Sodium Intake Influences Hemodynamic and Neural Responses to Angiotensin Receptor Blockade in Rostral Ventrolateral Medulla

Gerald F. DiBona, S.Y. Jones

Abstract—To determine the effects of physiological alterations in endogenous angiotensin II activity on basal renal sympathetic nerve activity (RSNA) and its arterial baroreflex regulation, angiotensin II type 1 receptor antagonists were microinjected into the rostral ventrolateral medulla of anesthetized rats consuming a low, normal, or high sodium diet that were instrumented for simultaneous measurement of arterial pressure and RSNA. Plasma renin activity was increased in rats fed a low sodium diet and decreased in those fed a high sodium diet. Losartan (50, 100, and 200 pmol) decreased heart rate and RSNA (but not mean arterial pressure) dose-dependently; the responses were significantly greater in rats fed a low sodium diet than in those fed a high sodium diet. Candesartan (1, 2, and 10 pmol) decreased mean arterial pressure, heart rate, and RSNA dose-dependently; the responses were significantly greater in rats fed a low sodium diet than in those fed a normal or high sodium diet. [D-Ala7]Angiotensin-(1-7) (100, 200, and 1000 pmol) did not affect mean arterial pressure, heart rate, or RSNA in rats fed either a low or a high sodium diet. In rats fed a low sodium diet, candesartan reset the arterial baroreflex control of RSNA to a lower level of arterial pressure, and in rats with congestive heart failure, candesartan increased the arterial baroreflex gain of RSNA. Physiological alterations in the endogenous activity of the renin-angiotensin system influence the bradycardic, vasodepressor, and renal sympathoinhibitory responses to rostral ventrolateral medulla injection of antagonists to angiotensin II type 1 receptors but not to angiotensin-(1-7) receptors. (Hypertension. 2001;37:1114-1123.)

Key Words: angiotensin II | angiotensin antagonist | brain | renal nerves | sodium, dietary

Angiotensin II (Ang II), via a central site of action, increases the basal level of renal sympathetic nerve activity (RSNA) and impairs arterial baroreceptor control of RSNA by shifting the curve relating RSNA to mean arterial pressure (MAP) to a higher level of MAP.1 This is a tonic effect and is dependent on the endogenous level of activity of the renin-angiotensin system. Intracerebroventricular administration of losartan, an Ang II type 1 (AT1) receptor antagonist, decreased the basal level of RSNA and shifted the arterial baroreceptor control of RSNA to a lower level of MAP in rats in balance on a low sodium diet (LNa) and a normal sodium diet (NNa) but not on a high sodium diet (HNa). The effects during LNa were greater than those during NNa and were proportional to the relative degree of activation of the renin-angiotensin system, as reflected by increases in plasma renin activity (PRA). During HNa, for which no effects were seen, PRA was suppressed. In addition, in pathophysiological conditions characterized by the activation of both the renin-angiotensin system and the sympathetic nervous system (eg, congestive heart failure [CHF], hepatic cirrhosis, and nephrotic syndrome), intracerebroventricular losartan exerts similar effects to lower basal RSNA and to improve both the arterial and cardiac baroreceptor reflex regulation of RSNA.2–5

Major central nervous system sites that participate in the setting of basal RSNA and its arterial baroreflex regulation are located in the medulla oblongata.6–8 The nucleus of the solitary tract (NTS) is the initial synaptic site for arterial baroreceptor afferents. The rostral ventrolateral medulla (RVLM) participates in arterial baroreflex function via its exchange of information with the NTS and is part of the final common pathway determining peripheral sympathetic nerve activity via its descending projections to the sympathetic preganglionic neurons in the intermediolateral column of the spinal cord. Both the NTS and RVLM contain large numbers of AT1 receptors.9–13 Of interest, both of these areas are considered beyond the influence of blood-borne Ang II because they lie behind a blood-brain barrier that, unlike the situation with the circumventricular organs, is nonfenestrated. Although our previous studies using the intracerebroventricular route of administration provided strong evidence for an effect within the central nervous system, they could not
provide information concerning anatomic location of the effect within the central nervous system. The present study was undertaken with the use of direct microinjection techniques to examine the role of the RVLM in assessing the effect of changes in the endogenous activity of the renin-angiotensin system on the basal level of RSNA and its arterial baroreflex regulation.

**Methods**

Male Sprague-Dawley rats, weighing 250 to 300 g, were used for all experiments. All animal procedures were performed in compliance with the University of Iowa Policies and Guidelines Concerning the Use of Animals in Research and Teaching and the US Public Health Service Guide for the Care and Use of Laboratory Animals.

