Blockade of Angiotensin Receptors in the Anterior Hypothalamic Preoptic Area Lowers Blood Pressure in DOCA-Salt Hypertensive Rats

Takao KUBO, Hiroaki YAMAGUCHI, Masaki TSUJIMURA, Yukihiko HAGIWARA, and Ryuji FUKUMORI

It has been established that deoxycorticosterone acetate (DOCA)-salt hypertensive rats have an overactive brain angiotensin-system. The purpose of the present study was to identify the brain sites showing enhanced angiotensin-system activity responsible for the pathogenesis of hypertension in DOCA-salt hypertensive rats. The angiotensin receptor antagonist, losartan, was injected into brain ventricles or into tissues around the rostral parts of the third ventricle in conscious DOCA-salt hypertensive rats. Losartan (1 μg) injection into the lateral ventricle or into the rostral parts of the third ventricle produced a depressor response, whereas the agent did not affect blood pressure when injected into the caudal parts of the third ventricle or into the fourth ventricle. Losartan (0.1 μg) injection into the anterior hypothalamic preoptic area, anterior (AHA) produced a depressor response. Angiotensin II (0.1–1 ng) injection into the AHA produced a pressor response in sham-operated and DOCA-salt hypertensive rats, and the pressor response to angiotensin II (1 ng) was greater in DOCA-salt hypertensive rats than that in sham-operated rats. Release of angiotensin peptides in the AHA was greater in DOCA-salt hypertensive rats than that in sham-operated rats. These findings suggest that the angiotensin-system in the AHA is enhanced, and that this enhancement is involved in the maintenance of hypertension in DOCA-salt hypertensive rats. Both increased pressor reactivity to angiotensin II and increased release of angiotensin peptides in the AHA appear to be related to this enhancement of the angiotensin-system in DOCA-salt hypertensive rats.

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Key Words: angiotensin system, anterior hypothalamic preoptic area, DOCA-salt hypertensive rats, losartan, blood pressure

Introduction

The central renin-angiotensin system plays a critical role in the regulation of cardiovascular functions and body water balance (1-7). The necessary precursors and enzymes required for the formation and degradation of the biologically active forms of angiotensins have been identified in the brain tissues.

It has been suggested that an overactive brain renin-angiotensin system is one of the factors contributing to the pathogenesis of hypertension in some models of hypertension. For example, in spontaneously hypertensive rats (SHR), brain renin activity (8) and angiotensin levels are increased (2, 9, 10). Elevated numbers of angiotensin binding sites have been measured even in neuronal cultures prepared from SHR brains as compared with those from Wistar Kyoto rats (11-13). Similarly, deoxycorticosterone acetate (DOCA)-salt hypertensive rats have also been demonstrated to possess a hyperactive central renin-
Materials and Methods

Little is known regarding the exact sites at which enhanced activity of the central renin-angiotensin system is related to the pathogenesis of hypertension, although in SHR, endogenous angiotensin II in the anterior hypothalamic area is suggested to be involved in the maintenance of hypertension (18). The purpose of the present study was to identify the brain sites showing enhanced angiotensin-system activity responsible for the pathogenesis of hypertension in DOCA-salt hypertensive rats, by using the angiotensin AT\textsubscript{1} receptor antagonist losartan. Possible mechanisms of the angiotensin-system enhancement were also examined.

Implantation of Chronic Catheterization for Cardiovascular Recording

Rats were anesthetized with pentobarbital (50 mg/kg, i.p.). For recording arterial pressure, a polyethylene cannula (Natsume, Tokyo, Japan) (0.5 mm, i.d., connected to the polyethylene tubing of 0.85 mm, i.d.) filled with 0.9% saline containing heparin (50 units/ml), was inserted into the abdominal aorta via the left femoral artery. The other end was passed subcutaneously to emerge at the back of the neck, and the catheter was held in place with wound clips. In some experiments, another polyethylene cannula (0.5 mm, i.d.) filled with 0.9% saline was inserted into the femoral vein for intravenous injection. During experiments, arterial pressure was recorded continuously by connecting the arterial catheter to a pressure transducer.

