Angiotensin II Receptors in the Solitary-Vagal Area of Hypertensive Rats

Dennis P. Healy and Nan Zhang

Angiotensin II (Ang II) has been proposed to be an endogenous neuromodulator of the baroreceptor reflex at the level of the brain stem solitary-vagal area. Elevated activity of the brain Ang II system has been implicated in the development and maintenance of hypertension in spontaneously hypertensive rats and deoxycorticosterone acetate–salt hypertensive rats. In the present study, we sought to determine if Ang II receptors in the solitary-vagal area exhibited altered binding kinetics in spontaneously hypertensive rats or deoxycorticosterone-salt hypertensive rats. Ang II receptors were examined by quantitative autoradiographic analysis of iodine-125–labeled [Sar1,Ile8]Ang II binding in the solitary-vagal area in six groups of animals: 1) spontaneously hypertensive rats, 2) normotensive Wistar-Kyoto rats, 3) uninephrectomized rats, 4) uninephrectomized rats with a 1% solution of saline for drinking water, 5) uninephrectomized and deoxycorticosterone-treated rats, and 6) uninephrectomized and deoxycorticosterone-treated rats given a 1% solution of saline for drinking water. Blood pressure was significantly elevated in the spontaneously hypertensive rats and deoxycorticosterone-salt rats relative to control animals. There was a significant decrease in the binding affinity (increased Kd) for 125I-[Sar1,Ile8]Ang II and a significant increase in the maximum binding density for 125I-[Sar1,Ile8]Ang II in the solitary-vagal area of spontaneously hypertensive rats relative to Wistar-Kyoto rats. Deoxycorticosterone-salt rats also exhibited significantly higher Kd and maximum binding density values compared with controls. These results indicate that Ang II receptor binding is altered in the solitary-vagal area of two different models of experimental hypertension and suggest that these changes could contribute to the expression of the hypertensive state. (Hypertension 1992;19:355–361)

KEY WORDS • angiotensin II • nucleus tractus solitarii • deoxycorticosterone • spontaneously hypertensive rats • brain

Numerous lines of evidence indicate angiotensin II (Ang II) may be an endogenous neuropeptide in the central nervous system. All the components of the biosynthetic pathway for Ang II are present in the brain, and Ang II immunoreactivity is distributed throughout the central nervous system. Ang II receptors are also widely distributed in the central nervous system. Intracerebroventricular injections of Ang II increase blood pressure, vasopressin release, and drinking, suggesting that the activity of the endogenous brain Ang II system may be linked to cardiovascular and volume homeostasis. Components of the Ang II biosynthetic system, as well as Ang II turnover, have been reported to be elevated in the brains of spontaneously hypertensive rats (SHR). In addition, Ang II receptor density has been shown to be increased in SHR brain, and intracerebroventricular Ang II produces greater responsiveness to Ang II in SHR than Wistar-Kyoto (WKY) rats. Moreover, the demonstrated effective-ness of intracerebroventricular Ang II antagonists or converting enzyme inhibitors in lowering blood pressure in the SHR has led to speculation that elevated activity of the brain Ang II system may contribute to the etiology of hypertension.

There is also evidence that brain Ang II may be involved in the deoxycorticosterone acetate (DOCA)–salt model of hypertension. Although plasma renin activity is depressed in DOCA-salt hypertensive rats, DOCA-salt rats have elevated brain renin activity and increased responsiveness to intracerebroventricular Ang II. The increased responsiveness to intracerebroventricular Ang II is consistent with the demonstration that binding of 125I-[Sar1]Ang II is also elevated in the brains of DOCA-salt rats. In addition, intracerebroventricular administration of captopril, a converting enzyme inhibitor, lowers blood pressure in DOCA-salt animals.

The dorsomedial medulla is a possible site at which Ang II could influence cardiovascular activity centrally. The nucleus tractus solitarii (NTS) receives primary baroreceptor afferents, and cardioinhibitory vagal efferents originate largely from the dorsal motor nucleus of the vagus (DMV) in the rat. Lesions of the NTS produce severe fulminating hypertension. The NTS and DMV contain Ang II immunoreactivity and a high density of Ang II receptors. Ang II receptors are also present within the area postrema. Low doses of Ang II injected into the NTS, DMV, or area postrema have
been reported to decrease blood pressure, whereas higher doses of Ang II increase blood pressure. Injections of Ang II into the NTS have also been reported to inhibit the reflex bradycardia in response to elevations in arterial pressure and to produce greater increases in blood pressure in the SHR than in the WKY rats. In addition, SHR exhibit a decreased sensitivity of the baroreceptor reflex, a condition that is reversed by intracerebroventricular captopril. Thus, altered activity of the brain Ang II system or Ang II receptors in the solitary-vagal area (SVA) of SHR may be involved in the expression of hypertension in this model.