**Dietary Preparation**

Before experimentation, the rats were allowed to equilibrate in individual metabolism cages for a minimum of 1 week while consuming 1 of 3 diets; the average time on the diets was 13 days. HNa rats had free access to a normal sodium rat pellet diet (163 meq/kg sodium, Harlan Teklad No. 7001) and 0.9% NaCl drinking fluid; NNa rats had free access to a normal sodium rat pellet diet (163 meq/kg sodium, Harlan Teklad No. 7001) and tap water drinking fluid; LNa rats had free access to a sodium-deficient rat pellet diet (1.6 meq/kg sodium, ICN) and tap water drinking fluid. With the exception of sodium content, the diets were similar.

**Anesthesia**

Rats were anesthetized with methohexital (50 mg/kg IP) for short-duration procedures (sinoaortic baroreceptor denervation [SAD] and preparation of rats with CHF) or pentobarbital (50 mg/kg IP) for long-duration procedures (RVLM microinjections).

**Sinoaortic Baroreceptor Denervation**

SAD was performed by methods previously used and validated in this laboratory. SAD was verified by noting the absence of decreases in RSNA after the intravenous administration of 3 μg/kg phenylephrine.

**Congestive Heart Failure**

By use of techniques previously described and validated for this laboratory, left coronary ligation was performed to produce chronic CHF. In rats consuming the NNa diet. After recovery from anesthesia, rats were returned to individual metabolism cages with free access to the NNa rat pellet diet and tap water. All subsequent studies were performed between 3 and 4 weeks thereafter, a time when ongoing renal sodium retention and edema formation are present.

**Procedures**

**Catheterization**

After anesthesia, the rats were placed on a heated surgical table that was thermostatically regulated to maintain rectal temperature at 37°C. An endotracheal tube was placed, and the rats were mechanically ventilated with room air. Polyethylene catheters were placed in a jugular vein and a carotid artery. The jugular vein catheter was connected to a syringe pump, which delivered isotonic saline at 0.05 mL/min for the duration of the experiment. The arterial catheter was connected to an electronic strain-gauge pressure transducer (Statham P23Db), the pulsatile output of which drove a cardiotachometer. The rat was placed in the prone position with the head mounted in a stereotaxic apparatus. By use of techniques previously described and validated for this laboratory, a branch of the left renal nerve was prepared for measurement of RSNA.

**RVLM Microinjections**

A midline surgical incision was made in the dorsum of the neck, the muscles were reflected, and an occipital craniotomy was performed to expose the dorsal surface of the medulla oblongata. Agents were dissolved in 0.9% NaCl and microinjected into the RVLM with use of a Hamilton syringe connected to the needle via polyethylene tubing. Agents were the AT1 receptor antagonists (losartan and candesartan) and [D-Ala]{\textsuperscript{1}}-angiotensin-(1-7) (A-779), an antagonist of the effects of angiotensin-(1-7).

In preliminary experiments, (1) we tested the effect of serial injections, both symmetrical (ie, both in the same side) and asymmetrical (ie, on opposite sides); the responses to the subsequent injection were similar to that of the initial injection; (2) we chose 20 minutes as the time interval between microinjections because this was sufficient time for the various responses to return to baseline; and (3) we found that vehicle injections produced no effects. In addition, injections of L-glutamate (0.3 nmol) were made into the RVLM of some of the rats, with a pressor response used as a confirmatory functional assessment of micropipette placement.

**Experimental Protocol**

**Microinjections**

HR, MAP, and RSNA were continuously recorded. After a control period of 5 minutes, a microinjection was made into the RVLM (unilateral). Experimental period data were recorded for 5 minutes after each microinjection. Twenty minutes was allowed between microinjections. Doses were given in random order, and RVLM microinjections (right versus left) were randomized. At the end of each experiment, the injection sites were marked with 5% Alcian blue (100 nL). Before euthanasia with an overdose of pentobarbital, an aortic blood sample was taken, and the plasma was separated and frozen for later determination of PRA. Postmortem RSNA was recorded for 30 minutes; this value was subtracted from all experimental values of RSNA. The brain was removed and placed in a solution of 0.1 mmol/L phosphate buffer (pH 7.4) containing 4% paraformaldehyde for 24 hours. Subsequently, 50-μm-thick coronal sections of frozen brain tissue were made. Microinjection sites were identified by the deposition of Alcian blue dye and referred to standard anatomic structures of the brain stem according to the atlas of Paxinos and Watson.

