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Ventrolateral Medulla AT₁ Receptors Support Blood Pressure in Hypertensive Rats

Satoru Ito, Kazutoshi Komatsu, Kazuyoshi Tsukamoto, Katsuo Kanmatsuse, Alan F. Sved

Abstract—Angiotensin within the central nervous system appears to be important for the maintenance of hypertension in spontaneously hypertensive rats. This study addresses the hypothesis that blockade of AT₁ receptors in the rostral ventrolateral medulla would decrease blood pressure in spontaneously hypertensive rats and that this tonically active AT₁-mediated input to the rostral ventrolateral medulla arises from the hypothalamic paraventricular nucleus. Injection of the nonpeptide AT₁ receptor antagonist valsartan bilaterally into the rostral ventrolateral medulla of chloralose-anesthetized adult spontaneously hypertensive rats produced a dose-related decrease in mean arterial pressure, with a maximal effect of ≈ 30 mm Hg. Inhibition of the paraventricular nucleus by local injection of muscimol elicited a similar response, which was inhibited by prior injection of valsartan into the rostral ventrolateral medulla. In contrast, in control Wistar-Kyoto rats, neither valsartan injected into the rostral ventrolateral medulla nor muscimol injected into the paraventricular nucleus had a substantial effect on arterial pressure. These data indicate that in spontaneously hypertensive rats but not in Wistar-Kyoto rats, rostral ventrolateral medulla vasomotor neurons are tonically excited by endogenous stimulation of AT₁ receptors, and this input is apparently driven from the hypothalamus. These results suggest that the rostral ventrolateral medulla is one site that the brain renin-angiotensin system acts to maintain elevated blood pressure in spontaneously hypertensive rats. (*Hypertension*. 2002;40:552-559.)

Key Words: brain ■ receptors, angiotensin ■ central nervous system ■ hypertension, experimental ■ hypothalamus ■ angiotensin

Angiotensin II (Ang II) acting within the brain can influence arterial pressure (AP),^{1,2} and the central actions of Ang II have been implicated in several forms of experimental hypertension.³⁻⁵ The data supporting a role of brain Ang II in hypertension in the spontaneously hypertensive rats (SHR) are particularly convincing. Specifically, pharmacological disruption of brain angiotensin signaling decreases AP in SHR.⁶⁻¹⁰ Furthermore, there appear to be alterations in SHR in the amount of angiotensin or its receptors in discrete brain regions.^{3,4,11,12}

Despite this important role of brain Ang II in hypertension, the site (or sites) within the brain at which Ang II acts in this regard is not fully worked out. Certainly, Ang II can act within the hypothalamus to influence cardiovascular regulation.¹³ However, Ang II can also act in the caudal brain stem to increase AP and sympathetic outflow.¹⁴ One region in the caudal brain stem that probably mediates some of the central cardiovascular effects of Ang II is the rostral ventrolateral medulla (RVLM).^{15,16} The RVLM appears to be the brain site responsible for the maintenance of basal sympathetic vasomotor activity, and inhibition of RVLM neurons reduces AP to the same extent as total inhibition of sympathetic vasomotor activity. Conversely, stimulation of neurons in the RVLM increases AP. There is evidence that increased activity of the

RVLM supports the elevated AP in SHR.¹⁷⁻¹⁹ The RVLM also has a high density of angiotensin AT₁ receptors.²⁰ Furthermore, microinjection of Ang II into the RVLM increases AP and sympathetic nerve activity,²¹⁻²⁴ and stimulation of AT₁ receptors increases the activity of identified RVLM spinal neurons studied *in vitro*.^{25,26}

There is evidence that the input to these RVLM AT₁ receptors arises from the hypothalamic paraventricular nucleus (PVN). Though injection of AT₁ receptor antagonists into the RVLM has minimal effects on AP in normotensive rats,^{21,24,27-30} the increase in AP evoked by disinhibition of the PVN can be attenuated by injection of AT₁ antagonists into the RVLM.³⁰ The existence of a projection from PVN to RVLM containing angiotensin is supported by anatomic data.³¹⁻³⁴

Taken together, the data lead to the hypothesis that elevated AP in SHR may result from increased stimulation of AT₁ receptors in the RVLM, and this may reflect increased activity of an AT₁-mediated input to RVLM vasomotor neurons derived from the hypothalamus. Indeed, it was recently reported that injection of an AT₁ antagonist into the RVLM decreased AP in SHR but not control Wistar-Kyoto (WKY) rats.³⁵ The current experiments sought to more fully evaluate this hypothesis and to further examine this issue in

