

Vasomotor Actions of Angiotensin II in the Ventrolateral Medulla of Spontaneously Hypertensive and Wistar Kyoto Rats

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I. Introduction

The ventrolateral medulla regulates the intrinsic activity of spinal preganglionic sympathetic neurons through a counterbalancing action of pressor and depressor circuits situated in the rostral (RVLM) and caudal (CVLM) regions of the ventral medulla, respectively (1–6). Briefly, barosensitive information is conveyed from intrinsic neurons of the nucleus of the tractus solitarius (nTS) to neurons of the CVLM. Gordon (7) has shown that *N*-methyl-D-aspartic acid (NMDA) receptors in the CVLM mediate the effect of barosensitive input to CVLM neurons. More recently, electrophysiological studies have revealed that the firing properties of CVLM neurons are consonant with the idea that these neurons are excited by baroreceptor afferent input (6,8–10). CVLM neurons project monosynaptically to pressor neurons of the RVLM (6); the effect of this projection from the CVLM is to inhibit RVLM neurons. Current evidence supports the idea that release of gamma-aminobutyric acid (GABA) from CVLM nerve terminals mediates this inhibition (11–13). Finally, it is well appreciated that pressor neurons of the RVLM project to the intermediolateral cell column of the thoracic and lumbar segments of the spinal cord and, presumably, this

projection provides monosynaptic excitatory input to preganglionic sympathetic neurons.

It was within this construct of the VLM that we became interested in the potential role that Ang II might play in the regulation of cardiovascular function. High-affinity binding sites for Ang II have been demonstrated in the VLM of a number of different species (14–18). We originally showed in the cat that Ang II applied topically to the glycine-sensitive area of the ventral medulla elicited dose-dependent increases in arterial pressure (19). At the same time Allen et al. (16) found that microinjection of Ang II into the RVLM of cats produced a dose-dependent increase in blood pressure. These findings were subsequently confirmed in the rabbit by Sasaki and Dampney (20), who showed that microinjection of Ang II in the RVLM increased blood pressure, whereas microinjection of the peptide in the CVLM caused blood pressure to decrease. In addition, these investigators demonstrated that blockade of Ang II receptors in the VLM by [Sar¹, Thr⁸]-Ang II produced changes in blood pressure that were directionally opposite to the effects evoked by local injection of the agonist peptide. These latter findings suggested that Ang II in the VLM could influence the tonic activity of caudal depressor and rostral pressor neurons responsible for the control of sympathetic nerve activity.

These observations then implicate Ang II in the VLM as playing a role in the regulation of cardiovascular function. However, we decided to pursue the issue of whether Ang II might influence the responsiveness of VLM neurons in the pathogenesis of hypertension. To investigate this issue, we have focused our attention on the actions of Ang II in the VLM of spontaneously hypertensive rats (SHR). The evolution of hypertension in SHR is associated with an augmented activity of the sympathetic nervous system (21–23). Another factor is enhanced activity of the brain renin-angiotensin system. Intraventricular injection of either Ang II blockers (24) or Ang I converting enzyme inhibitors (25,26) decreases the blood pressure in SHR but not that of normotensive Wistar-Kyoto (WKY) rats. Evidence also suggests that centrally administered Ang II evokes a pressor response, at least in part via enhancement of sympathetic outflow to the peripheral circulation (27,28). This chapter, therefore, will review the results of our work, which has attempted to characterize the role that Ang II acting in either the RVLM or CVLM plays in the maintenance of blood pressure in SHR and also to determine the receptor subtype at which Ang II acts in the VLM to influence the activity of vasomotor neurons.

II. Methods

A. Animal Preparation

Experiments were done in adult male SHR (13 to 16 weeks old) and age-matched WKY rats obtained from Taconic Farms (Germantown, NY). All experiments were carried out in accordance with the guiding principles in the care and use

of animals as mandated by the American Physiological Society. Rats were anesthetized either with halothane (0.9% to 1.1%) and breathed a mixture of 65% room air/35% oxygen, which served as the carrier for the anesthetic agent, or with urethane (1.3 to 1.5 g/kg, IP). A femoral artery and vein were cannulated for the measurement of arterial pressure and the injection of drugs, respectively. Arterial blood gases and pH were kept within physiological limits (pH 7.35 to 7.45, PO₂ 100 to 140 mm Hg, PCO₂ 40 to 45 mm Hg). Body temperature was maintained at 37.5 ± 0.5°C by a heating pad.

Anesthetized rats were placed in a supine position with their heads fixed in a stereotaxic frame (David Kopf Instruments). The trachea and esophagus were transected in the lower neck and reflected rostrally. The distal trachea was cannulated to facilitate ventilation. After retraction of the bilateral longus capitis muscles, the inferior occipital bone was removed to provide a 5 × 6 mm window onto the surface of the ventral medulla oblongata. After incision and retraction of the dura, the ventral surface of the medulla was kept moist either by artificial cerebrospinal fluid (aCSF; pH 7.4) or by production of endogenous CSF. In some experiments, rats were paralyzed with *d*-tubocurarine (0.8 to 1.0 mg/kg, IV). In these experiments the tracheal cannula was connected to a ventilator (model 681, Harvard Apparatus, MA) and animals were artificially ventilated at a rate of 60 strokes/min and a tidal volume of 2.5 mL. For experiments in which sympathetic nerve activity was recorded, the abdominal plexus was exposed through a transverse incision of the lateral abdominal wall, and the inferior nerve bundle accompanying a superior mesenteric artery was placed over a bipolar silver electrode. Nerves and electrode tips were immersed in mineral oil to preserve integrity of the nerve bundle. Nerve activity was amplified and filtered (bandwidth 100 to 3000 Hz). At the end of each experiment, the noise level associated with the recording of sympathetic activity was determined following intravenous injection of hexamethonium bromide (30 mg/kg).

B. Microinjection Procedures

Microinjections were made from multibarrel micropipettes with tip diameters of 20 to 50 μm. The pipettes were made from calibrated microbore capillary glass tubing (Accu-Fill 90, Clay Adams, NJ). Tips were drawn on a micropipette puller (model PE-2, Narishige Scientific Instruments, Japan). The inner surface of the pipettes was coated with silicone (Sigmacote, Sigma Chemical Co., St. Louis, MO). Injections (50 nL) were made over a 30-s period with a hand-held syringe, as described elsewhere (29). The injected volume was measured by observing the movement of the fluid meniscus along a reticule in a microscope. Microinjections were made on one side of the brainstem unless noted otherwise.