**Arterial Baroreflex Curves**

HR, MAP, and RSNA were continuously recorded. After a control period of 5 minutes, MAP was lowered from the control level to ~50 mm Hg with an infusion of nitroprusside (0.4 μg/min IV for 40 to 45 seconds) and increased from that level to ~200 mm Hg with an infusion of phenylephrine (2 to 5 mg/min IV for 45 to 60 seconds). The phenylephrine infusion was stopped, and 15 minutes was allowed for MAP and RSNA to return to their respective control levels. Then, candesartan (1 pmol) was microinjected into the RVLM. Thirty minutes thereafter (when MAP and RSNA had stabilized), the nitroprusside-phenylephrine maneuver was repeated. In the CHF rats, the carotid arterial catheter was then advanced into the left ventricle for measurement of left ventricular end-diastolic pressure. The rats were euthanized with an overdose of pentobarbital. Postmortem RSNA was recorded for 30 minutes; this value was subtracted from all experimental values of RSNA. In the CHF rats, the heart was removed and weighed.
Analytical Assessment

HR, MAP, and RSNA were digitized at 5 Hz. For each microinjection, the absolute values of HR, MAP, and RSNA during the control period were averaged and set to 100%. Experimental period data were expressed as a percentage of the control period value. To take into account maximum change in HR, MAP, and RSNA from control and the duration of the response, the data were calculated as the area under the curve (AUC) from the time of injection (60 seconds) to the end of the recording period (300 seconds) with units of percent×seconds.

PRA was measured by determining the generation rate of angiotensin I per milliliter plasma per hour by a radioimmunoassay using 125I-labeled angiotensin I as a tracer (Rianen, DuPont Medical Products). The sensitivity of the PRA assay was 0.05 pg/mL per hour. The analytic coefficients of variation were 4.8% within assay and 12.6% between assays.

For analysis of the arterial baroreflex control of RSNA, the absolute values of RSNA during the control period were averaged and set to 100%; experimental period RSNA values were expressed as a percentage of this control period value. Percent control RSNA was plotted against MAP over the MAP range beginning at the nadir of the MAP response to nitroprusside (~50 mm Hg) and extending to the peak of the MAP response to phenylephrine (~200 mm Hg). The resultant sigmoidal relationship was analyzed by use of the 4-parameter logistic regression equation

\[ y = p_4 + \frac{p_1}{1 + \exp[p_2(x - p_3)]} \]

where y is percent control RSNA, and x is MAP. The parameters are range of change in y (p_1), coefficient for calculation of maximal gain (p_2), midrange of curve (p_3), minimum value of y (lower plateau, p_4), and maximum value of y (upper plateau, p_1 + p_4). The maximal gain (where MAP=p_3) is \( \frac{p_1}{4} \). Values of p_1−p_4 were derived for each relationship in each rat. They were then used to generate values of percent control RSNA for a standardized MAP range of 50 to 200 mm Hg in 10 mm Hg steps; these were subsequently averaged to produce a mean curve for the group. We have previously demonstrated that this mean curve is a close fit to the mean±SE of the raw data for percent control RSNA versus MAP.14

Statistical analysis was performed by ANOVA with subsequent use of the Scheffé method for simultaneous comparisons within groups and with subsequent use of the F ratio and modified statistic for nonsimultaneous comparisons between groups.25 A significance level of 5% was chosen. Data in the text, tables, and figures are expressed as mean±SE.

Results

Plasma Renin Activity

PRA was 28.1±1.8 ng/mL per minute in LNa rats (n=32), 3.2±0.9 ng/mL per minute in NNa rats (n=20), 0.4±0.1 ng/mL per minute in HNa rats (n=21), and 12.1±1.8 ng/mL per minute in CHF rats (n=7). The PRA values in LNa, HNa, and CHF rats were significantly different from the PRA value in NNa rats.

Basal MAP and HR

The basal MAP and HR values were not different among the various groups of rats used for the different experimental protocols; the data are presented in the figure legends for Figures 1A, 2, 3A, and 5. In aggregate, MAP was 116±4 mm Hg, and HR was 331±12 bpm.