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the context of reports that (1) the injection of the nonselective angiotensin antagonist sarthran into the RVLM markedly decreases AP in normotensive control rats, though apparently not as the result of an action on AT₁ receptors^{27,28} and (2) that the excitatory amino acid receptor antagonist kynurenic acid (KYN) injected into the RVLM decreases AP in SHR but not WKY.¹⁷

Methods

These studies were conducted with the use of adult male SHR/Izm and WKY/Izm, 14 to 16 weeks of age and weighing between 280 and 350 g (Funabashi Farm, Chiba, Japan).¹⁷ For measuring AP, mean AP (MAP), and heart rate (HR) during injections of substances into the ventrolateral medulla, rats were prepared as previously described,^{17,36} except that the RVLM was exposed through a ventral approach.³⁷ Rats were initially anesthetized with halothane, and cannulas were inserted into a femoral artery and a femoral vein. Then, rats were placed in a stereotaxic frame in a supine position with the incisor bar positioned at the level of the interaural line. The trachea was cannulated and the rat was paralyzed with tubocurarine (0.5 mg/kg, supplemented hourly with 0.2 mg/kg) and ventilated with 100% oxygen for the remainder of the experiment. After completion of this surgery, the rat was injected with α -chloralose (60 mg/kg IV) and the halothane was terminated; additional chloralose (20 mg/kg IV) was administered hourly. The upper trachea, larynx, esophagus, and surrounding musculature were cut and retracted, exposing the occipital foramen and occipital bone. The basal aspect of the occipital bone was removed to expose the ventral surface of the rostral medulla.³⁷

Drugs were microinjected into the RVLM in 100 nL of aCSF (in mmol/L: 144 NaCl, 1.2 CaCl₂, 2.8 KCl, 0.9 MgCl₂; except for KYN, which was first dissolved in 100 mmol/L sodium bicarbonate and then diluted 10-fold in aCSF) with single-barrel glass micropipettes ($\approx 50 \mu\text{m}$ od tip). Coordinates for drug microinjections into the RVLM were 3.5 to 4.5 mm rostral to the caudal tip of the occipital foramen, 1.5 to 2.0 mm lateral to the basilar artery, and 0.7 mm below the surface of the medulla.³⁷ In each rat, initial test injections of 1 nmol of L-glutamate were made into the RVLM on each side to confirm that the coordinates identified a functional pressor site.³⁷ Baseline MAP and HR were then recorded for 60 minutes before additional RVLM injections. All protocols involved multiple injections into the same site (eg, the injections of glutamate to localize the site followed by a test substance); all injections into a specific site used the same pipette. After the initial injection, the pipette was removed, rinsed, refilled, and reinserted at the same coordinates. In protocols requiring bilateral injections, these injections were made one side at a time, with ≈ 30 seconds separating the two injections. In experiments examining the effect of valsartan on the response to Ang II, an initial injection of Ang II was followed approximately half an hour later by the injection of valsartan and a second injection of Ang II ≈ 8 minutes later. (In preliminary experiments in Sprague-Dawley rats, injections of Ang II into the RVLM at 30-minute intervals elicited similar responses.) In the experiment testing the combined effect of valsartan and KYN, these two drugs were mixed together before injection. At the conclusion of experiments, ≈ 20 nL of 1% fast green dye was injected at the coordinates used during the experiment to allow for histological verification of the center of the microinjection site. All RVLM injection sites were determined to be located in the rostral medulla, just ventral to the nucleus ambiguus, similar to the injection sites that we have published previously,³⁸ except that the present injections tended to be closer to the ventral medullary surface and the dye was distributed more ventrally.

For experiments involving injections into the PVN, rats were placed in a prone position in a stereotaxic instrument with the incisor bar positioned 19 mm ventral to the interaural point. Holes were drilled in the skull to allow pipettes to be inserted 1.8 mm posterior and 0.5 mm lateral to bregma, to a depth of 7.8 mm below the dura.^{30,39} If a pressor response of at least 10 mm Hg in response to

TABLE 1. Effect of Ang II and Glutamate Injected Into RVLM of SHR and WKY

Treatment Group	MAP (mm Hg)		HR (bpm)	
	Baseline	Change	Baseline	Change
Ang II				
WKY (n=4)	104 \pm 10	16 \pm 2	360 \pm 21	19 \pm 6
SHR (n=6)	167 \pm 6*	35 \pm 5*	375 \pm 15	25 \pm 3
Glutamate				
WKY (n=5)	98 \pm 10	45 \pm 7	350 \pm 11	33 \pm 6
SHR (n=7)	175 \pm 5*	51 \pm 3	383 \pm 18	45 \pm 6

Ang II (100 pmol) or glutamate (1 nmol) were injected unilaterally into the RVLM of SHR or WKY anesthetized with chloralose. Baseline values were the measurements taken just before injection, and change is expressed as the maximal change following injection. Both Ang II and glutamate increased MAP and HR compared with baseline in each strain ($P < 0.05$).