The rostral pressor and caudal depressor areas were identified by the injection of 2 nmol of L-glutamate (L-Glu) into the RVLM or CVLM, respectively. Identification of either the rostral pressor or caudal depressor area at

the time of the experiment conformed to the following modification of criteria described by Willette et al. (2): (a) the latency to the onset of change in blood pressure produced by L-Glu was no more than 5 s; (b) the response plateau occurred within 20 s after microinjection of the amino acid; and (c) the change in blood pressure was at least 30 mm Hg. In our experiments we found that the rostral pressor area was restricted to L-Glu injection at sites located 0.6 to 1.0 mm rostral to the most rostral rootlet of the hypoglossal nerve; 1.7 to 1.9 mm lateral to the midline; and 0.5 to 0.8 mm below the ventral surface. The caudal depressor area corresponded to L-Glu injection sites located between the second and third rostral rootlets of the hypoglossal nerve, 1.9 to 2.1 mm lateral to the midline, and 0.7 to 0.9 mm below the ventral surface.

Ang II (5–100 pmol), the Ang II analogue antagonist [Sar¹,Thr⁸]-Ang II (100–1000 pmol), the nonpeptide Ang II antagonist losartan (10 pmol to 10 nmol), muscimol (500 pmol), and L-Glu (2 nmol) were dissolved in aCSF (in mM: 133.3 NaCl, 3.4 KCl, 1.3 CaCl₂, 1.2 MgCl₂, 0.6 NaH₂PO₄, 32.0 NaHCO₃, and 3.4 glucose). Alcian blue dye (10 nL) was injected from a separate barrel of the pipette to mark the site of injection after completing injections at a particular site.

C. Histological Analysis

At the completion of an experiment, rats were deeply anesthetized with either 4% halothane or pentobarbital sodium (50 mg, IV). The animals were then perfused transcardially with 150 mL of 0.9% NaCl followed by 150 mL of 10% phosphate-buffered formaldehyde solution. The brainstem was removed, stored overnight in 10% phosphate-buffered formaldehyde solution, and then transferred to a fixative containing 30% sucrose. Frozen brain tissue was sectioned in the coronal plane (50 μ m) and stained with neutral red. Microinjection sites were identified by the deposition of Alcian blue dye and referred to standard anatomical structures of the caudal brainstem according to the atlas of Paxinos and Watson (30).

D. Statistical Analysis

Data are expressed as means \pm SEM. A general linear model two-way analysis of variance followed by multiple comparisons with a Duncan's multiple range test was employed for analysis of the hemodynamic and sympathetic neural effects of Ang II or Ang II antagonist microinjection. One-way analysis of variance was also employed when appropriate. Student's *t*-test was used for comparisons of the magnitudes of blood pressure and sympathetic nerve responses to L-Glu, Ang II, and Ang II antagonist microinjection between SHR and WKY rats (31). *P* values less than 0.05 were considered statistically significant.

III. Results

A. Hemodynamic and Sympathetic Effects of Angiotensin II Microinjection in the VLM

Previously, we had shown that topical application of Ang II to the glycine-sensitive area of the cat elicited a dose-related increase in blood pressure (19). Now, we sought to investigate the potential role of this peptide in the pathogenesis of hypertension of SHR via actions of the peptide at vasomotor neurons. Thus, the first objective was to characterize the cardiovascular responses produced by microinjection of Ang II into regions of the ventrolateral medulla functionally identified as either the rostral pressor or caudal depressor area. Microinjection of either L-Glu or Ang II into the RVLM increased blood pressure, whereas injection of either substance into the CVLM decreased blood pressure. Although both Ang II and L-Glu produced directionally similar changes in blood pressure, depending upon whether the substances were injected into either the RVLM or CVLM, the time course of the cardiovascular response to Ang II differed distinctly from that produced by L-Glu injection. The hemodynamic response produced by Ang II was characterized by a slow onset and a gradual change from baseline. In contrast, injection of L-Glu into the RVLM caused a rapidly developing and transient increase in blood pressure. Table 1 summarizes the onset times and peak latencies of the blood pressure responses to Ang II (20 and 100 pmol) and L-Glu (2nmol) microinjections into the RVLM and CVLM. The average onset time and the latency to the peak of

Table 1 Onset Times and Latencies of Blood Pressure Responses to Ang II and L-Glu Microinjection into RVLM and CVLM of Rats^a

	Spontaneously hypertensive rats		Wistar-Kyoto rats	
	Ang II	L-Glu	Ang II	L-Glu
Rostral ventrolateral medulla				
Onset time	7.8 \pm 0.5 ^b	2.3 \pm 0.1	9.4 \pm 0.9 ^b	2.6 \pm 0.2
Peak latency	46.0 \pm 3.3 ^b (13)	13.8 \pm 0.6 (21)	42.7 \pm 3.1 ^b (12)	15.4 \pm 0.8 (15)
Caudal ventrolateral medulla				
Onset time	11.5 \pm 1.1 ^b	2.0 \pm 0.1	10.8 \pm 0.8 ^b	2.2 \pm 0.1
Peak latency	45.3 \pm 3.6 ^b (13)	12.4 \pm 0.7 (17)	42.7 \pm 2.4 ^b (14)	12.7 \pm 0.6 (20)

^aValues are means \pm SEM of time factors measured in seconds; no. of rats studied under different conditions is given in parentheses. Ang II, angiotensin II (20 and 100 pmol); L-Glu, L-glutamate (2 nmol).

^b*P* < 0.01 for Ang II versus L-Glu.

Source: Ref. 46.

the blood pressure changes to Ang II were similar in SHR and WKY rats. L-Glu injected into the RVLM and CVLM evoked blood pressure responses that had similar onset times and latencies in both strains. However, Table 1 documents that the onset times and latencies to peak responses associated with L-Glu injections were significantly ($P < 0.01$) shorter than those associated with Ang II injections.

Microinjections of Ang II into the RVLM elicited significant ($P < 0.01$) dose-dependent increases in blood pressure that were of comparable magnitude in SHR and WKY rats (Fig. 1). Pressor responses were significant at the lowest dose of Ang II (5 pmol) and reached maximal effects at a dose of 20 pmol. Although injection of the peptide into the RVLM increased heart rate, these responses were more variable than those observed for blood pressure. Administration of L-Glu (2 nmol) into the RVLM produced comparable pressor responses in both strains. The L-Glu-evoked pressor responses were significantly ($P < 0.01$) larger than the increases in blood pressure produced by Ang II injections at the same sites (Fig. 1).