Using quantitative autoradiography, we have shown that the radiolabeled Ang II antagonist \([\text{Sar}^1,\text{Ile}^8]\text{Ang-II} (\text{\[^{125}\text{I}-\text{SI-Ang-II}\]}) binds to sites within the NTS and DMV (i.e., SVA) with a pharmacological profile consistent with labeling of the Ang II receptor. We sought to examine in greater detail the Ang II receptor in the SVA of two different models of hypertensive rats, namely, SHR and DOCA-salt rats. We report here that Ang II receptor binding is altered in a similar fashion in both models of hypertensive rats relative to their normotensive controls.

**Methods**

**Animals**

Ten 12-week-old SHR and WKY rats (Charles River Breeding Laboratories, Wilmington, Mass.) were housed on a 12-hour light/dark schedule and allowed free access to food and water. After 1 week, animals were acclimated to the restraining tube for blood pressure monitoring for 1-hour intervals for 3 consecutive days. On the fourth day, blood pressures were measured using an indirect tail-cuff blood pressure-measuring device (ITTC, Inc., Woodland Hills, Calif.) with the animals resting quietly. Individual blood pressures were taken from the average of five separate tail-cuff readings of animals resting quietly. Blood pressures were measured, as above, after 4 weeks of treatment.

**Receptor Autoradiography**

Animals were processed for receptor autoradiography as described previously. Briefly, animals were anesthetized with pentobarbital (50 mg/kg i.p.) and perfused transcardially with 1 ml/g body wt phosphate-buffered saline (PBS) containing 0.1% formalin. The brains were immediately removed and frozen on dry ice to microtome chucks. Serial transverse sections (10 μm) of the brain stem including the SVA were cut in a cryostat (–20°C) and collected on slides, two sections per slide. The sections were vacuum desiccated briefly and then stored at –20°C.

\([\text{Sar}^1,\text{Ile}^8]\text{Ang II} was radioiodinated with \([\text{\[^{125}\text{I}\]}}\text{SI-Ang-II}\]) sodium iodide (sp. act. 17 Ci/mg New England Nuclear, Boston, Mass.) as described previously according to the procedure of Glossmann et al. The iodinated peptide was separated from the unlabeled peptide by high-performance liquid chromatography. The specific activity of the purified peptide was estimated to be approximately 2,200 Ci/mmol.

Saturation binding of \([\text{\[^{125}\text{I}\]}}\text{SI-Ang-II}\] binding was conducted using 10-μm serial sections from individual animals. Two slides (two sections per slide) were used for total binding and two slides for nonspecific binding (radioligand plus 1.0 μM Ang II) at six concentrations of \([\text{\[^{125}\text{I}\]}}\text{SI-Ang-II}\] (0.2–3 nM) for a total of 48 sections per animal. The mid portion of the NTS and DMV (centered 13.80 mm caudal to bregma) was selected for study. Ang II binding in the area postrema was not examined because an insufficient number of sections were obtainable. Preliminary studies indicated that the NTS and the adjacent DMV exhibited similar Ang II receptor densities. Because of the similarities in receptor density and the lack of a clear demarcation between the NTS and DMV in the autoradiographic images of \([\text{\[^{125}\text{I}\]}}\text{SI-Ang-II}\] binding, these structures were analyzed together, and the area was termed the SVA.

Slide-mounted sections were preincubated in buffer minus radioactivity for 15 minutes at room temperature. Sections were then transferred to fresh incubation buffer (30 mM sodium phosphate, pH 7.2, 150 mM NaCl, 5 mM EGTA, 10 mM MgCl₂, 0.1 mM phenylmethylsulfonyl fluoride, and 0.4% bovine serum albumin) containing increasing concentrations of \([\text{\[^{125}\text{I}\]}}\text{SI-Ang-II}\] to define nonspecific binding; specific binding (total minus nonspecific) was used to determine concentrations of \([\text{\[^{125}\text{I}\]}}\text{SI-Ang-II}\] in the presence and absence of 1.0 μM Ang II to define nonspecific binding; specific binding (total minus nonspecific) was two to three times higher than the nonspecific binding. Sections were incubated for 45 minutes at room temperature and then transferred through three 2-minute rinses in cold PBS rinse buffer. The sections were then dried and exposed to Hyperfilm (Amersham, Arlington Heights, Ill.) for a period of 3–7 days.