*Indicates significant difference between SHR and WKY.

injection of bicuculline (100 pmol) was not obtained, the pipette was moved 0.2 mm rostral or caudal. In these particular experiments, dye was not included to allow for histological assessment of the injection site. However, in other experiments in Sprague-Dawley rats in which cardiovascular responses to bicuculline were observed at these coordinates, the microinjection site was consistently found to be located within the boundaries of the PVN, typically centered laterally within the medial parvocellular division (unpublished observations). Nonetheless, the assertion that injections from this experiment were localized to the PVN is based only on the coordinates of the injection and the functional response; therefore, the anatomic localization of this response must be interpreted with caution.

In one set of experiments, injections were made into both the PVN and the RVLM. Animals were prepared for injections into the PVN, and then the dorsal surface of the medulla was surgically exposed.³⁶ Injections were made into the RVLM as described previously.³⁶

All drugs used in these studies were purchased from Sigma Chemical Co, except for valsartan, which was a gift from Novartis Pharma AG (Basel, Switzerland). Doses of drugs were selected on the basis of published studies, with the exception of valsartan, for which dose data are presented.

Data are expressed as mean \pm SEM. Responses in SHR and WKY rats were compared by *t* test or ANOVA, with the use of Systat 10 software (SPSS, Inc). The time course of the responses was analyzed by repeated-measures ANOVA.

Results

Ang II Injected Into the RVLM Increases MAP in SHR to a Greater Extent Than in WKY

Unilateral injection of Ang II (100 pmol) into the RVLM rapidly increased MAP, and this response was significantly greater in SHR compared with WKY (Table 1). In contrast, glutamate (1 nmol) injected into the RVLM elicited a similar increase in MAP in the two strains (Table 1), as noted previously.¹⁷

Valsartan Injected Into the RVLM Decreases MAP in SHR But Not in WKY

Bilateral injection of valsartan (100 pmol) into the RVLM of chloralose-anesthetized WKY rats had no effect on MAP or HR (Figures 1 and 2). In contrast, bilateral injection of this dose of valsartan into the RVLM in SHR decreased AP by ≈ 30 mm Hg (Figures 1 and 2). This response developed rather slowly; MAP began to decrease in 1.0 ± 0.1 minute and then gradually reached a peak response at 10.3 ± 0.7 minutes (Figure 2, bottom). The depressor response in SHR was