Losartan

Generally, losartan microinjected into the RVLM elicited sustained decreases in HR and RSNA but not MAP (Figures 1A and 1B). The decreases in HR were dose dependent in both LNa and HNa rats, but the magnitude of the decreases was significantly greater in LNa rats than in HNa rats at each dose. In LNa rats, MAP was slightly (nonsignificant) decreased by the lowest and highest dose of losartan, whereas it was significantly increased by the middle dose of losartan. In HNa rats, MAP was significantly increased by all doses of losartan. The MAP responses to losartan were significantly different between LNa and HNa rats at the lowest and highest doses. The decreases in RSNA were dose dependent in both LNa and HNa rats, but the magnitude of the decreases was significantly greater in LNa rats than in HNa rats at each dose.
To exclude changes in MAP from contributing to the decreases in HR and RSNA via arterial baroreceptor stimulation, studies were performed in LNa rats 2 hours after SAD (Figure 2). After RVLM administration of losartan (100 pmol), the decrease in RSNA was associated with a parallel decrease in MAP; thereafter, both RSNA and MAP returned to the control level. HR showed a slow continuous decrease. For comparison, the responses of LNa rats with intact sinoaortic baroreceptor innervation to the same dose of losartan (data taken from Figure 1A) are also shown. The HR responses were not significantly different. Although it appears that MAP fell to a greater extent in the SAD group, this difference was not significant when the entire response was analyzed as AUC (note overlapping SE bars). The decrease in RSNA was, if anything, less for SAD, but this difference was not significant when the entire response was analyzed as AUC (note overlapping SE bars). Thus, the magnitude and time course of the HR, MAP, and RSNA responses were not significantly different between the intact and SAD rats. This suggests that there was little effect of intact sinoaortic baroreceptor function on these responses. The SE bars shown in Figure 2 are representative of the variation present in the data sets shown in Figures 1A, 3A, 4A, and 5, wherein the SE bars have been omitted for clarity.

Candesartan

Generally, candesartan (10 pmol) microinjected into the RVLM elicited prompt and sustained decreases in HR, MAP, and RSNA that were dependent on dietary sodium intake (Figures 3A and 3B). The decreases in HR were similar between LNa, NNa, and HNa rats. However, candesartan decreased MAP in LNa rats, a response that was significantly different from the small increases in MAP observed in both NNa and HNa rats. In addition, the marked decrease in RSNA produced by candesartan in LNa rats was significantly different from the responses observed in both NNa and HNa rats.

Because MAP and RSNA responses to candesartan were greater in LNa rats than in either NNa or HNa rats, the effects of various doses of candesartan were tested in LNa rats (Figures 4A and 4B). HR and RSNA were significantly decreased at all doses, whereas MAP was significantly decreased at the 2 and 10 pmol doses. Decreases in MAP and
RSNA were greater at the 10 pmol dose than at either the 2 or 1 pmol doses ($P<0.05$). The HR responses were biphasic with initial prompt decreases followed by a partial return toward normal and a subsequent more gradual decline; the responses were similar in magnitude at all 3 doses.

[D-Ala$^7$]Angiotensin-(1-7)

Generally, A-779 microinjected into the RVLM produced small ($<5\%$) changes in HR, MAP, and RSNA (Figure 5). Overall, when the data were analyzed as AUC, A-779 had no significant effect on HR, MAP, or RSNA in either LNa or NNa rats at any dose. At the 100 pmol dose, HR and MAP increased in LNa rats and decreased in NNa rats, whereas RSNA decreased similarly in both LNa and NNa rats. At the 200 and 1000 pmol doses, there were similar increases in HR, MAP, and RSNA in both LNa and NNa rats.

Arterial Baroreflex Function in LNa

Candesartan (1 pmol) microinjected into the RVLM had no significant effect on basal MAP ($111\pm4$ versus $108\pm5$ mm Hg) or HR ($316\pm13$ bpm versus $309\pm10$ bpm) but significantly decreased RSNA ($-214\pm6\%$, $P<0.05$). After candesartan administration, the arterial baroreceptor reflex curve (Figure 6) was shifted to a lower level of MAP, as reflected by a significant decrease in $p_3$ (MAP at midrange) from $119\pm6$ to $109\pm3$ mm Hg ($P<0.05$) (Table 1). Neither
the maximal gain (−3.13±0.15% versus −2.96±0.14% per mm Hg) nor other aspects (p1, p2, or p1+p4) of the arterial baroreflex relationship were significantly affected.

**Arterial Baroreflex Function in CHF**

Candesartan (1 pmol) microinjected into the RVLM had no significant effect on basal MAP (108±5 versus 103±5 mm Hg) or HR (360±14 versus 363±15 bpm) but significantly decreased basal RSNA (−22±4%, P<0.05). After candesartan administration, the arterial baroreflex (Figure 7) was substantially affected. The minimum RSNA during increases in MAP, p4, was significantly lowered from 42±4% to 19±2% RSNA (P<0.01), and maximal gain was significantly increased from −1.98±0.16% to 3.29±0.23% per mm Hg (P<0.01) (Table 2). Left ventricular end-diastolic pressure averaged 11.6±0.6 mm Hg in CHF rats compared with normal values of ≈3.0 mm Hg in sham-operated or control rats in our laboratory.2,14,16 Heart weight–to–body weight ratios averaged 0.71±0.03% compared with normal values of ≈0.40% in sham-operated or control rats in our laboratory.2,14,16

Histological examination of the brain indicated that the RVLM injection sites (Figure 8) were located ventral to the compact portion of the nucleus ambiguous corresponding to the ventral portions of the rostral ventrolateral reticular nucleus and overlapping the lateral paragigantocellular nucleus at bregmata 11.96 to 12.30 mm according to the atlas of Paxinos and Watson.24 In addition, the injection of l-glutamate (0.3 nmol) into the RVLM produced a pressor response of 20±3 mm Hg (n=15).