Quantitative analysis of \([\text{\[^{125}\text{I}\]}}\text{SI-Ang-II}\] binding to brain sections was conducted by mixing increasing amounts of \([\text{\[^{125}\text{I}\]}}\text{SI-Ang-II}\] into a brain paste that was then sectioned and exposed simultaneously with the brain sections. The inclusion of the radioactive standards permitted calibration of the optical density versus radioactive relation for each film. Standard curves were expressed as optical density versus microcuries of \([\text{\[^{125}\text{I}\]}}\text{SI-Ang-II}\]. Optical density readings from the SVA were converted to femtomoles per milligram protein. Data was analyzed using the MCID image analysis system (Imaging Research, Inc., St. Catherine's, Canada) and the EBDA and LIGAND binding analysis programs.

**Statistics**

Values were expressed as the mean±SEM. Statistical comparisons between the SHR and WKY groups were performed using two-way analysis of variance and the Bonferroni-Dunn test.
FIGURE 1. Bar graph shows indirect tail-cuff blood pressure measurements of conscious, restrained rats. Each column represents the mean ± SEM of seven animals. Recorded blood pressure value for each animal was the average of five readings. Note that the spontaneously hypertensive rats (SHR) had significantly higher pressure than the Wistar-Kyoto (WKY) rats (*p < 0.01, Student’s t test, SHR vs. WKY) and the deoxycorticosterone acetate (DOCA)-salt rats had significantly higher pressures than the nephrectomized animals receiving normal tap water (H2O group) (#p < 0.01, Dunnett’s t test, DOCA-salt vs. H2O group).

Results

SHR had significantly elevated blood pressures compared with the WKY rats (Figure 1). Four weeks of treatment with DOCA-salt also resulted in a significant increase in blood pressure, compared with control UNX rats, whereas UNX rats on 1% saline or UNX rats implanted with DOCA pellets did not have significantly altered blood pressures (Figure 1).

125I-SI-Ang II binding was concentrated in the NTS and DMV (Figure 2). The area postrema was also labeled, but to a lesser extent. The pattern of labeling with 125I-SI-Ang II in the SVA was similar to that reported previously.31

Autoradiographic images of 125I-SI-Ang II suggested that binding was increased in the SVA of SHR (Figure 2). Examination of 125I-SI-Ang II binding at different levels of the SVA indicated that binding density was increased in SHR at all levels (Table 1). Further detailed kinetic analysis of 125I-SI-Ang II binding was conducted with sections through the mid portion of the SVA since this region contains the highest density of Ang II receptors (Table 1) and is the site used previously for microinjection studies.24-29 Binding of 125I-SI-Ang II in the SVA was not completely saturable at the highest concentrations used (Figure 3). However, at all concentrations there was greater binding of 125I-SI-Ang II in the SVA from SHR than from WKY (Figure 3). Scatchard transformations of the 125I-SI-Ang II binding isotherms were best fit to a single binding site (Figure 3). There was a significant decrease in 125I-SI-Ang II binding affinity (increased Kd) and significant increase

in maximum binding density (Bmax) in the SHR relative to the WKY (Figure 4).

Binding of 125I-SI-Ang II was also elevated in the SVA of DOCA and DOCA-salt rats compared with UNX control animals (Figure 5). Scatchard analysis of the

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TABLE 1. 125I-[Sar1,Ile8]Angiotensin II Binding in the Solitary-Vagal Area of Adult Wistar-Kyoto Rats and Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Animals</th>
<th>Caudal</th>
<th>Mid</th>
<th>Rostral</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>1.78±0.48</td>
<td>2.91±0.11</td>
<td>1.79±0.44</td>
</tr>
<tr>
<td>SHR</td>
<td>2.75±0.32</td>
<td>3.86±0.14*</td>
<td>2.91±0.54</td>
</tr>
</tbody>
</table>

Values represent mean±SEM of specific binding in femtomoles per milligram protein from three animals. Sections of brain stem including the solitary-vagal area (SVA) were incubated with 0.17 nM 125I-[sarcosine, isoleucine]angiotensin II (125I-SI-Ang II) in the presence and absence of 1.0 μM Ang II to define nonspecific binding. The sections corresponded to Paxinos and Watson atlas coordinates -14.3 mm (caudal), -13.8 mm (mid), and -13.3 mm (rostral) relative to bregma. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

*p<0.05, Student’s t test.