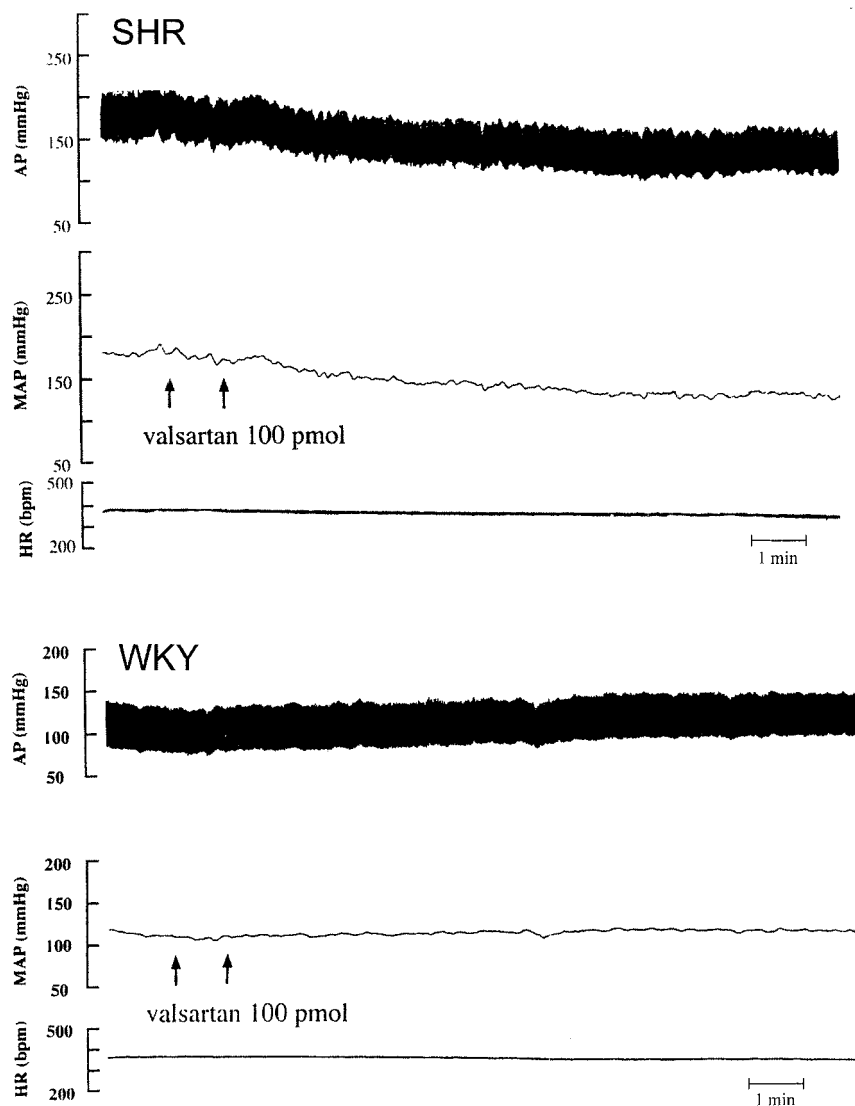


Figure 1. Representative polygraph recordings from SHR (top) and WKY (bottom) in which 100 pmol of valsartan was injected bilaterally into the RVLM. Arrows indicate points at which valsartan was injected, first on left side and then on right. Recordings are typical of data included in Figure 2.

consistently accompanied by a slight bradycardia (see legend to Figure 2). A smaller dose of valsartan (10 pmol) in SHR elicited a smaller decrease in MAP (Figure 2, top).

These dose-response data are consistent with the ability of valsartan to block the pressor action of injection of Ang II into the RVLM. Bilateral injection of 100 pmol of valsartan into the RVLM of SHR or WKY completely abolished the response to injection of 100 pmol Ang II \approx 8 minutes later (Table 2). In contrast, injection of 10 pmol valsartan only reduced the pressor response elicited by 100 pmol Ang II by \approx 50% (Table 2). The specificity of valsartan for blocking AT_1 receptors is suggested by the lack of an effect of valsartan on the pressor response produced by injection of glutamate into the RVLM (Table 2).

Bilateral injection into the RVLM of Sprague-Dawley rats of sarthran or sarile, peptide antagonists of angiotensin receptors, reduces MAP to the same extent as total ganglionic blockade,^{38,40} though this action appears to be independent of blocking AT_1 receptors.^{27,28} Injection of sarthran (1 nmol) into the RVLM markedly decreased MAP in both SHR and WKY (from 121 ± 3 to 64 ± 4 mm Hg in WKY [$n=5$] and

from 184 ± 5 to 77 ± 4 mm Hg in SHR [$n=7$]). The effect of sarthran injected into the RVLM on MAP is considerably larger than with valsartan. However, like valsartan, sarthran eliminated the pressor response to RVLM injection of Ang II in SHR (34 ± 4 mm Hg before, 5 ± 2 mm Hg after; $n=5$) without altering the pressor response to glutamate (50 ± 3 mm Hg before, 52 ± 3 mm Hg after; $n=5$). A fragment of Ang II, Ang 3–8, which is inactive at the AT_1 receptor, can reverse the decrease in MAP caused by injection of sarthran into the RVLM.²⁸ Therefore, we tested the ability of Ang 3–8 to reverse the effects of sarthran and valsartan injected into the RVLM in SHR. Injection of valsartan bilaterally into the RVLM of SHR decreased MAP by \approx 35 mm Hg, and this was not altered by subsequent injection of Ang 3–8 (Table 3). In contrast, bilateral injection of sarthran in SHR decreased MAP by >100 mm Hg, and this was totally reversed by the subsequent injection of Ang 3–8 (Table 3).

Inhibition of PVN Decreases MAP in SHR But Not in WKY

Tagawa and Dampney³⁰ previously reported that disinhibition of the PVN by local injection of bicuculline produced an