**Discussion**

When losartan and candesartan, AT1 receptor antagonists, were microinjected into the RVLM, losartan decreased HR and RSNA (but not MAP), whereas candesartan decreased HR, MAP, and RSNA. The responses were dose dependent and were significantly greater in LNa rats than in HNa rats. These effects are substantially influenced by the level of dietary sodium intake, which may exert its action via regulating the activity of the endogenous (central) renin-angiotensin system, which may be reflected by alterations in PRA. The decreases in HR, MAP, and RSNA were greater in LNa rats with activation of the renin-angiotensin system than in HNa rats with suppression of the renin-angiotensin system. Because these studies involved microinjection into a brain region that is less likely to be accessed by circulating Ang II and contains abundant AT1 receptors, these results suggest that it is the activity of the local tissue renin-angiotensin system in the RVLM that is involved in modulating these responses. In LNa rats, it is likely that there is abundant local Ang II binding to AT1 receptors, which is effectively competed for by the microinjected AT1 receptor antagonists, resulting in the observed changes in HR, MAP, and RSNA. Conversely, in HNa rats, it is likely that there is greatly reduced local Ang II binding to AT1 receptors and, accordingly, only minimal changes in HR, MAP, and RSNA. Because antagonism of AT1 receptors in the RVLM produces bradycardia and depressor and renal sympathoinhibitory responses, this suggests that Ang II has a tonic action in the RVLM to increase HR, MAP, and RSNA.

The effect of LNa, NNa, and HNa on the reactivity of RVLM neurons to l-glutamate has been determined.20 Compared with NNa, pressor responses were increased by HNa and decreased by LNa. It has been suggested that alterations in dietary sodium intake may change the responsiveness of RVLM neurons to excitatory (ie, l-glutamate) input. If LNa results in increased functional action of Ang II on AT1 receptors in the RVLM, as suggested by the present study, then an inhibitory interaction between this Ang II effect and the reactivity to l-glutamate can be considered.

LNa is known to increase both the plasma and the cerebrospinal concentrations of Ang II in human subjects.26 That the levels are not correlated suggests that the regulation

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**TABLE 1. Summary Data on Arterial Baroreflex Control of RSNA in 6 Rats on LNa Before and After 1 pmol Candesartan Into RVLM**

<table>
<thead>
<tr>
<th>Group</th>
<th>p1</th>
<th>p2</th>
<th>p3</th>
<th>p4</th>
<th>p1+p4</th>
<th>Gmax, %/mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>127±5</td>
<td>0.098±0.0038</td>
<td>119±4</td>
<td>7±2</td>
<td>134±7</td>
<td>−3.13±0.15</td>
</tr>
<tr>
<td>Candesartan</td>
<td>116±5</td>
<td>0.102±0.0044</td>
<td>109±3*</td>
<td>6±2</td>
<td>122±7</td>
<td>−2.96±0.14</td>
</tr>
</tbody>
</table>

Values are mean±SE. p1 indicates range of change in % control RSNA; p2, coefficient for calculation of gain; p3, midrange of curve; p4, minimum value of % control RSNA; p1+p4, maximum value of % control RSNA; Gmax, maximal gain; control, before treatment; and candesartan, after treatment. Basal MAP was 111±4 mm Hg for control and 108±5 mm Hg for candesartan.

*P<0.05 vs candesartan.
of cerebrospinal fluid Ang II concentration is independent of that of plasma, raising the possibility that it reflects the activity of the brain renin-angiotensin system. In dogs with 2-kidney, 1-clamp hypertension, there were parallel increases in plasma and cerebrospinal fluid Ang II concentrations. However, in dogs made hypertensive by intravenous administration of Ang II over a 7-day period, cerebrospinal fluid Ang II concentrations were unaffected, whereas plasma Ang II concentrations were markedly increased. Thus, the extent to which measurement of components of the renin-angiotensin system in peripheral blood provides information on the activity of the brain renin-angiotensin system is unclear. There is little information on the effect of LNa on Ang II concentration in the RVLM. An additional unexplored possibility is that LNa, in association with the increased plasma and cerebrospinal fluid concentrations of Ang II, may result in an increased permeability of the blood-brain barrier, facilitating access of circulating Ang II to local brain areas.