Intracerebroventricular Surgery

For injections into the lateral brain ventricle, each rat was anesthetized with pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic apparatus. A 10 mm guide cannula (23-gauge stainless steel tubing) was then inserted into the brain (20). Through a burr hole, the cannula guide was placed 2.0 mm below the cortical surface so that the tip was 1.0 mm above the surface of the lateral ventricle (0.5 mm caudal and 1.5 mm lateral to the bregma). A steel screw with a covering of acrylic cement was used to anchor the guide in place, and a 30-gauge wire was used to plug the guide. Injections into the third ventricle were performed at two sites: the first was 1.8 mm caudal and 0 mm lateral to the bregma and 4.5 mm below the cerebral surface, and the second was 3.8 mm caudal and 0 mm lateral to the bregma and 4.5 mm below the cerebral surface. Injections into the fourth ventricle were made at one site (1.0 mm caudal and 0 mm lateral to the lamda and 4.0 mm below the cerebellar surface). Three days after surgery, the arterial catheter was connected to a pressure transducer under a conscious state. After a 60-min stabilization period, the obturator was removed from the guide cannula and replaced with an inner cannula (30-gauge stainless-steel tubing) filled with the agent to be administered. The tip of the inner cannula extended 1.0 mm beyond the guide cannula. The inner cannula was connected to a 25-\textmu{l} Hamilton syringe through tubing filled with saline. Inner cannula insertion itself usually caused no change in blood pressure. In some cases, only a small transient increase in blood pressure appeared. In such cases, we waited for at least 5 more min after blood pressure returned to the initial level before the cannula insertion. Then, rats were injected with 0.1-1 \mu{g} of losartan or with 0.1-1 ng of angiotensin II in 10 \mu{l} buffered saline (pH 7.4). Blood pressure was calculated by averaging mean blood pressures of 4 points every 1 min for 3
min.

At the end of each experiment, 10 μl of 5% Pontamine sky blue was administered into the brain ventricle through the cannula. Rats were killed by an overdose of pentobarbital and perfused through the heart with 200 ml of saline, followed by 600 ml of phosphate buffered solution containing 4% paraformaldehyde. The brain was removed from the skull and sectioned at 50 μm on a freezing microtome. Sections were mounted and stained with cresyl violet, and the extent of the injected dye was examined.

**Microinjection into Tissues around the Rostral Parts of the Third Ventricle and into the Anterior Hypothalamic Preoptic Area, Anterior**

To examine exact sites responsible for the depressor response to losartan, we microinjected losartan into tissues around the rostral parts of the third ventricle. Rats were anesthetized with pentobarbital (50 mg/kg, i.p.), and a catheter was implanted into the abdominal aorta. After placing each rat in a stereotaxic apparatus, the skin overlying the midline of the skull was incised, and a small hole was drilled through the skull. A guide cannula (26-gauge stainless-steel tubing) was lowered to a position 1.0 mm dorsal to an injection site, at areas around the rostral parts of the third ventricle. A 32-gauge stainless-steel wire was inserted into the guide cannula after implantation. Three days after surgery, the arterial catheter was connected to a pressure transducer. After a 60-min stabilization period, the obturator was removed from the guide cannula and replaced with an inner cannula (32-gauge stainless-steel tubing) filled with the agent to be administered. The tip of the inner cannula extended 1 mm beyond the guide cannula. The inner cannula was connected to a 5-μl Hamilton syringe and a microinjector (IM-1; Narishige, Tokyo, Japan). Rats were injected with losartan or angiotensin II in 100 nl buffered saline (pH 7.4) or with buffered saline vehicle. Each rat received only a single injection.

In some experiments, drugs were microinjected into the anterior hypothalamic preoptic area, anterior (AHA) (1.1 mm caudal and 0.8 mm lateral to the bregma, and 8.2 mm below the cerebral surface).

At the end of each experiment, the injection site was marked by injecting 100 nl of concentrated solution of Pontamine sky blue. The brain was fixed and removed, and frozen sections were cut for identification of the injection site.

**Brain Microdialysis in the Anterior Hypothalamic Preoptic Area, Anterior**

Rats were anesthetized with pentobarbital (50 mg/kg, i.p.), and a guide cannula was placed just above the anterior hypothalamic preoptic area, anterior, (AHA) and fixed to the skull with dental cement as in the microinjection experiments. Animals were allowed to recover for at least three days before dialysis experiments were performed. The dialysis probe (CMA/10; cut-off, 20 kDa; o.d., 0.5 mm; membrane length, 2 mm; CMA/Microdialysis AB, Stockholm, Sweden) was lowered via the guide cannula into the hypothalamic area while the animals were awake.

The hypothalamic area was perfused with Ringer solution (NaCl 147 mM, KCl 4 mM, CaCl2 3.4 mM) at a rate of 3 μl/min (21). Perfusates were successively collected every 40 min from 60 min after the start of perfusion. Angiotensin peptides in perfusates were measured by radioimmunoassay directly from the perfusate without extraction. The antisera was equivalently reactive to angiotensin II and angiotensin III, and as a result reflected the total levels of biologically active angiotensin in samples (6). The detection limit for immunoreactive angiotensin peptides was 1.8 pg/tube. The cross-reactivity of the antibody with angiotensin I was <0.5%, with an atrial natriuretic polypeptide was 0, and with [Arg8]vasopressin was 0 (Peninsula Laboratories, Inc., Belmont, California).