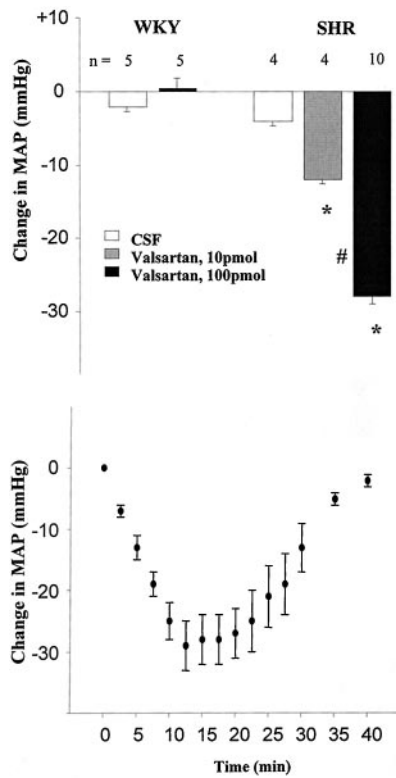


Figure 2. Effect of valsartan injected into the RVLM on MAP in SHR and WKY. Groups of chloralose-anesthetized SHR (n=10) and WKY (n=5) received bilateral injections of 100 pmol valsartan into the RVLM. Baseline MAP in these rats was 118±3 mm Hg in WKY and 183±7 mm Hg in SHR. A separate group of SHR (n=4) received bilateral injections of 10 pmol valsartan; baseline MAP in this group was 175±6 mm Hg. Other SHR (n=4) and WKY (n=4) received bilateral injections of CSF into the RVLM. Top, Maximal change from baseline. *Significant change from baseline and significant difference from WKY rats; #significant difference between 10- and 100-pmol dose in SHR. Bottom, Full time course of response in SHR injected with 100-pmol dose; at all time points between 2.5 and 35 minutes, MAP is significantly different from baseline. In rats receiving 100 pmol of valsartan, baseline HR was 390±7 bpm and 409±17 bpm in SHR and WKY, respectively. HR did not change significantly in WKY (+3±4 bpm) but decreased by 18±3 bpm in SHR (P<0.05 compared with baseline and WKY). In SHR injected with 10 pmol valsartan, HR decreased to a similar extent (-17±5 bpm).

increase in MAP that was attenuated by injection of losartan into the ipsilateral RVLM. If the response to bicuculline injected into PVN is mediated, at least in part by excitation of AT₁ receptors in RVLM, and the response to excitation of AT₁ receptors in the RVLM is exaggerated in SHR compared with WKY, then the response to bicuculline injected into PVN should similarly be enhanced in SHR. Indeed, unilateral injection of bicuculline (100 pmol) into the PVN increased MAP by 33±2 mm Hg in SHR (n=6; baseline 170±2 mm Hg), compared with 19±2 mm Hg in WKY (n=6; baseline 103±3 mm Hg; response different from SHR, P<0.05). This difference between SHR and WKY is similar to the difference between these strains in the response to Ang II injected into the RVLM (P>0.1 for the interaction term in a 2-way ANOVA comparing Ang II and bicuculline across rat strains).

TABLE 2. Effect of Valsartan Injected Into RVLM on the Pressor Response to Ang II or Glutamate Injected Into RVLM

Rat Strain	Ang II Pressor Response		Glutamate Pressor Response	
	Pre-Valsartan	Post-Valsartan	Pre-Valsartan	Post-Valsartan
WKY (n=7)	22±3	2±1†	45±7	46±4
SHR (n=4)	36±4*	4±2†	51±4	49±2

Data represent change from baseline (in mm Hg). Ang II (100 pmol) or glutamate (1 nmol) were injected into the RVLM in WKY and SHR rats before, and approximately 8 minutes after, injection of 100 pmol valsartan. Values are the maximal change in MAP elicited by either Ang II or glutamate. The baseline MAP values for the SHR and WKY rats used in these experiments were 178±6 mm Hg and 108±7 mm Hg, respectively.

*Indicates significant difference between SHR and WKY, P<0.05.

†Indicates no significant change from baseline and significant difference from before valsartan injection (P<0.05). In other SHR the pressor response to Ang II was tested after injection of 10 pmol valsartan; MAP increased 19±2 (n=4), which is significantly less than the control response to Ang II in SHR (P<0.05).

If the depressor response caused by injection of valsartan into the RVLM of SHR results from blockade of an Ang II-mediated input arising from the PVN, then inhibition of the PVN in SHR should produce a similar response. Bilateral injection of muscimol (100 pmol) directed at the PVN decreased MAP by ≈40 mm Hg in SHR compared with ≈10 mm Hg in WKY (Figure 3). In SHR, the response to bilateral injection of valsartan into the RVLM was similar in magnitude to the response to bilateral injection of muscimol into the PVN (2-way ANOVA of treatment and rat strain). Also like the response to valsartan, the decrease in MAP elicited by injection of muscimol directed at the PVN in SHR was slow to develop, with an onset latency of 1.0±0.2 minutes and a peak response at 12±1.2 minutes. The smaller response in WKY had a similar onset (1.0±0.3 minutes), but the maximal response occurred sooner (3.7±0.4 minutes). Unilateral injection of muscimol directed at the PVN also significantly decreased MAP in SHR by approximately half as much as bilateral injections (Figure 3).

If the depressor responses elicited by injection of valsartan into the RVLM and muscimol into the PVN in SHR share a similar mechanism of action, then one response should occlude the other. Therefore, we tested the effect of injecting

TABLE 3. Effect of Ang 3–8 on the Decrease in MAP Produced by Injection of Sarthran and Valsartan into RVLM in SHR

Treatment Group	MAP (mm Hg)		
	Baseline	Post-Antagonist	Post-Ang 3–8
Sarthran	190±6	78±3*	187±9
Valsartan	193±11	158±12*†	162±14*†

Sarthran (1 nmol; n=4) or valsartan (100 pmol; n=4) were injected bilaterally into the RVLM of SHR. Approximately 8 minutes later, Ang 3–8 was injected bilaterally into the RVLM.

