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Symposium 3: Role of neural angiotensin II in regulation of cardiovascular function

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Chair: Roger Dampney and Simon Malpas

Angiotensin in the ventrolateral medulla

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The ventrolateral medulla contains groups of neurons that regulate autonomic functions such as respiration and cardiovascular control. This presentation will concentrate on two of these groups, the caudal and rostral ventrolateral medulla (CVLM and RVLM respectively). These nuclei contain catecholaminergic and non-catecholaminergic cells, which regulate sympathetic vasomotor nerve activity and neuroendocrine function. The CVLM contains a group of GABA-ergic interneurons that are involved in the sympathetic component of the baroreceptor reflex, and noradrenergic A1 neurons that project to the magnocellular neurosecretory neurons of the hypothalamus to modulate vasopressin release. The RVLM contains spinally-projecting neurons (some of which are adrenergic (C1 cells)) whose activity is essential for the tonic and reflex regulation of sympathetic vasomotor tone.

Angiotensin AT_1 receptors occur throughout the ventrolateral medulla in all mammals, including humans. In the human the AT_1 receptors are associated with the catecholaminergic neurons. Angiotensin II-like immunoreactivity also occurs in the region of the catecholaminergic neurons in the rat suggesting that neuronally released angiotensin might act in this region (see Allen *et al.*, 1992).

Microinjections of angiotensin into the ventrolateral medulla of anesthetized animals induce decreases in blood pressure and vasopressin release in the CVLM and increases in blood pressure from the RVLM. Studies *in vitro* support this observation with angiotensin II increasing the activity of presumed C1 RVLM neurons via activation of an AT_1 receptor (Li & Guyenet, 1995).

Microinjections of the selective AT_1 receptor antagonists into the RVLM of anesthetized animals have little effect under basal conditions. Interestingly these agents elicit a pressor response from the RVLM of conscious animals – the mechanism responsible for this is not yet elucidated (Fontes, *et al.*, 2000). However, there are some situations in which endogenous angiotensin does elicit an excitatory action in the RVLM. These include the sympathetic excitation following airjet stress (Mayorov & Head, 2003) or activation of the hypothalamic paraventricular nucleus (Tagawa & Dampney, 1999), and in several models of hypertension including the spontaneously hypertensive rat (Allen, 2001), the transgenic (mREN2) rat (Fontes, *et al.*, 2000) and the Dahl salt sensitive rat (Ito, *et al.*, 2003). Interestingly microinjection of an AT_1 receptor antagonist into the RVLM of the L-NAME-induced hypertensive rat does not affect blood pressure indicating that not all forms of hypertension involve activation of AT_1 receptors in the RVLM (Bergamaschi *et al.*, 2002).

Thus, via activation of AT_1 receptors, angiotensin acts as an excitatory neuromodulator in the ventrolateral medulla to regulate cardiovascular function. The physiological role of this angiotensinergic input is still being elucidated but the input to the RVLM is activated in several forms of hypertension, contributing to the sympathetic activation observed in some forms of this disease.

Allen, A.M., Paxinos, G., Song, K. & Mendelsohn F.A.O. (1992) *Handbook of Chemical Neuroanatomy,* Vol 11, Elsevier Science B.V. Amsterdam, 1-35.

Allen, A.M. (2001) *Journal of the Renin, Angiotensin, Alodosterone System,* 2, S120-S124.

Bergamaschi, C.T., Biancardi, V.C., Lopes, O.U. & Campos, R.R. (2002) *Brain Research,* 927, 195-199.

Fontes, M.A., Baltatu, O., Caligiorni, S., Campagnole-Santos, M.J., Ganten, D., Bader, M. & Santos, R.A. (2000) *Physiological Genomics,* 33: 643-652.

Ito, S., Hiratsuka, M., Komatsu, K., Tsukamoto, K., Kanmatsuse, K. & Sved, A.F. (2003) *Hypertension,* 41, 744-750.

Li, Y-W. & Guyenet, P.G. (1995) *American Journal of Physiology,* 268, R272-R277.

Mayorov, D.N. & Head, G.A. (2003) *Hypertension,* 41, 1168-1173.

Tagawa, T. & Dampney, R.A.L. (1999) *Hypertension,* 34, 1301-1307.