Ang II microinjections into the CVLM elicited significant ($P < 0.01$) dose-dependent decreases in blood pressure in both SHR and WKY rats. The depressor responses were significant at the lowest dose of the peptide (5 pmol) and reached a maximum at a dose of 20 pmol (Fig. 2). In contrast to the pressor effects produced in the RVLM, the magnitude of the decreases in blood pressure caused by Ang II was significantly greater ($P < 0.01$) in SHR than in WKY rats. Decreases in blood pressure were accompanied by falls in heart rate that did not differ between SHR and WKY rats. Injection of L-Glu (2 nmol) into the CVLM elicited significantly ($P < 0.01$) greater depressor responses in SHR than in WKY rats (Fig. 2).

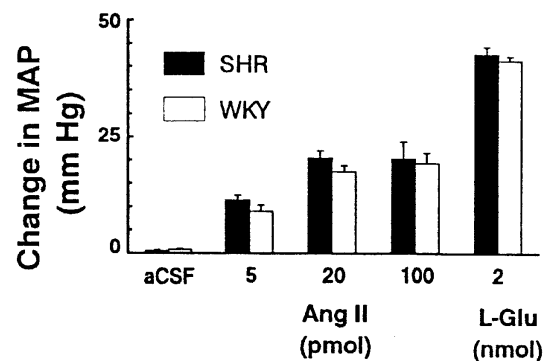


Figure 1 Changes in mean arterial pressure (MAP) in response to microinjection of artificial cerebrospinal fluid (aCSF), L-glutamate (L-Glu), and graded doses of angiotensin II (Ang II) into the rostral ventrolateral medulla of 14- to 16-week-old SHR and WKY rats. Injection volumes were 50 nL.

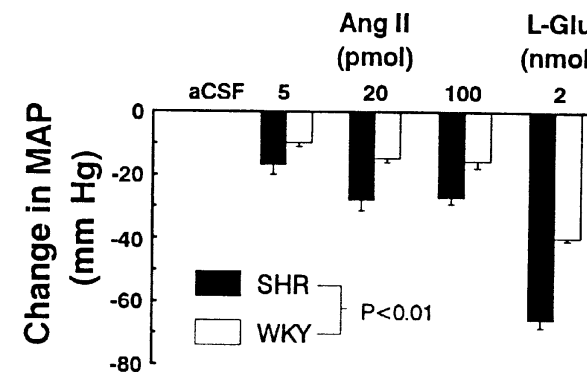


Figure 2 Changes in mean arterial pressure (MAP) in response to microinjection of artificial cerebrospinal fluid (aCSF), L-glutamate (L-Glu), and graded doses of angiotensin II (Ang II) into the caudal ventrolateral medulla of 14- to 16-week old SHR and WKY rats. Injection volumes were 50 nL. SHR showed significantly greater depressor responses than WKY rats.

Although the blood pressure changes elicited by Ang II injections into either the CVLM or RVLM were consistently smaller than the blood pressure changes produced by L-Glu injections at the same sites, these findings suggested that Ang II acted to stimulate the same vasomotor neurons which could be activated by L-Glu. Figure 3 is a composite of the locations where both Ang II and L-Glu produced either pressor or depressor responses. In both SHR and WKY rats, depressor responses were produced by injections of the peptide restricted to a region ventral to the nucleus ambiguus and dorsal to the lateral reticular nucleus, approximately at the level of the obex. In both strains, pressor responses were produced by injection of the peptide in an area encompassing the dorsolateral aspect of the lateral paragigantocellular nucleus (LPGi) and a region dorsolateral to the LPGi. This area lies at the caudal end of the facial nucleus and is consistent with the region known as the subretrofacial nucleus based upon pressor responses evoked by L-Glu injections.

Previous studies have suggested that the pressor response evoked by Ang II in the RVLM may be mediated by the sympathetic nervous system (16,19,20). Two separate sets of experiments were conducted to establish that the pressor responses evoked by Ang II injections in the RVLM were sympathetically mediated. First, Ang II was injected into the RVLM of SHR and WKY rats before and after ganglionic blockade with hexamethonium bromide (40 mg/kg, IV). The initial injection of Ang II increased blood pressure by an average of 17 ± 4 mm Hg in SHR ($n = 3$) and 16 ± 3 mm Hg in WKY rats ($n = 5$). Delivery of hexamethonium bromide 60 min after the first injection of Ang II caused blood pressure in SHR to fall from 135 ± 3 to 65 ± 2 mm Hg in SHR and from 91 ± 4 to 49 ± 2 mm Hg in WKY rats. However, injection of Ang II (20 pmol) into the

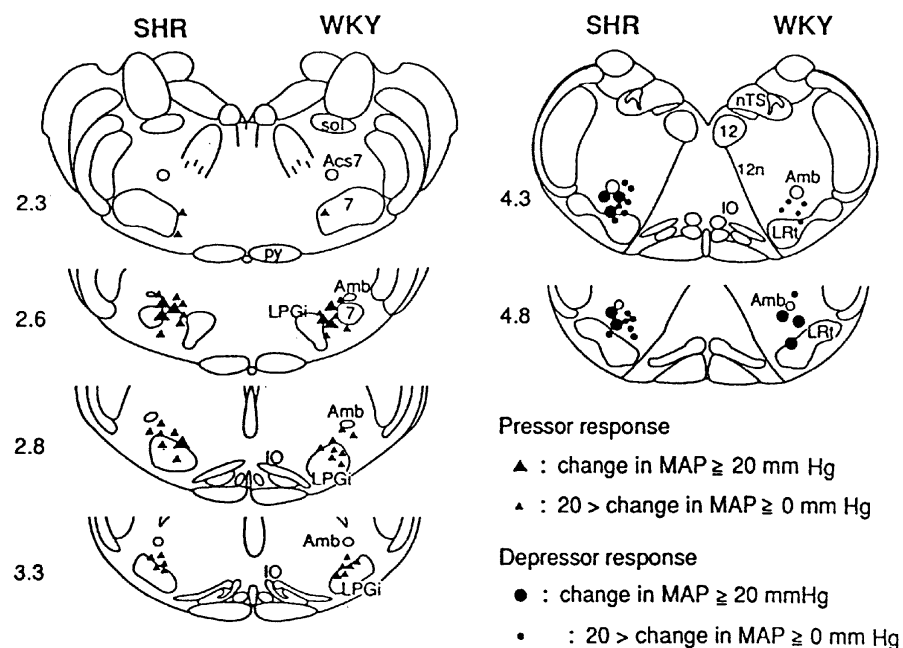
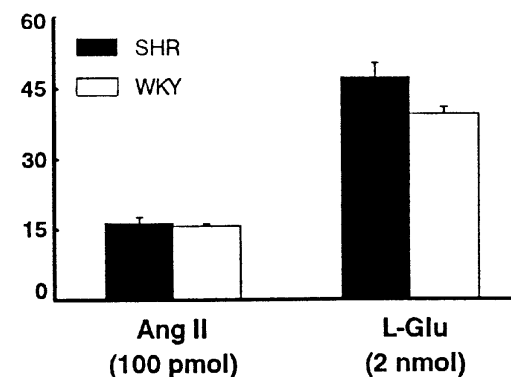