HR and RSNA were decreased by both losartan and candesartan, whereas MAP was decreased by candesartan but unaffected by losartan. Whether the failure of losartan to decrease MAP represents a differential effect on peripheral sympathetic outflow with the decrease in RSNA being offset by increases in sympathetic nerve activity to other vascular resistance beds is not known. Aside from the difference in potency between losartan and candesartan, the receptor binding characteristics of candesartan are different from those of losartan, leading to its characterization as an insurmountable antagonist.

Two mechanisms of action whereby Ang II of central nervous system origin acting on brain sites may increase peripheral sympathetic nerve activity have received attention. One postulates an inhibition of arterial baroreflex regulation of peripheral sympathetic nerve activity wherein neuronal Ang II originating from the paraventricular nucleus (PVN) and released in the NTS inhibits neurotransmitter release at the first synapse in the arterial baroreflex pathway via presynaptic AT1 receptors. In the NTS, Ang II injection decreases, whereas the nonselective peptide Ang II receptor antagonist [Sar1, Thr8]Ang II increases arterial baroreflex gain.

A second postulates that Ang II originating from neurons in the PVN and released in the NTS, RVLM, or the intermediolateral column of the spinal cord leads to activation of sympathetic preganglionic neurons. The RVLM plays a central role in the autonomic neural control of the circulation, including setting the basal level of peripheral sympathetic nerve activity and participating in its arterial baroreflex regulation. The RVLM contains Ang II immunoreactive nerve terminals and predominant AT1 receptor mRNA and AT1 receptor binding sites, which, however, are less numerous in rats than in rabbits or humans. Microinjection of Ang II into the RVLM increases arterial pressure and/or peripheral sympathetic nerve activity and facilitates arterial baroreflex modulation of RSNA; these effects of exogenous Ang II are blocked by AT1 but not by Ang II type 2 (AT2) receptor blockers.

Because, under normal circumstances, circulating Ang II does not have direct access to the RVLM, endogenous (central) Ang II excitation is likely derived from either angiotensinergic neural inputs (see above) or from paracrine secretion of angiotensin peptides within the brain stem. More significant, therefore, are the findings that microinjection of Ang II receptor antagonists into the RVLM produces a decrease in arterial pressure and/or peripheral sympathetic nerve activity. Such observations suggest that centrally derived Ang II causes tonic excitation of RVLM neurons with increased peripheral sympathetic nerve activity. Many of these studies used nonselective (peptide) Ang II receptor blockers, which have partial agonist properties, and although they measured MAP and HR, they did not include measurements of peripheral sympathetic nerve activity. In the anes-

### Table 2. Summary Data on Arterial Baroreflex Control of RSNA in 7 Rats With CHF Before and After 1 pmol Candesartan Into RVLM

<table>
<thead>
<tr>
<th>Group</th>
<th>p1 (%)</th>
<th>p2 (%)</th>
<th>p3 (%)</th>
<th>p4 (%)</th>
<th>p1+p4 (%)</th>
<th>Gmax, %/mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>122±7</td>
<td>0.0646±0.0039</td>
<td>130±5</td>
<td>42±4</td>
<td>164±7</td>
<td>−1.98±0.16</td>
</tr>
<tr>
<td>Candesartan</td>
<td>129±6</td>
<td>0.1021±0.0042*</td>
<td>131±7</td>
<td>19±2*</td>
<td>148±7*</td>
<td>−3.29±0.23*</td>
</tr>
</tbody>
</table>

Values are mean±SE. MAP was 108±5 mm Hg for control and 103±5 mm Hg for candesartan. *P<0.05 vs candesartan.

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**Figure 8. Diagrammatic representation of coronal sections of lower brain stem showing distribution of centers of microinjection sites in RVLM as determined by deposition of Alcian blue dye in each experiment. Because microinjections were made in 96 rats, points represent >1 microinjection site. Maps and coordinates are from the atlas of Paxinos and Watson.**

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Angiotensin Receptors in RVLM

RVLM derives from studies in conscious and anesthetized animals. Angiotensin-(1-7) as an endogenous angiotensin peptide in the rabbit RVLM might possibly be stimulated by endogenous angiotensin peptides in the rabbit RVLM. These responses to microinjection of Ang II and angiotensin III. PD123319 blocked the pressor and sympathoexcitatory responses to microinjection of Ang II and angiotensin III. These results suggest that the tonic excitation of RVLM neurons as well as excitatory inputs into the RVLM arising from activation of PVN could be mediated by AT1 receptors, likely being stimulated by centrally derived Ang II.