Brain angiotensin and body fluid homeostasis

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The angiotensin AT1 receptor is expressed in many regions of the mammalian brain. High concentrations of AT1 receptors are found in the subfornical organ, organum vasculosum of the lamina terminalis (OVLT) and area postrema, regions of the brain that lack a blood-brain barrier. The endogenous ligand for these AT1 receptors is angiotensin II derived from the blood. Circulating angiotensin II is prevented from having access to the AT1 receptors in other of the brain regions such as the hypothalamic paraventricular nucleus, median preoptic nucleus, lateral parabrachial nucleus, nucleus of the solitary tract or ventrolateral medulla by the blood-brain barrier. It seems likely that the endogenous ligand for these AT1 receptors may be angiotensin synthesised within the brain. We have utilised pharmacological agents to investigate possible roles of brain angiotensin in body fluid homeostasis and cardiovascular control in sheep. Intracerebroventricular (ICV) administration of the AT1 receptor antagonist losartan in conscious sheep has been shown to block water drinking, vasopressin secretion, reduced renin secretion, reduced renal sympathetic nerve activity, and the pressor response to centrally administered hypertonic saline as well as to ICV angiotensin II, suggesting that an angiotensinergic pathway within the brain may have a role in osmoregulation. However, when we tested the effect of the same dose of ICV losartan on the water drinking response to systemic infusion of hypertonic saline which gradually increased plasma osmolality over 30 min, there was no inhibition of the water drinking response, which challenges the idea that a central angiotensinergic pathway mediates physiological osmoregulatory drinking. Moreover, we have recently observed in mice (Agt-/- mice) in which the angiotensinogen gene had been deleted by gene targetting techniques, that they are able to respond to osmotic challenges (water deprivation for 24 hours or intraperitoneal injection of hypertonic saline) with appropriate increases in water intake. In rats, we observed that ICV administration of an 18-mer antisense oligonucleotide directed against part of the angiotensinogen gene in order to reduce angiotensinogen synthesis in the brain caused a large reduction in the water drinking response to ICV renin (administered 24 h later), suggesting that brain angiotensinogen levels had fallen. However, such antisense treatment did not reduce water drinking in response to systemically administered hypertonic saline or to water deprivation, also suggesting that brain angiotensinergic mechanisms are not mediating osmoregulatory thirst. Thus, our data in several species does not favour a major role for brain angiotensinergic mechanisms in osmoregulation.

Superoxide mediates excitatory actions of angiotensin II in the rostral ventrolateral medulla during acute stress

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Reactive oxygen species (ROS) are thought to be important intracellular mediators of angiotensin II (AII) actions in the brain (Zimmerman *et al*., 2002). We recently found that AII in the rostral ventrolateral medulla (RVLM) mediates the blood pressure (BP) response to emotional stress in rabbits (Mayorov & Head, 2003). In the current study, we examined the role of the superoxide radical ($\bullet O_2^-$) and nitric oxide (NO) in this action of AII in the RVLM.

We first evaluated the role of superoxide in the stress-induced neuronal excitation in the RVLM. We tested the cardiovascular response to airjet stress before and after injections of cell permeable superoxide dismutase (SOD) mimetics tempol, tiron or 3-carbamoyl proxyl (3-CP) into this region in conscious rabbits. Eight minute airjet stress evoked a sustained increase in BP (+12±2 mmHg). Bilateral microinjections of equimolar doses (20 nmol; n=7-9) of tempol, tiron or 3-CP into the RVLM did not alter resting BP. Tempol and tiron attenuated the pressor response to airjet by 57±12% and 52±8%, respectively. By contrast, 3-CP which is structurally similar to tempol but has a lower superoxide scavenging activity, did not alter the stress response. The SOD mimetics did not affect the renal sympathetic nerve activity (RSNA) baroreflex or the pressor response to local microinjection of glutamate.

In another series of experiments, we determined whether NO is important in mediating the circulatory stress reactions in the RVLM. Microinjections of NO donors, sodium nitroprusside or S-nitroso-N-acetylpenicillamine (1-20 nmol), dose-dependently increased BP, indicating that NO predominantly plays an excitatory role in the RVLM of the conscious rabbit. Microinjection of N(G)-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor (10 nmol), did not affect the pressor stress response, measured 10-20 min after injection. However, this response was diminished by 55±13% one hour later. Notably, L-NAME decreased the gain of the RSNA baroreflex by 38±12%, suggesting that NO is involved in modulating baroreflexes.