Figure 3 Sites in the ventrolateral medulla where Ang II (20 pmol) elicited either a pressor (left) or a depressor (right) response. According to the atlas of Paxinos and Watson (30), medullary sections extended from 2.30 to 4.80 mm caudal to interaural line. Abbreviations: Acs7, accessory facial nucleus; Amb, nucleus ambiguus; IO, inferior olivary nucleus; LPGi, lateral paragigantocellularis nucleus; LRGi, lateral reticular nucleus; py, pyramidal tract; nTS, nucleus of tractus solitarius; sol, solitary tract; 7, facial nucleus; 12, hypoglossal nucleus; 12n, root of hypoglossal nerve; MAP, mean arterial pressure. (From Ref. 46.)

same sites after ganglionic blockade had no effect upon blood pressure or heart rate in either strain. However, ganglionic blockade was associated with a dramatic reduction in the baseline blood pressure. Therefore, in additional experiments, we assessed the effects of administration of L-Glu and Ang II into the RVLM upon splanchnic sympathetic nerve activity in SHR and WKY rats. Figure 4 shows that microinjection of Ang II (100 pmol) on average increased splanchnic sympathetic nerve activity by approximately 10% in both SHR and WKY rats. In contrast, L-Glu (2 nmol) injection at the same sites increased splanchnic sympathetic nerve activity by approximately 35% and 40% in WKY and SHR, respectively. Just as L-Glu injected into the RVLM acted as a more potent stimulus than Ang II to increase blood pressure, so did injection of the excitatory amino acid increase sympathetic nerve activity to the same relevant extent when compared to the sympathetic activation produced by Ang II injection in the RVLM. Thus, two different sets of experiments support the idea

Change in MAP (mm Hg)



Change in SpSNA (%)

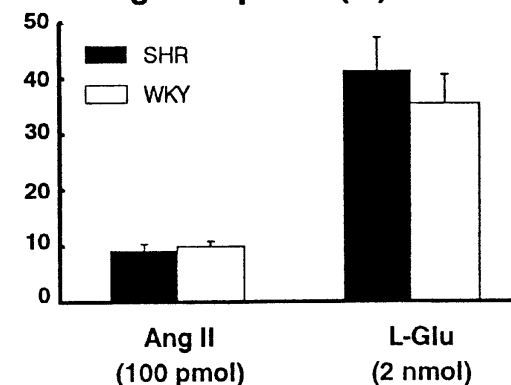


Figure 4 Changes in mean arterial pressure (MAP) and splanchnic sympathetic nerve activity (SpSNA) produced by microinjection of angiotensin II (Ang II) and L-glutamate (L-Glu) into the rostral ventrolateral medulla of SHR and WKY rats. Injection volumes were 50 nL.

that Ang II stimulates RVLM neurons, providing excitatory input to sympathetic preganglionic neurons.

B. Blockade of Angiotensin II Receptors in the Ventrolateral Medulla

The experiments reviewed above as well as those performed in cats (16,19) and rabbits (20) demonstrate that the activity of vasomotor neurons is enhanced by stimulation of Ang II receptors. These findings provided some insight into the ability of Ang II to influence the activity of vasomotor neurons. Although Ang II was less potent than L-Glu in exciting vasomotor neurons, our data

suggested that this peptide could make a significant but quantitatively modest contribution to the tonic activity of a counterbalancing set of ventrolateral medullary neurons involved in the regulation of sympathetic nerve activity. Thus, it became of paramount importance to assess whether activation of Ang II receptors in the caudal depressor and rostral pressor regions by endogenous Ang II contributed to the tonic activity of the counterbalancing pools of vasomotor neurons. Investigation of this concept was pursued in both SHR and WKY rats to determine whether endogenous Ang II had a quantitatively different influence upon the tonic activity of vasomotor neurons in hypertensive rats compared to normotensive animals. To determine the physiological role that Ang II might play in the activity of vasomotor neurons in hypertensive and normotensive rats, we blocked Ang II receptors in the CVLM and RVLM with the peptide analogue antagonist [Sar¹,Thr⁸]-Ang II.

Microinjection of [Sar¹,Thr⁸]-Ang II into either the RVLM or CVLM produced changes in blood pressure and heart rate that were directionally opposite to the cardiovascular effects which we had previously observed for Ang II injection into each region. Injection of [Sar¹,Thr⁸]-Ang II into the CVLM of SHR and WKY rats increased blood pressure and heart rate. In contrast, injection of this Ang II antagonist into the RVLM of both strains decreased blood pressure and heart rate. The pressor responses produced by [Sar¹,Thr⁸]-Ang II injection in the CVLM and the depressor responses evoked by this antagonist in the RVLM developed more slowly and were more sustained than the changes in blood pressure produced by L-Glu injection in the CVLM or RVLM.