*Indicates significant difference from baseline value, P<0.05.

†Indicates significant difference between the groups treated with sarthran and valsartan, P<0.05.

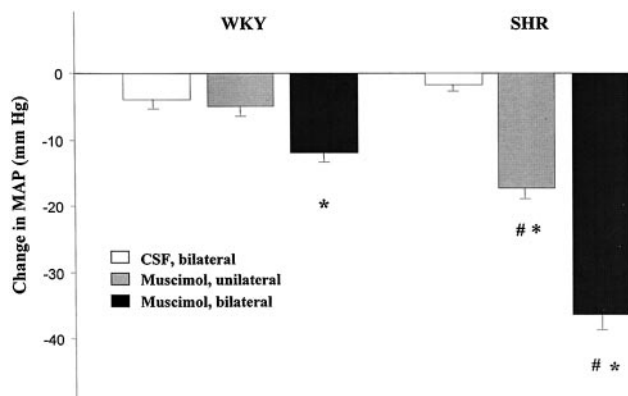


Figure 3. Effect of muscimol injected into PVN on MAP in SHR and WKY. Groups of chloralose-anesthetized SHR ($n=4$) and WKY ($n=4$) received unilateral or bilateral injections of 100 pmol muscimol or bilateral injections of CSF into the PVN. Baseline MAP in these rats before bilateral injection of muscimol was 103 ± 3 mm Hg in WKY and 178 ± 3 mm Hg in SHR. Values represented are maximal change from baseline. *Significant change from baseline; #significant difference between SHR and WKY. In rats receiving bilateral injections of muscimol into the PVN, baseline HR was 416 ± 6 bpm and 425 ± 12 bpm in SHR and WKY, respectively. HR decreased in response to muscimol injections in both strains; though the response tended to be larger in SHR (-40 ± 11 bpm), it was not significantly greater than in WKY (-20 ± 4 bpm; $P > 0.05$).

muscimol into the PVN after injection of valsartan into the RVLM. In 5 SHR, injection of 100 pmol valsartan into the RVLM (approached from the dorsal surface) reduced MAP by 30 ± 3 mm Hg from a baseline of 175 ± 4 mm Hg. MAP decreased slowly, with a latency of 1.3 ± 0.2 minutes and reaching a peak response at 9.4 ± 0.8 minutes. At ≈ 10 minutes after injection of valsartan, when MAP was 144 ± 6 mm Hg, muscimol was injected bilaterally into the PVN. There was a small decrease in MAP, reaching a maximum response of -9 ± 2 mm Hg at ≈ 4 minutes after injection. At 12 minutes after injection of muscimol directed at the PVN, the time at which the depressor response was greatest in rats just receiving muscimol directed at the PVN, MAP was not different from that before injection of muscimol. Thus, after injection of valsartan into the RVLM, the response to injection of muscimol into the PVN closely mimicked the response seen in WKY in terms of magnitude and time course.

Effect of Valsartan in RVLM Is Additive With Kynurenic Acid

Injection of the excitatory amino acid antagonist KYN into the RVLM of SHR but not WKY decreases MAP.¹⁷ To determine whether these responses share a similar mechanism, the two drugs were injected together into the RVLM in SHR. Bilateral injection into the RVLM of valsartan plus KYN decreased MAP by 66 ± 6 mm Hg ($n=4$), a significantly larger response than elicited by either drug alone (valsartan: 28 ± 1 mm Hg, see Figure 2; KYN: 40 ± 5 mm Hg¹⁷). In fact, the response to the two drugs injected together was similar to what would be expected from adding the responses of the individual drugs.

Discussion

The major finding of the current study is that blockade of AT_1 receptors in the RVLM decreases AP in SHR but not in WKY. A similar response is evoked by injections of muscimol targeting the PVN, which is consistent with previous reports that the PVN can excite RVLM sympathoexcitatory neurons through an AT_1 -mediated input to the RVLM.³⁰