In further experiments, we tested whether the inhibitory action of tempol in the RVLM depends on local NO levels. Co-injections of L-NAME and tempol (n=4) did not affect resting BP, but attenuated the pressor stress response by 31±8%, indicating that the SOD mimetic acted, at least in part, via a NO-independent mechanism. Finally, we determined whether ROS in the RVLM mediate the pressor action of exogenously applied AII. Unilateral microinjections of AII (100 pmol) increased BP by 12±3 mmHg. Tiron and tempol attenuated by the pressor response to AII by 59-64%. By contrast, L-NAME tended to increase the pressor response to AII.

It is plausible that stress-induced activation of the AII – superoxide signalling pathway is not confined to the RVLM. We have found, in a pilot study, that microinjections of either AT_1-receptor antagonist candesartan (500 pmol) or the SOD mimetics into the dorsomedial hypothalamus also attenuated the pressor response to airjet by 30-46%.

Overall, these results suggest that the stress-induced neuronal excitation in the RVLM involves activating a redox sensitive signaling pathway in rabbits. Local superoxide, but not NO is critically important in mediating the acute pressor effects of emotional stress in rabbits. Together with our previous findings (Mayorov & Head, 2003), these results also indicate that superoxide is a key intracellular signaling molecule in the acute excitatory action of AII on the RVLM vasomotor neurons.

Mayorov & Head (2003). *Hypertension*, 41, 1168-1173.

Zimmerman, M.C., Lazartigues, C., Lang, J.A., Sinnayah, P., Ahmad, I.M., Spitz, D.R. & Davisson R.L. (2002). *Circulation Research*, 91, 1038-1045.

Modulation of neurohumoral effector gain as a novel mechanism for the long term regulation of blood pressure

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There is increasing evidence for the importance of the sympathetic nervous system in a number of disease states such as hypertension, congestive heart failure and renal failure. While it is recognised that the sympathetic nervous system consists of differentially regulated outflow to a variety of organs, perhaps the most relevant to the long term regulation of blood pressure (BP) and also to hypertension is renal sympathetic nerve activity (RSNA). By regulating renal hemodynamics, tubular function, and renal renin release, RSNA has the potential to contribute to the initiation, development and maintenance of hypertension. DiBona and colleagues suggest that renal sympathetic activity at low frequencies affects renin release, moderate frequencies inhibits sodium excretion and only at high stimulation frequencies reduces renal blood flow (RBF) (DiBona & Kopp, 1997). They suggest that in conscious animals resting RSNA is too low to affect RBF. We developed a combined nerve electrode and flowprobe for conscious rabbits and examined the relationship between RSNA and RBF. Initial studies showed that RSNA can influence RBF especially when activated by physiological stimuli (Janssen *et al.*, 1997). In further studies we examined the effect of acute sympathetic inhibition with rilmenidine, and acute angiotensin converting enzyme inhibition with captopril. Rilmenidine produced hypotension but no change in renal vascular conductance in normal rabbits, renal vasodilatation in barodenervated rabbits and some renal vasoconstriction in renal denervated rabbits. By contrast captopril produced similar renal vasodilatation whether the RSNA increased or was absent. These results suggest that while RSNA can influence short term fluctuations in RBF, the renin angiotensin system is the major influence on RBF and can override the influence of RSNA. From these studies we might expect there to be little role for RSNA in conditions where there is elevated plasma renin such as in renovascular hypertension. However, there is a large body of evidence to suggest an important role of the sympathetic nervous system and the central nervous system in this form of hypertension (Fink, 1997). We assessed the contribution of the sympathetic nervous system using acute and chronic sympathetic inhibition with rilmenidine in 2K1C hypertensive rabbits. After establishing a stable level of hypertension, rilmenidine or vehicle (saline) was infused by osmotic minipump. After a further 2 weeks, an electrode for recording renal sympathetic nerve activity (RSNA) and a RBF probe was implanted under halothane anaesthesia. Five weeks after renal artery clipping, mean arterial pressure (MAP) was 34% higher and renin was elevated 5 fold, but rabbits treated with rilmenidine were normotensive with less elevated renin. RSNA, heart rate and blood flow to the unclipped left kidney were similar in both groups of rabbits. The acute response to rilmenidine was greater in the hypertensive group with a lesser fall in RSNA such that the change in BP per change in RSNA was 5 fold greater than in normotensive rabbits. Also the relative renin release in response to increased RSNA was increased 3 fold in 2K1C rabbits. By contrast the pressor response to airjet stress was similar in hypertensive and chronic rilmenidine treated (normotensive) rabbits indicating that vasoconstrictor neuroeffector mechanisms were not altered by high renin states. These studies suggest that in the long term the RSNA may make an increasing contribution to the maintenance of BP in renovascular hypertension, possibly though an amplification of the neural release of renin. The long term modulation of the renin neuroeffector mechanism which in our case took at least 4 weeks may be an important way in which the sympathetic nervous system contributes to the long term setting of BP.