Figure 5 summarizes the changes in blood pressure produced by injection of either L-Glu (2 nmol), Ang II (20 pmol), or [Sar¹,Thr⁸]-Ang II (100 pmol) into the RVLM of SHR and WKY rats. The most dramatic effect of [Sar¹,Thr⁸]-Ang II injections into the RVLM was the significant ($P > 0.01$) larger reduction in baseline blood pressure measured in SHR compared to WKY rats. This was in marked contrast to the repeated demonstration that Ang II elicited similar pressor responses in SHR and WKY rats and that L-Glu produced larger pressor responses than Ang II, which were of similar magnitude in SHR and WKY rats. To ascertain that the reduction in blood pressure produced by [Sar¹,Thr⁸]-Ang II injection into the RVLM was caused by a reduction in sympathetic nerve activity, splanchnic sympathetic nerve activity (SpSNA) was recorded in another set of SHR and WKY rats. Injection of [Sar¹,Thr⁸]-Ang II (1 nmol) into the RVLM decreased SpSNA by $21 \pm 2\%$ in SHR and by $18 \pm 4\%$ in WKY rats. These reductions in baseline SpSNA were accompanied by depressor responses in SHR (-39 ± 5 mm Hg) and WKY rats (-25 ± 4 mm Hg).

Figure 6 summarizes the changes in blood pressure produced by injection of L-Glu (2 nmol), Ang II (20 pmol), or [Sar¹,Thr⁸]-Ang II (100 pmol) into the CVLM of SHR and WKY rats. When compared to WKY rats, SHR had significantly larger decreases in blood pressure in response to L-Glu ($P < 0.01$) and Ang II ($P < 0.05$) injections into the CVLM. In contrast, [Sar¹,Thr⁸]-Ang

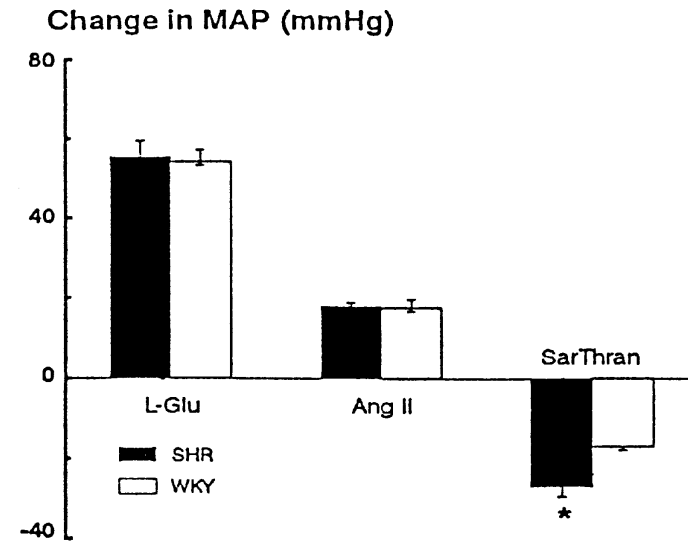


Figure 5 Changes in mean arterial pressure (MAP) produced by microinjection of L-glutamate (L-Glu), angiotensin II (Ang II), and [Sar¹,Thr⁸]-Ang II (SarThran) into the rostral ventrolateral medulla of SHR and WKY rats. Injection volumes were 50 nL. * $P < 0.01$.

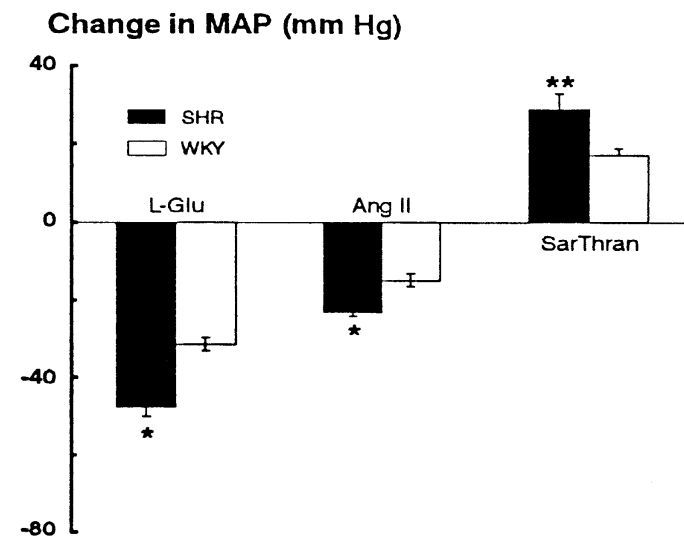


Figure 6 Changes in mean arterial pressure (MAP) produced by microinjection of L-glutamate (L-Glu), angiotensin II (Ang II), and [Sar¹,Thr⁸]-Ang II (SarThran) into the caudal ventrolateral medulla of SHR and WKY rats. Injection volumes were 50 nL. * $P < 0.05$, ** $P < 0.01$.

II injection into the CVLM increased baseline blood pressure of both strains, and this pressor response was significantly ($P < 0.05$) larger in SHR.

Recent studies have shown that GABAergic projections from the CVLM provide inhibitory input to presympathetic neurons of the RVLM (11–13). We hypothesized that the increase in blood pressure produced by microinjection of [Sar¹,Thr⁸]-Ang II into the CVLM was due to a reduction of GABAergic input to presympathetic neurons of the RVLM. This hypothesis was supported by the finding that [Sar¹,Thr⁸]-Ang II injection into the CVLM increased SpSNA in SHR and WKY rats. However, to address more directly the concept that blockade of Ang II receptors in the CVLM reduced the tonic activity of CVLM GABAergic projections to the RVLM, we determined whether the pressor response to [Sar¹,Thr⁸]-Ang II injection into the CVLM could be abolished by inhibition of RVLM neurons. Figure 7 summarizes the results of experiments in which the blood pressure changes produced by [Sar¹,Thr⁸]-Ang II injection into the CVLM were determined before and after bilateral injection of the GABA_A agonist muscimol into the RVLM. Injection of [Sar¹,Thr⁸]-Ang II into the CVLM before delivery of muscimol into the RVLM caused pressor responses in both SHR (23 ± 1 mm Hg) and WKY rats (18 ± 2 mm Hg; $P < 0.05$ SHR versus WKY).