Drugs used were losartan (gift of du Pont Merck Pharmaceutical Co., Wilmington, DE), angiotensin II acetate salt, d(CH2)5Tyr(Me)arginine vasopressin (Sigma, St. Louis, MO), PD123319 ditrifluoroacetate (Research Biochemicals International, Natick, MA), and hexamethonium bromide (Yamanouchi Pharmaceutical, Tokyo).

The results are expressed as mean ± SEM. All results were analyzed by either Student's t-test or one-way analysis of variance combined with Dunnett's test for post hoc analysis for intergroup comparison. Differences were considered significant at p < 0.05.

**Results**

**Blood Pressure Responses to Losartan Injected into the Lateral Ventricle, the Third Ventricle and the Fourth Ventricle in DOCA-Salt Hypertensive Rats**

The basal mean blood pressure of conscious sham-operated rats was 109 ± 1 mmHg (n = 52) and of conscious DOCA-salt hypertensive rats was 167 ± 2 mmHg (n = 72) (p < 0.001). Injection of losartan (0.3-1 μg) into the lateral ventricle caused a dose-dependent depressor response in DOCA-salt hypertensive rats (Fig. 1A and Table 1), whereas in sham-operated rats, losartan (1 μg) similarly injected did not affect blood pressure (Table 1). The depressor response to losartan (1 μg) in DOCA-salt hypertensive rats began within 1 min, reached a plateau within 20 min and lasted for more than 1 h. Neither losartan (0.1 μg) nor saline injected affected blood pressure in DOCA-salt hypertensive rats (Table 1). Losartan (1 μg) injected intraarterially did not affect blood pressure in DOCA-salt hypertensive rats.
hypertensive rats and postmortem histo-
logical examination demonstrated that Pontamine sky
blue injected into the lateral ventricle diffused
throughout the forebrain to the spinal cord.

To localize the sites of action of losartan for the
depressor response, we next injected losartan into the third
ventricle and fourth ventricle in DOCA-salt hypertensive
rats. When losartan (1 μg) was injected into the rostral
parts of the third ventricle, it produced a great depressor
response (10–17 mmHg) in five animals (Fig. 1B and
Table 2) and a moderate depressor response (5–9 mmHg)
in three animals. Microinjection of losartan (1 μg) into
the caudal parts of the third ventricle produced only a
small (less than 4 mmHg) or no depressor response in six
animals (Fig. 1C and Table 2). Losartan (1 μg) injected
into the fourth ventricle did not affect blood pressure in
seven animals (Fig. 1D and Table 2). Postmortem histo-
logical examination demonstrated that Pontamine sky
blue injected into the rostral parts of the third ventricle
diffused to tissues around the rostral parts of the third
ventricle to the spinal cord, but that dye injected into the
diffused to tissues around the rostral parts of the third
ventricle to the spinal cord, but that dye injected into the
caudal parts of the third ventricle did not diffuse to tis-
sues around the rostral parts of the third ventricle. The
Pontamine sky blue injected into the fourth ventricle also
did not diffuse into the third ventricle.

Table 1. Maximal Changes in Blood Pressure After In-
jection of Losartan into the Lateral Ventricle

<table>
<thead>
<tr>
<th>Dose</th>
<th>Basal BP (mmHg)</th>
<th>Maximal changes in BP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOCA-salt hypertensive rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>172 ± 5</td>
<td>+1 ± 1</td>
</tr>
<tr>
<td>Losartan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 μg</td>
<td>165 ± 6</td>
<td>+2 ± 1</td>
</tr>
<tr>
<td>0.3 μg</td>
<td>176 ± 8</td>
<td>-6 ± 2*</td>
</tr>
<tr>
<td>1.0 μg</td>
<td>164 ± 5</td>
<td>-17 ± 3*</td>
</tr>
<tr>
<td>1.0 μg*</td>
<td>170 ± 6</td>
<td>+1 ± 1</td>
</tr>
<tr>
<td>Sham-operated rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Losartan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 μg</td>
<td>105 ± 3</td>
<td>+2 ± 1</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM from 5–6 animals. *p<0.05,
compared to saline. BP: blood pressure, a: intraarterial
injection.
Blood Pressure Responses to Losartan Injected into Tissues around the Rostral Parts of the Third Ventricle in DOCA-Salt Hypertensive and Sham-Operated Rats