DiBona, G.F. & Kopp, U.C. (1997) *Physiological Reviews,* 77(1): 75-197.

Fink, G.D. (1997) *Clinical and Experimental Pharmacolology and Physiology,* 24(1):91-95.

Janssen, B.J.A., Malpas, S.C., Burke, S.L. & Head G.A. (1997) *American Journal of Physiology,* 273(2 Pt 2):R597-R608.

Role of angiotensin II in regulating long term levels of sympathetic activity

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Angiotensin II is recognised to play a critical role in the regulation of arterial pressure. In addition to its direct vasoconstrictive actions there is strong evidence to indicate that angiotensin II maintains arterial pressure through an excitatory action on the sympathetic nervous system. This action may be via a direct action on central nervous system pathways involved in generating and regulating sympathetic nerve activity (SNA) or via an action on a pathway such as the arterial baroreflex, that plays an important role in regulating short term SNA. One concern is that the data used to base such hypotheses are generally taken from short-term recordings of SNA (generally less than 3 hours). Thus the mechanisms that regulate SNA under such conditions may not necessarily reflect those seen under more chronic conditions and thus be reflective of the human condition.

To address the question of how angiotensin II regulates SNA chronically we developed technology which enables us to record SNA for up to 50 days via telemetry in rabbits. We made continuous recordings of renal SNA before, during and after one week of angiotensin II based hypertension in rabbits living in their home cages. Angiotensin II infusion (50 $ng.kg^{-1}.min^{-1}$) caused a sustained increase in arterial pressure (18 ± 3 mmHg). There was a sustained decrease in SNA, from 18 ± 2 normalised units (n.u.) before angiotensin II to 8 ± 2 n.u. on day 2 and 9 ± 2 n.u. on day 7 of the angiotensin II infusion ($P < 0.01$) before recovering to 17 ± 2 n.u. after ceasing angiotensin II. Analysis of the baroreflex response showed that while angiotensin II induced hypertension led to resetting of the MAP-HR relationship, there was no evidence of resetting of the MAP-SNA relationship. We propose that the lack of resetting of the MAP-SNA curve, with the resting point lying near the lower plateau suggests the sustained decrease in SNA during angiotensin II is baroreflex mediated.

Subsequently, to address whether the action of angiotensin II was solely via a sustained non-resetting of arterial baroreflexes or via a central action, we followed the same protocol as above but in sino-aortically denervated animals. Under these conditions the increase in arterial pressure was the same as previously observed in intact animals however there was no evidence of a reduction in SNA. Indeed mean SNA was unchanged after 7 days for angiotensin II infusion. These results suggest that the action of peripheral angiotensin II on SNA appear to be determined primarily via an arterial pressure dependent action through non-resetting of arterial baroreflexes. While a central action of angiotensin II on SNA may exist, we suggest that the lack of alteration in SNA levels in baroreceptor denervated animals indicates that this effect may be relatively minor.

Overall these results suggest two surprising findings; firstly that angiotensin II is sympathoinhibitory and not sympathoexcitatory as previously indicated, and that baroreflex control of renal SNA and thus renal function is likely to play a significant role in the control of arterial pressure in the long-term (Barrett *et al.*, 2003; Lohmeier, 2003).

Barrett C.J, Ramchandra R., Guild S.J., Lala A., Budgett D.M. & Malpas S.C. (2003). *Circulation Research,* 92, 1330-1336.

Lohmeier, T.E. (2003). *Circulation Research,* 92, 1282-1284.