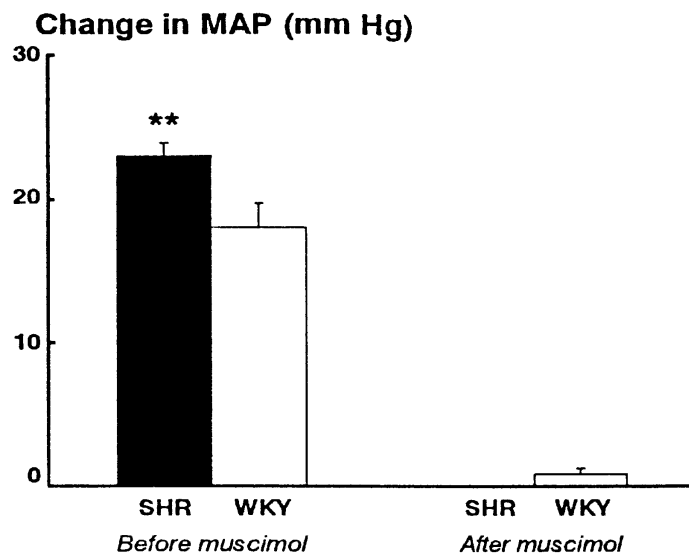


Figure 7 Changes in mean arterial pressure (MAP) produced by microinjection of [Sar¹,Thr⁸]-Ang II into the caudal ventrolateral medulla before and after bilateral injection of muscimol (500 pmol each side) into the RVLM of SHR and WKY rats. The increases in MAP in response to [Sar¹,Thr⁸]-Ang II injection into the caudal ventrolateral medulla after bilateral injection of muscimol into the rostral ventrolateral medulla were significantly different from the pressor responses produced by [Sar¹,Thr⁸]-Ang II injection into the caudal ventrolateral medulla before muscimol injection. ** $P < 0.05$.

Bilateral injection of muscimol (500 pmol each side) into the RVLM decreased mean arterial pressure (MAP) from 123 ± 2 to 51 ± 3 mm Hg in SHR and from 92 ± 3 to 45 ± 2 mm Hg in WKY rats. A second injection of [Sar¹,Thr⁸]-Ang II into the CVLM after bilateral injection of muscimol into the RVLM completely abolished in both strains the pressor response that previously was produced by Ang II antagonist injection into the CVLM.

C. Angiotensin II Receptor Subtypes in the Rostral Ventrolateral Medulla

The development of new agents selective for different receptor subtypes responsive to Ang II has revealed the presence of AT₁ and AT₂ receptors in the brainstem of rats. Recently, we showed that Ang II acts at AT₁ receptors in the nTS to produce decreases in blood pressure in response to microinjection of the peptide in this dorsal medullary nucleus (32). Thus, the next objective of our studies investigating the actions of Ang II in the ventrolateral medulla of SHR and WKY rats was to determine the receptor subtype at which Ang II acts in the RVLM. To investigate the role of AT₁ receptors in the RVLM, we used losartan, a 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[2'-(1H-tetrazol-5-yl) biphenyl-4-yl] methyl] imidazole potassium salt, as an antagonist selective for AT₁ receptors (33).

A range of doses (0.01, 0.1, 1, and 10 nmol) of losartan was used to assess the effect of AT₁ receptor blockade on the actions of Ang II in the RVLM. The lowest dose (0.01 nmol) injected into the RVLM caused a partial attenuation of the pressor response that could be elicited by Ang II (100 pmol) injection at the same site. However, higher doses (0.1, 1, and 10 nmol) of losartan attenuated by two-thirds the pressor responses that could be produced by Ang II injection in SHR and WKY rats. Since our previous studies had shown that [Sar¹,Thr⁸]-Ang II could also attenuate the pressor action of Ang II injected into the RVLM, we compared the abilities of losartan and [Sar¹,Thr⁸]-Ang II to block the effects of Ang II. Figure 8 illustrates the increases in blood pressure and SpSNA that were elicited by Ang II injected into the RVLM of SHR and WKY rats after prior injection of artificial cerebrospinal fluid (aCSF). When losartan (1 nmol) was injected into the RVLM immediately before injection of Ang II at the same site, the pressor response and sympathetic activation produced by Ang II were significantly ($P < 0.01$) attenuated. In contrast, prior injection of [Sar¹,Thr⁸]-Ang II into the RVLM essentially abolished the pressor response and sympathetic activation produced by Ang II injections in both SHR and WKY rats. Comparisons of the residual pressor responses produced by Ang II observed after injection of either losartan or [Sar¹,Thr⁸]-Ang II revealed a significant ($P < 0.05$) difference in SHR. An important feature of the doses (0.1 to 10 nmol) of losartan that significantly attenuated the actions of Ang II in the RVLM was that baseline blood pressure was not changed 5 min after injection of this Ang II antagonist. Figure 9 shows that lack of effect of

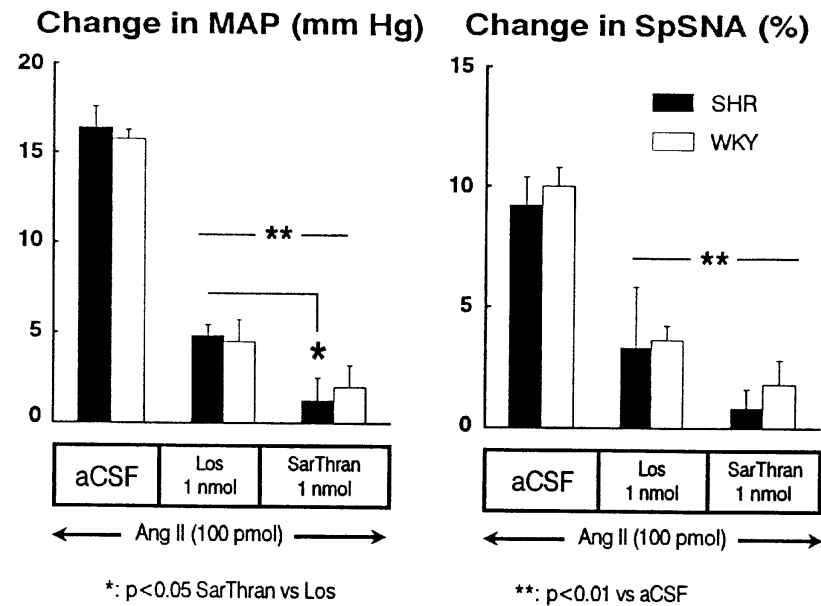


Figure 8 Changes in mean arterial pressure (MAP) and splanchnic sympathetic nerve activity (SpSNA) in response to angiotensin II (Ang II) injection into the rostral ventrolateral medulla after injection of artificial cerebrospinal fluid (aCSF), losartan (Los), or [Sar¹,Thr⁸]-Ang II (SarThran) at the same site. * $P < 0.05$ for comparison of responses in SHR between losartan and [Sar¹,Thr⁸]-Ang II. ** $P < 0.01$ for comparison of responses produced by Ang II after either losartan or [Sar¹,Thr⁸]-Ang II to that after aCSF.