In the anesthetized rabbit with basal RSNA elevated by the stress of surgery and anesthesia, neither resting arterial pressure nor RSNA was affected by losartan or PD123319, a selective nonpeptide AT2 receptor antagonist, but they were both significantly decreased by the nonselective Ang II receptor antagonist [Sar1, Thr8]Ang II. Losartan but not PD123319 blocked the pressor and sympathoexcitatory responses to microinjection of Ang II and angiotensin III. These results suggest that the tonic sympahtoexcitation produced by endogenous angiotensin peptides in the rabbit RVLM might be mediated by receptors other than AT1 or AT2 receptors and possibly be stimulated by endogenous angiotensin peptides other than Ang II or angiotensin III. Evidence in support of angiotensin-(1-7) as an endogenous angiotensin peptide in RVLM derives from studies in conscious and anesthetized rats showing that RVLM microinjection of A-779, a selective antagonist of angiotensin-(1-7) receptors, decreased resting arterial pressure (no measurements of peripheral sympathetic nerve activity). These responses to A-779 are similar to those observed with RVLM microinjection of [Sar1, Thr8]Ang II. There was either no effect (anesthetized) or a pressor effect (conscious) with AT1 or AT2 receptor blockers, emphasizing the substantial influence that anesthesia may have on these various responses. Thus, studies in both rabbits and rats suggest a role for angiotensin-(1-7).

The present study provides additional information concerning the role of angiotensin peptides in the RVLM and their influence on the basal level of RSNA and its arterial baroreflex regulation. First, by making direct measurements of RSNA, direct information is obtained concerning peripheral postganglionic sympathetic neural outflow. This obviates the need to make indirect interpretations and conclusions concerning peripheral sympathetic neural outflow from measurements of MAP and HR alone. Second, by making measurements in rats in balance on varying dietary sodium intake, the effects of alterations in endogenous renin-angiotensin activity could be observed. For example, it was observed that losartan did not affect MAP in either LNa or HNa rats. If one assumes that changes in MAP directly reflect peripheral sympathetic nerve activity, these findings could be interpreted as agreeing with those of other investigators who concluded that “the tonic sympathoexcitatory action of endogenous Ang II in the rat is not mediated by the classical AT1 receptor.” However, we found that losartan did decrease directly measured RSNA, an effect that was greater in LNa than HNa rats and was paralleled by decreases in HR. Furthermore, by use of the 100-fold more potent AT1 receptor antagonist candesartan, a marked decrease in RSNA was observed only in LNa (not NNa or HNa) rats, and this decrease was accompanied by decreases in both MAP and HR. Thus, these 2 important elements of the experimental design, direct measurement of RSNA and variations in endogenous renin-angiotensin activity as reflected by alterations in PRA, resulted in a more precise understanding of the effect of Ang II operating via AT1 receptor antagonists on RVLM regulation of basal RSNA.

These results suggest that local modification of components of the renin-angiotensin system within the central nervous system may affect responses to Ang II injected into the RVLM. It has been demonstrated that chronic oral candesartan treatment of SHR decreased MAP, increased ACE activity in the brain stem, and blocked the pressor and tachycardia responses to Ang II injection into the RVLM. Acute arterial hemorrhage has been used to acutely increase the activity of the renin-angiotensin system activity. In these studies, RVLM injection of losartan increased MAP, whereas [Sar1, Thr8]Ang II decreased MAP; these responses were not different after hypotensive hemorrhage. However, RVLM injection of A-779 decreased MAP before but increased MAP after hypotensive hemorrhage. It is important to emphasize that although acute arterial hemorrhage may have acutely increased renin-angiotensin system activity, it also decreased MAP so that the basal MAP before RVLM injections of the various agents was higher during the control period than after hemorrhage. The effect of a lower basal level of MAP on the response to RVLM injection of the agents used is unknown. It is also possible that the relatively large injection volume used (200 nL) may have resulted in some spread to the nearby caudal ventrolateral medulla. In the LNa rats with chronically increased renin-angiotensin system activity, RVLM injection of candesartan decreased HR, MAP, and RSNA.

A-779 did not affect HR, MAP, or RSNA in either LNa, NNa, or HNa rats. These results differ from those of others, who found that A-779 decreased both MAP and HR. Doses of 100, 200, and 1000 pmol were used, in excess of the 100 pmol reported to be effective. It is possible that the larger doses of A-779 had effects other than to antagonize the effects of angiotensin-(1-7). Other differences, including rat strain (Wistar versus Sprague-Dawley), anesthesia (urethane versus pentobarbital), and injection (bilateral versus unilateral), may contribute to the conflicting results.

