated by 5-HT_{2B} receptors whilst inhibitory effects are mediated by 5-HT_{2C} receptors (Sévoz-Couche et al., 2000). Blockade of brainstem 5-HT_3 receptors attenuates the reflex bradycardia evoked by upper airway and cardiopulmonary afferent simulation. This is compatible with electrophysiological data at the level of both the DVN and NTS where activation of 5-HT_3 receptors excites neuronal activity by facilitating release of glutamate from a presynaptic site (Wang et al, 1998). Whether the glutamate is of neuronal or possibly glial origin remains to be determined. However, the latter is a possibility since vagal-

and baro-sensitive NTS neurones have been shown to receive few direct synaptic contacts from 5-HT containing boutons (Llewellyn-Smith et al., 2004). Most recently, blockade of 5-HT_7 receptors has been demonstrated to markedly attenuate the falls in heart rate evoked by stimulating cardiopulmonary receptors, arterial baroreceptors and chemoreceptors (Kellett et al., 2004). Thus, 5-HT plays a critical role in the control of vagal outflow to the heart, however, why so many different receptors seem to be involved, and their relative functional roles remains to be resolved.

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SA6

SYMPATHO-RENAL INTERACTIONS IN THE REGULATION OF BLOOD PRESSURE: ROLE IN HYPERTENSION

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The kidney and the sympathetic nervous system importantly contribute to the development and maintenance of arterial hypertension. Clinical observations in kidney transplant recipients and renal cross-transplantation experiments in rodents provided data supporting the pathogenetic importance of the kidney in primary or "essential" hypertension. On the other hand, sympatholytic drugs effectively lower arterial pressure in hypertensive patients. Primary hypertension in experimental animals can be mitigated by renal denervation or neonatal sympathectomy.

Renal transplants from spontaneously hypertensive rats (SHR) induce arterial hypertension in normotensive histocompatible recipients (renal post-transplantation hypertension). Kidney grafts from normotensive histocompatible donors normalize arterial pressure in SHR.

Analysis of arterial pressure regulating systems did not provide evidence for transient volume and electrolyte retention, activation of the sympathetic nervous system, as well as increased activity of the plasma renin-angiotensin-aldosterone system during the early phase of renal post-transplantation hypertension in rats. When recipients of an SHR kidney were sympathectomized prior to induction of renal post-transplantation hypertension, the rise in arterial pressure was less than in sham-sympathectomized recipients.

Neonatal sympathectomy causes a chronic arterial pressure reduction in SHR. When kidneys from neonatally sympathectomized SHR were transplanted into untreated SHR chronic arterial pressure was less than in SHR transplanted with a kidney from hydralazine-treated SHR donors. Sodium sensitivity of arterial pressure was less in recipients of kidney grafts from sympathectomized donors than in recipients of kidneys from hydralazine-treated donors. These data indicate that neonatal sympathectomy produces chronic changes in SHR renal function which contribute to arterial pressure reduction even when extrarenal sympathetic tone is restored. This finding is not specific for reduction of sympathetic activity during early life since other investigators found that ACE-inhibitor treatment in juvenile SHR gave similar results. Detailed investigation of proximal renal resistance artery function did not provide results which could explain the beneficial effect of neonatal sympathectomy. Gene expression analyses in the renal cortex and medulla with variations in NaCl intake showed a consistently reduced expression level of the NADPH oxidase subunits gp91phox and p47phox in the renal medulla of sympathectomized SHR compared to hydralazine-treated SHR with similar arterial pressure. The functional significance of this finding for renal medullary function remains to be investigated.

Conclusions: 1) Elevated sympathetic activity does not mediate the development of renal post-transplantation hypertension in recipients of an SHR kidney. 2) The level of extrarenal sympathetic activity modulates the severity of renal post-transplantation hypertension. 3) Neonatal reduction of sympathetic tone induces chronic changes in renal function which contribute to long-term reduction in arterial pressure in SHR.

It is suggested that the timing during ontogeny and the efficiency of blocking rather than the specific nature of the blocked "pressure system" determine the long-term effects of this type of interventions on arterial pressure in SHR.