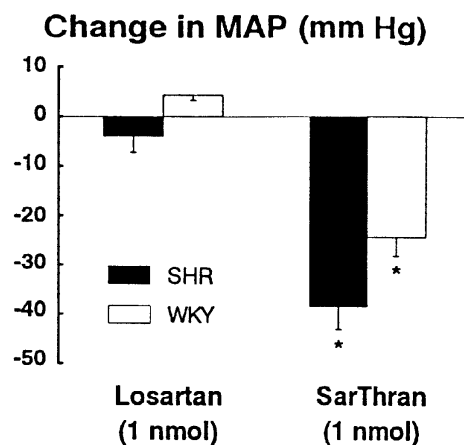


Figure 9 Changes in baseline mean arterial pressure (MAP) produced by microinjection of either losartan or [Sar¹,Thr⁸]-Ang II (SarThran) into the rostral ventrolateral medulla of SHR and WKY rats. * $P < 0.01$ for comparison of responses between losartan and [Sar¹,Thr⁸]-Ang II.

losartan (1 nmol) upon baseline blood pressure was in marked contrast to the ability of [Sar¹,Thr⁸]-Ang II (1 nmol) injected into the RVLM to significantly reduce baseline blood pressure. Based upon these results, we conclude that AT₁ receptors play a role in mediating vasomotor actions of Ang II in the RVLM of SHR and WKY rats. However, the residual increases in blood pressure and sympathetic nerve activity produced by Ang II after losartan and the lack of effect of losartan upon baseline blood pressure suggest that Ang II may also act at non-AT₁ or AT₂ receptor subtypes in the RVLM.

IV. Discussion

The data presented above provide evidence that Ang II contributes to the tonic activity of vasomotor neurons of the ventrolateral medulla. Initially, we showed that this peptide acted as a neuroexcitatory agent to increase the activity of rostral pressor and caudal depressor neurons. The neuroexcitatory action of Ang II is also supported by the finding that iontophoretic application of Ang II to RVLM neurons increases the firing rate of these vasomotor neurons (34). However, the neuroexcitatory action of Ang II appeared less potent than the effects that could be evoked by L-Glu. On average, the increases in blood pressure that could be produced by peptide injection into the RVLM ranged from 32% to 48% of the magnitude that could be elicited by L-Glu injection in either SHR or WKY rats. In the CVLM, the depressor responses produced by Ang II injection ranged from 39% to 49% of the magnitude of the decreases in blood pressure evoked by L-Glu injections in either SHR or WKY rats. Another characteristic difference between the blood pressure responses evoked by Ang II and L-Glu in the VLM was that the onset times and latencies to the peak change in blood pressure were significantly longer in response to Ang II in comparison to L-Glu. The smaller changes in blood pressure and the longer time course of the changes in blood pressure evoked by Ang II might suggest that the mode of action of the peptide upon vasomotor neurons is different from that of L-Glu. Electrophysiological recordings from nTS neurons of an *in vitro* brainstem slice have shown that, for the same neuron, L-Glu evoked a larger change in firing rate than did Ang II, and this occurred with a shorter latency (K. L. Barnes, personal communication). These electrophysiological studies also showed that the increase in firing rate produced by Ang II was substantially more prolonged than that elicited by L-Glu. These latter findings are consistent with the notion that Ang II and L-Glu act through different neuronal mechanisms. However, one must consider that L-Glu may have neuronal effects mediated either through ligand-gated ion channels or by effects upon second-messenger systems involving changes in intracellular Ca²⁺ concentration (35). It is interesting to note that Ang II acting at AT₁ receptors evokes a similar constellation of effects upon intracellular Ca²⁺ as those produced by L-Glu acting at metabotropic excitatory amino acid receptors. Thus, an important objective for future in-

vestigations will be to determine the biophysical and biochemical mechanisms by which Ang II affects excitability of vasomotor neurons.

The changes in blood pressure produced by Ang II injection into the RVLM and CVLM provide functional evidence for Ang II receptors in these regions of the rat brainstem. However, the physiological role of Ang II in these two regions of the VLM was revealed by the changes in blood pressure produced by blockade of Ang II receptors with [Sar¹,Thr⁸]-Ang II. Blockade of Ang II receptors in the RVLM reduced baseline blood pressure, whereas Ang II receptor blockade in the CVLM increased baseline blood pressure. The changes in baseline blood pressure produced by local injection of [Sar¹,Thr⁸]-Ang II were directionally opposite to the effects produced by injection of the agonist peptide. Qualitatively similar effects upon baseline blood pressure were observed in both SHR and WKY rats, albeit the magnitude of the changes in blood pressure was significantly larger in SHR. These data agree with our previous finding in the cat that [Sar¹,Thr⁸]-Ang II applied to the ventral surface overlying the RVLM reduced blood pressure (19). Sasaki and Dampney also found that injection of [Sar¹,Thr⁸]-Ang II in the VLM of rabbits produced changes in blood pressure that were in a direction opposite to those produced by the parent peptide (20). More recently, Chan et al. (36) have provided preliminary evidence that microinjection of [Sar¹,Thr⁸]-Ang II reduced the firing rate of individually recorded presympathetic neurons of the RVLM. These results are taken as evidence that Ang II in the CVLM and RVLM contributes to the tonic activity of vasomotor neurons in these two regions of the VLM. In the RVLM, we have attempted to assess the relative contribution of Ang II to the tonic activity of presympathetic neurons. We compared the reduction of blood pressure produced by [Sar¹,Thr⁸]-Ang II injection into the RVLM. In both SHR and WKY rats, the reduction in baseline blood pressure evoked by [Sar¹,Thr⁸]-Ang II was on average 50% of the reduction in blood pressure observed when RVLM neurons were inhibited by the GABA_A agonist muscimol.