In addition to studying the effects of RVLM injection of AT1 receptor antagonists on basal values of HR, MAP, and RSNA in rats with various levels of renin-angiotensin system activity, we also examined the effects on arterial baroreflex control of RSNA. Basal RSNA is increased in LNa and decreased in HNa rats compared with that in NNa rats. In addition, arterial baroreflex regulation of RSNA is set at a higher level of MAP in LNa than in NNa or HNa rats. In rats on different dietary sodium intakes, we found that intravenous or intracerebroventricular administration of losartan had effects on arterial baroreflex regulation of RSNA: (1) at unchanged MAP, a decrease in basal RSNA, and (2) a shift in
arterial baroreflex regulation of RSNA to a lower level of MAP without changing the reflex gain. These 2 effects were more pronounced in LNa rats (increased PRA) than in NNa rats and were not detectable in HNa rats (decreased PRA). These results indicated that tonic levels of central nervous system renin-angiotensin activity, as present in NNa rats, increased basal RSNA and reset the arterial baroreflex regulation of RSNA to a higher level of MAP. In the present study, RVLV injection of candesartan in LNa rats decreased the basal level of RSNA without changing MAP and shifted the arterial baroreflex regulation of RSNA to a lower level of MAP without changing the reflex gain. These results were similar to those seen with intracerebroventricular losartan in LNa rats (see above); therefore, the RVLV is identified as one site of action where Ang II increases basal RSNA and resets arterial baroreflex regulation of RSNA to a higher level of MAP in LNa rats.

CHF is a pathological state in which there is increased activity of both the renin-angiotensin system and the sympathetic nervous system, with defective arterial and cardiac baroreflex regulation of RSNA. The defect in arterial baroreflex regulation of RSNA was characterized by an inability to fully suppress RSNA during increases in MAP and a decrease in reflex gain. In previous studies, it has been found that intracerebroventricular administration of losartan produces 3 beneficial effects on arterial baroreflex regulation of RSNA in CHF: (1) at unchanged MAP, basal RSNA was decreased; (2) during increases in MAP, RSNA was more fully inhibited; and (3) reflex gain was increased toward normal. In the present study, RVLV injection of candesartan in CHF produced the same beneficial effects on arterial baroreflex regulation of RSNA: (1) at unchanged MAP, basal RSNA was decreased; (2) during increases in MAP, RSNA was more fully inhibited; and (3) reflex gain was increased toward normal. These results identify the RVLV as one site of action where Ang II increases basal RSNA and contributes to defective arterial baroreflex regulation of RSNA in CHF. These favorable effects on arterial baroreflex regulation of RSNA are similar to those previously observed when CHF rats were treated with intracerebroventricular losartan. The beneficial effects of improvement in both arterial and cardiac baroreflex regulation of RSNA in CHF are reflected in an improved ability of the kidney to dispose of both acute and chronic sodium loads.

Although both LNa and CHF rats exhibit an increase in basal RSNA, the abnormalities in arterial baroreflex regulation of RSNA are different in LNa and CHF rats. In LNa rats, arterial baroreflex gain is normal, and the arterial baroreflex curve is parallel, reset to a higher level of MAP. In CHF, arterial baroreflex gain is decreased, and RSNA is not maximally suppressed at high MAP. It is likely that these differences in arterial baroreflex regulation of RSNA in LNa and CHF rats involve complex and distinct mechanisms. Microinjection of losartan into the RVLV decreased basal RSNA and favorably affected its arterial baroreflex regulation in both LNa and CHF rats, suggesting that altered Ang II neurotransmission within the RVLV is a uniform important contributory factor. However, the fact that the reduction in basal RSNA was accompanied by a change in arterial baroreflex gain in CHF but not LNa suggests that there is unlikely to be a fixed relationship between the tonic level of basal RSNA and arterial baroreflex gain.

In summary, AT1 receptor antagonists injected into the RVLV of pentobarbital-anesthetized rats decreased HR, MAP, and RSNA. These effects are greatest during LNa, with increased activity of the renin-angiotensin system, and least during HNa, with decreased activity of the renin-angiotensin system. In LNa rats and rats with CHF, AT1 receptor antagonists injected into the RVLV decrease the basal level of RSNA and improve arterial baroreflex regulation of RSNA. The present study identifies the RVLV as one site of action where Ang II increases basal RSNA and influences arterial baroreflex regulation of RSNA.

These observations lend increasing support to the notion that angiotensin peptides of brain origin exert a local paracrine or autocrine action on sites that regulate RSNA. That this local action is influenced by alterations in dietary sodium intake, long known to modulate the activity of the renin-angiotensin system, suggests a potentially important compensatory adaption in the overall neural regulation of renal function.

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References
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