Previous studies have demonstrated that Ang II can act on AT_1 receptors in the RVLM to increase AP. AT_1 receptors are present in RVLM,²⁰ and the activity of RVLM spinal neurons studied in vitro is stimulated by Ang II in a losartan-sensitive manner.^{25,26} Previous studies have found that microinjection of Ang II into the RVLM, in the dose range of 10 to 100 pmol, increases AP.^{21–24} One notable exception to this action of Ang II in the RVLM is a previous report in which we did not observe an increase in AP in response to Ang II injection.²⁸ The difference between the current study and our previous results with Ang II may be related to the precise site of injection. Specifically, in the prior study the RVLM was approached from the dorsal side, whereas in the current study it was approached from the ventral surface. In both studies, functional pressor sites were identified by local injection of glutamate, and the center of the microinjection site appears to be located in a similar region in both studies. However, the distribution of dye injected into the RVLM appears to be different, depending on whether the pipette was inserted from the dorsal surface or the ventral surface; with injections from the dorsal side, dye was seen to run along the pipette track toward the nucleus ambiguus, whereas in the current study the dye spread along the rostral ventral medullary surface. The possibility that Ang II acts specifically in a ventral region of the RVLM is supported by studies showing that AT_1 receptors in the RVLM are localized very close to the ventral medullary surface.²⁰ Benarroch et al⁴¹ previously suggested that the RVLM, identified through a dorsal approach, and the rostral lateral region of the ventral medullary surface from which cardiovascular responses can be evoked were essentially the same; however, one implication of the current data, taken together with our previous studies, is that subtle differences may exist in the site of action of different substances in the RVLM. The increase in MAP evoked by injection of Ang II into the RVLM in the current study results from stimulation of AT_1 receptors in this region, since it can be antagonized in a dose-dependent manner by valsartan, a selective AT_1 receptor antagonist.⁴²

The current data show that stimulation of AT_1 receptors in the RVLM causes a greater increase in AP in SHR than in WKY. Specifically, we observed that the pressor response to 100 pmol Ang II injected into the RVLM is potentiated by $\approx 100\%$ in SHR compared with WKY. Chan et al¹⁸ also reported that the RVLM of SHR is more sensitive to Ang II than in WKY. However, other previous studies^{21,22} found no difference between SHR and WKY in the response to injection of Ang II into the RVLM. The reason for these conflicting results is unclear but may relate to technical differences such as the anesthetic used (chloralose in the current study versus halothane). In the current study, the distinction that the pressor response to glutamate injected into the RVLM is not different between SHR and WKY rats,

whereas the response to Ang II is larger in SHR than WKY, suggests that the exaggerated response to Ang II in SHR is not a nonselective difference in vascular responsiveness between these two strains.

A major finding of the current study is that valsartan injected into the RVLM produced a dose-related decrease in MAP in SHR but did not decrease MAP in WKY. The lack of an effect of valsartan in WKY is consistent with previous studies showing no effect on MAP of injecting other AT₁ antagonists into the RVLM of normotensive Sprague-Dawley rats,^{27–29,43} consistent with our own data that valsartan injected into the RVLM in Sprague-Dawley rats has no effect on MAP (unpublished observation). These results suggest that AT₁ receptors in the RVLM are not activated under baseline conditions in normotensive rats. In contrast, the marked fall in AP after injection of valsartan into the RVLM of SHR suggests that RVLM AT₁ receptors are tonically stimulated and acting to support sympathetic vasomotor tone under basal conditions in these animals. This finding is similar to the data in the recent report by Allen,³⁵ showing that candesartan injected into the RVLM in SHR decreased AP and lumbar sympathetic nerve activity by ≈20%; the fact that these studies were conducted in rats anesthetized with urethane rather than chloralose, as in the current study, suggests that this effect is not dependent on the specific anesthetic used. Also, in the TGR(mREN2)27 hypertensive rat, RVLM injection of an AT₁ antagonist, CV-11974, decreased AP,⁴³ and we have observed similar results with valsartan in Dahl salt-sensitive hypertensive rats.⁴⁴ Thus, in models of hypertension that may be associated with increased levels of angiotensin in the brain, blockade of AT₁ receptors in the RVLM appears to decrease AP.

These observations that stimulation of AT₁ receptors elicits a greater increase in AP in SHR and that blockade of AT₁ receptors decreases AP in SHR may relate to an increase in the number of AT₁ receptors in the brains of SHR. Though previous studies have not focused specifically on the RVLM, at least in some brain regions the number of angiotensin binding sites is increased in SHR.^{11,12}