The cardiovascular effects of Ang II receptor blockade in either the CVLM or RVLM appear to be the result of modulation of sympathetic outflow. First, the pressor response to [Sar¹,Thr⁸]-Ang II injection into the CVLM of SHR and WKY rats was abolished after inhibition of RVLM neurons by bilateral injection of muscimol. Thus, vasomotor neurons of the RVLM are required for the action of the Ang II antagonist in the CVLM, which was to reduce inhibitory drive to RVLM vasoconstrictor neurons. This pathway agrees with the demonstration by Sasaki and Dampney (20) that injection of [Sar¹,Thr⁸]-Ang II in the rabbit CVLM increased sympathetic nerve activity. We have also performed studies in Sprague-Dawley rats showing that [Sar¹,Thr⁸]-Ang II injection in the CVLM caused splanchnic sympathetic nerve activity to increase (37). Second, the data reviewed above show that injection of [Sar¹,Thr⁸]-Ang II into the RVLM of SHR and WKY rats reduced baseline levels of splanchnic sympathetic nerve activity. Sasaki and Dampney (20) have also shown in rabbits that injection of [Sar¹,Thr⁸]-Ang II reduced renal sympathetic nerve activity.

In the last few years, agents exhibiting selectivity for different Ang II receptor subtypes have been developed; these have shown that AT₁ and AT₂ receptors may be associated with brain pathways involved in neural or neuroendocrine control of the cardiovascular system (32,38–41). Therefore, we determined whether Ang II acted at AT₁ receptors in the RVLM. Local injection of the AT₁ receptor antagonist losartan into the RVLM of SHR and WKY rats significantly attenuated the pressor responses that could be evoked by Ang II injection at the same site. However, even at the highest dose of losartan (10 nmol) utilized, there remained a residual pressor response to Ang II injection. Thus, it appeared that blockade of AT₁ receptors could not abolish the neuroexcitatory action of Ang II in the RVLM. In contrast, [Sar¹,Thr⁸]-Ang II at a dose of 1 nmol essentially eliminated the pressor response that could be evoked by Ang II. These findings are in contrast to a recent study by Fow et al. (32), who showed that the depressor responses produced by a low dose of Ang II (200 fmol) injected into the nTS were blocked by losartan (100 pmol). In addition, when we compared the effects of losartan and [Sar¹,Thr⁸]-Ang II upon baseline blood pressure in SHR and WKY rats, we observed that losartan (1 nmol) did not reduce baseline blood pressure, whereas [Sar¹,Thr⁸]-Ang II (1 nmol) caused a substantial reduction in baseline blood pressure of SHR and WKY rats. This set of data suggests that Ang II injected into the RVLM may act on non-AT₁ or AT₂ receptors as well as on AT₁ receptors. In addition, we have the provocative finding that the actions of Ang II endogenous to the RVLM apparently are not affected by AT₁ receptor blockade. Future studies need to focus on the possible role of non-AT₁ receptors in the actions of Ang II or Ang II metabolites in the RVLM.

One of the goals of these experiments was to assess the role that Ang II in the VLM might have in the pathogenesis of hypertension. Since a number of studies have provided evidence that the elevated blood pressure of SHR is related to an enhanced activity of the sympathetic nervous system (21–23), we hypothesized that an action of Ang II at vasomotor neurons may be a contributing factor to the hypertension of SHR. In part, this hypothesis was based upon findings showing that central blockade of the renin-angiotensin system reduces the blood pressure of SHR (24–26). Injection of Ang II into the RVLM produced equivalent pressor responses in SHR and WKY rats, whereas injection of the peptide into the CVLM caused significantly larger decreases in blood pressure of SHR compared to WKY rats. This data set by itself did not resolve the potential role of Ang II in the VLM in the hypertension of SHR. In part, this was related to the fact that L-Glu injection in the RVLM caused equivalent pressor responses in SHR and WKY. Moreover, L-Glu injection into the CVLM evoked larger depressor responses in SHR, which were qualitatively similar to the responses evoked by Ang II. Our findings for the responses to L-Glu were in agreement with the reports of Smith and Barron (42,43). However, our data for the responses to Ang II in the RVLM did not appear consistent with the observations of Chan et al. (34). These investigators observed that application

of Ang II to individually recorded RVLM neurons produced significantly larger increases in firing rate, shorter latency of augmented firing rate, and a lower current threshold for iontophoretic application of Ang II in SHR compared to WKY rats. Examination of the effects of [Sar¹,Thr⁸]-Ang II injection into the VLM of SHR and WKY rats revealed that Ang II receptor blockade in the RVLM caused larger reductions in baseline blood pressure in SHR, and injection of this receptor antagonist into the CVLM produced larger increases in baseline blood pressure in SHR. The larger changes in blood pressure produced by [Sar¹,Thr⁸]-Ang II injection into the RVLM and CVLM of SHR cannot be explained easily by differences in vascular reactivity between SHR and WKY because L-Glu injection in this latter set of experiments caused equivalent pressor responses in SHR and WKY rats. The question remains whether Ang II has actions in either the RVLM or CVLM that contribute to the elevated blood pressure of SHR. Our data suggest that the increased action of Ang II in the RVLM of SHR may relate to higher tonic activity of RVLM neurons in this strain. On the other hand, the action of endogenous Ang II may be augmented in the CVLM of SHR, but this enhanced activity of CVLM inhibitory neurons may be insufficient to suppress an apparent greater intrinsic pressor activity of RVLM neurons. Additional insight into this issue is provided by the work of Chan et al. (34,44,45). These investigators showed that in comparison to WKY rats, SHR have a greater proportion of RVLM neurons that fire with doublet discharges. Most intriguing is their recent finding (45) that long-term treatment of SHR with captopril not only reduced the blood pressure of SHR but also altered the firing properties of RVLM neurons such that the proportion of RVLM neurons firing in doublets or singlets was essentially the same as that determined for WKY rats. In conclusion, it will be important to determine what other factors influence the firing properties of vasomotor neurons and to identify the contribution of singlet and doublet discharging RVLM neurons in the maintenance of sympathetic nerve activity.

V. Summary

This chapter has reviewed recent work done in the author's laboratory to define the role of Ang II in the function of RVLM and CVLM in SHR and WKY rats. Data are presented demonstrating that Ang II acts as an excitatory agent in the RVLM and CVLM of both strains of rats. In addition, the results of experiments employing the receptor antagonist [Sar¹,Thr⁸]-Ang II provide evidence that Ang II endogenous to both regions of the VLM contributes to the tonic activity of vasomotor neurons. Available data shows that Ang II acts at AT₁ receptors in the RVLM of SHR and WKY rats. Finally, results of experiments utilizing [Sar¹,Thr⁸]-Ang II as an Ang II antagonist suggest that endogenous Ang II may contribute to a greater tonic activity of CVLM and RVLM neurons of SHR.

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