Discussion

SHR and DOCA-salt animals exhibited similar differences in 125I-SI-Ang II binding compared with their respective normotensive controls, i.e., a significant decrease in binding affinity (increased Kd) and significant increase in Bmax in the DOCA-salt-treated animals (Figure 6). The DOCA-treated animals also had significantly increased levels of 125I-SI-Ang II binding compared with the control animals, but with no change in binding affinity (Figure 6).
pressure. Using a single concentration of \( ^{125}\text{I}-\text{Ang II} \), Wilson et al\(^\text{16} \) did not detect any difference in binding in the NTS in two-kidney, one clip hypertensive rats or one-kidney, one clip hypertensive rats compared with normotensive control rats. Therefore, the basis for the different responsiveness to Ang II after microinjection into the NTS in these two models of hypertension is not known at this time. A detailed analysis of Ang II receptors in the NTS of renal hypertensive rats may be required to determine whether Ang II receptors binding kinetics are altered in a similar or opposite direction than those seen in SHR and DOCA-salt rats.

Ang II has also been shown to influence the vagal component of the baroreceptor reflex. In the rat, cardioinhibitory vagal efferents originate largely from the DMV\(^\text{21} \). The DMV contains a high level of Ang II receptors, with a significant percentage of the Ang II receptors being localized directly on DMV neurons\(^\text{31} \). Microinjections of Ang II into the NTS or DMV of normotensive rats have been reported to produce a decrease in heart rate at low doses\(^\text{25-27} \), with higher doses resulting in either a modest increase in heart rate\(^\text{28} \) or no change in heart rate\(^\text{24-26} \). Microinjections of Ang II into the NTS of two-kidney, one clip hypertensive rats decrease heart rate\(^\text{27} \), whereas similar injections into the NTS of SHR increase heart rate\(^\text{29} \). The reason for these discrepancies is not clear. Microinjections of Ang II into the NTS have also been shown to inhibit the vagal-mediated bradycardia in response to elevations in blood pressure\(^\text{29} \). Interestingly, the decreased sensitivity of the baroreceptor reflex in SHR can be reversed by the intracerebroventricular captopril\(^\text{30} \). Since the NTS and DMV contain Ang II immunoreactive neurons and nerve terminals, we have speculated that increased activity of Ang II neurons in the SVA could result in decreased sensitivity of the baroreceptor reflex and contribute to development or maintenance of hypertension in the SHR\(^\text{31} \). The elevated density of Ang II receptors in the SVA of hypertensive animals is thus consistent with this hypothesis.

The results of the saturation binding experiments with \( ^{125}\text{I}-\text{Ang II} \) in the SVA of SHR shown here are at variance with an earlier study that reported an increase in the affinity (increased \( K_d \)) of \( ^{125}\text{I}-\text{Sar}^1\text{Ang II} \) binding in the NTS of SHR, with no change in \( B_{\text{max}} \).\(^\text{36} \) It is not clear whether the differences were due to the use of different radioligands (antagonist versus agonist) or different commercial sources of SHR (Charles River versus Taconic Farms\(^\text{37} \)). A decrease in binding affinity and an increase in \( B_{\text{max}} \) has been reported with \( ^{125}\text{I}-\text{Sar}^1\text{Ang II} \) in the subfornical organ of SHR.\(^\text{38} \)

As noted previously, 1) the increased synthesis and turnover of Ang II in the brain, 2) the increased responsiveness to intracerebroventricular Ang II, 3) the increase in brain Ang II receptors, and 4) the effectiveness of intracerebroventricular Ang II antagonists and converting enzyme inhibitors in reducing blood pressure\(^\text{5-13} \), all point to an alteration in the regulation of the brain Ang II system as playing a causative role in the development and maintenance of hypertension in the SHR.\(^\text{14} \) The role of the brain Ang II system in DOCA-salt hypertensive rats has not been studied as exten-
within the SVA when combined with the elevated increase in Ang II receptors (a DOCA effect) may then contribute to the development of the hypertension. In this case, the DOCA-mediated increase in Ang II receptor density in the brain may act as a permissive factor in the development of the hypertension.

In summary, these results indicate that Ang II receptor density is increased and Ang II receptor affinity is decreased in the SVA of SHR and DOCA-salt hypertensive rats. These receptor differences may contribute to the maintenance of the hypertension in these two experimental hypertensive models.

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Solitary-Vagal Area Angiotensin II Receptors  


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