The input to the RVLM AT₁ receptors appears to arise from the PVN, as suggested by the observation that the increase in MAP resulting from disinhibition of the PVN was attenuated by prior injection of losartan into the RVLM.³⁰ This is also consistent with anatomic evidence of angiotensin-immunoreactive neurons in the PVN,^{31,32} in the region that can be retrogradely labeled from the RVLM.³³ If this PVN-to-RVLM angiotensinergic pathway is tonically active in SHR but not WKY, and the response to Ang II at the level of the RVLM is enhanced in SHR, then the response to disinhibition of the PVN should be enhanced in SHR and inhibition of the PVN should decrease AP in SHR. Indeed, both predictions are supported by the current data. Bilateral injection of muscimol directed at the PVN, using a dose of muscimol that has been shown previously to inhibit activity of this region,^{45,46} decreased MAP by ≈35 mm Hg in SHR. In contrast, injection of muscimol into the PVN evoked only small responses in WKY, in agreement with previous studies in normotensive rats.^{45–47} Further evidence that the exaggerated depressor response to injection of muscimol directed at

the PVN of SHR results from inhibition of an AT₁-mediated input to the RVLM comes from the observation that it is eliminated by pretreatment of the RVLM with valsartan. A decrease in AP after injection of muscimol into the PVN has also been observed in renal hypertensive rats.⁴⁷ Thus, the PVN, through an angiotensin-mediated input to the RVLM, may contribute to the maintenance of baseline AP in certain models of hypertension.

Injection into the RVLM of sarthran, or similar peptide antagonists of angiotensin receptors, markedly decreases AP,^{38,40} though this action is unrelated to blockade of AT₁ receptors.^{27,28} Interestingly, the decrease in AP caused by sarthran injection into the RVLM can be reversed by fragments of Ang II, for example Ang 3–8, which have minimal activity on AT₁ receptors.²⁸ In SHR, as in normotensive rats, injection of sarthran into the RVLM decreased AP to approximately the same extent as does total autonomic blockade. Also in SHR, the decrease in AP caused by sarthran was totally reversed by injection of Ang 3–8, whereas the decrease in AP caused by injection of the AT₁ receptor selective antagonist valsartan was not influenced by Ang 3–8. We have previously noted that this dose of Ang 3–8 (1 nmol) has no effect on AP in Sprague-Dawley rats,²⁸ and this is supported in the current study by the observation that Ang 3–8 did not increase AP in SHR pretreated with valsartan. Presumably, the dose of sarthran used in these studies (1 nmol) effectively blocks AT₁ receptors; this is consistent with blockade of the Ang II response. However, this raises the question as to why sarthran plus Ang 3–8 does not decrease MAP to the same degree as valsartan, since AT₁ receptors should still be blocked. Though the answer to this question is unclear at present, it may simply reflect the very large changes in AP elicited by sarthran and the subsequent injection of Ang 3–8.

The time course of the response to valsartan injected into the RVLM compared with that of sarthran also deserves comment. Sarthran injected into the RVLM elicits a rapid decrease in MAP, whereas the response elicited in SHR by injection of either valsartan or candesartan³⁵ is considerably more gradual. This slowly developing depressor response to blockade of AT₁ receptors in the RVLM is consistent with the time course of the action of Ang II on RVLM spinal neurons studied *in vitro*.²⁶ Interestingly, injection of muscimol directed at the PVN elicits a similarly slow decrease in AP in SHR, consistent with the hypothesis that the PVN input to the RVLM is mediated by AT₁ receptors in the RVLM. Thus, it appears that removing excitation of AT₁ receptors in the RVLM in SHR results in a slowly developing disexcitation of RVLM sympathoexcitatory neurons.

We have previously reported that injection of KYN into the RVLM of SHR but not WKY reduces baseline AP.¹⁷ The similarity of the magnitude of the response to KYN and valsartan prompted the question of whether they might represent a single mechanism. To address this issue, we combined a maximally effective dose of KYN with valsartan. The response to the two drugs together was markedly greater than either drug alone. The apparently additive interaction between these two responses indicates that they represent distinct, independent mechanisms. Because the response to

each drug appears to be enhanced in SHR, either both excitatory amino acid-mediated and Ang II-mediated excitation of RVLM sympathoexcitatory neurons are enhanced in SHR, or a single mechanism results in enhancement of both of these inputs.

In summary, the current data demonstrate that blockade of AT₁ receptors in the RVLM or inhibition of neuronal activity in the PVN substantially decreases AP in SHR but not WKY. These data are consistent with the possibility that an input to the RVLM arising from the PVN and mediated by Ang II is enhanced in SHR and contributes to the maintenance of elevated AP in this model of hypertension.

Perspective

Data from a variety of sources suggest that in most models of experimental hypertension, as well as clinical primary hypertension, sympathetic vasomotor activity is inappropriately high and contributes to the elevation in blood pressure. Angiotensin within the central nervous system has been suggested to be involved in this central neurogenic component of hypertension and may partially underlie the efficacy of inhibitors of the renin-angiotensin system in the treatment of hypertension. Our observation that selective injection of an AT₁ antagonist into the RVLM of SHR produces a marked decrease in MAP, which may be due to blockade of an angiotensin input to the RVLM arising from the PVN, focuses attention on this pathway as a possible major substrate of central neurogenic hypertension.

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