To more precisely locate the sites responsible for the depressor response to losartan, we next microinjected losartan into tissues around the rostral parts of the third ventricle in DOCA-salt hypertensive rats. Losartan was injected at a dose of 0.1 μg, since this dose was previously shown not to affect blood pressure when injected intracerebroventricularly. When losartan (0.1 μg) was injected into the anterior hypothalamic preoptic area, anterior (AHA) (Fig. 2), it caused depressor responses of more than 10 mmHg (Fig. 3A and Fig. 4a). The depressor response to losartan began immediately after injection and lasted more than 1 h. Losartan (0.1 μg) injected more rostrally or more caudally to the AHA caused only a small or no decrease in blood pressure (Fig. 4a).

For comparison, we next microinjected losartan into tissues around the rostral parts of the third ventricle in sham-operated rats. In sham-operated rats, losartan (0.1 μg) did not affect blood pressure even when injected into the AHA (Fig. 3B and Fig. 4b).

Blood Pressure Responses to Angiotensin II Injected into Tissues around the Rostral Parts of the Third Ventricle

Next, to examine whether angiotensin receptors responsible for pressor responses exist in the AHA, we microinjected angiotensin II into tissues around the rostral parts of the third ventricle in sham-operated rats. Angiotensin II (0.1 ng) injected into the AHA caused a pressor response of more than 10 mmHg (Fig. 3C and Fig. 5). When injected more rostrally or more caudally to this area, angiotensin II (0.1 ng) caused only a small or no increase in blood pressure.

In normotensive rats, the pressor response to angiotensin II (1 ng) injected into the AHA was inhibited by intraarterial injection of either the ganglion blocking agent hexamethonium (20 mg/kg) or the vasopressin V1 antagonist, d(CH₂)₅Tyr(Me)arginine vasopressin (20 μg/kg), and markedly inhibited by combined administration of both agents (Table 3). In addition, the pressor response to angiotensin II (0.1 ng) injected into the AHA was markedly inhibited by pretreatment with the angiotensin AT₁ receptor antagonist losartan (0.1 μg) but not by the angiotensin AT₂ receptor antagonist PD123319 (0.1 μg) in the same sites (Table 3). The intraarterial injection of hexamethonium (20 mg/kg) decreased basal blood pressure. In other normotensive rats, we examined whether the decrease in blood pressure induced by hexamethonium caused a decrease of pressor reactivity to intravenous noradrenaline, the neurotransmitter of sympathetic postganglionic nerves. The pressor response to noradrenaline (0.25 μg/kg, i.v.) was 35 ± 4 mmHg after the hexamethonium treatment as compared with 18 ± 3 mmHg before the hexamethonium treatment (n = 5) (p < 0.05).

Table 2. Blood Pressure Responses to Losartan (1 μg) Injected into the Lateral Ventricle, Third Ventricle and Fourth Ventricle in DOCA-Salt Hypertensive Rats

<table>
<thead>
<tr>
<th>Injection sites</th>
<th>Basal BP (mmHg)</th>
<th>BP responses</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Latent period (s)</td>
<td>Maximal responses (mmHg)</td>
<td>(n)</td>
<td></td>
</tr>
<tr>
<td>Lateral ventricle</td>
<td>166±5</td>
<td>39±9</td>
<td>−15±3</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>Rostral parts of the third ventricle</td>
<td>171±7</td>
<td>32±7</td>
<td>−14±3</td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>Caudal parts of the third ventricle</td>
<td>162±6</td>
<td>−</td>
<td>−2±1</td>
<td>(6)</td>
<td></td>
</tr>
<tr>
<td>Fourth ventricle</td>
<td>170±5</td>
<td>−</td>
<td>+1±1</td>
<td>(7)</td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean±SEM. BP: blood pressure.

Fig. 2. A coronal section of the rat anterior hypothalamic preoptic area stained with Pontamine sky blue indicating the location of the injection site (arrow). Sch, supra-chiasmatic nucleus; the scale bar represents 1 mm.
Fig. 3. A: Effect of unilateral microinjection of losartan into the anterior hypothalamic preoptic area, anterior (AHA) on blood pressure (BP) in DOCA-salt hypertensive rats. B, C: Effects of unilateral microinjection of losartan (B) and angiotensin II (Ang II) (C) into the AHA on blood pressure in sham-operated control rats.

Fig. 4. Distribution of microinjection sites into tissues around the rostral parts of the third ventricle in DOCA-salt hypertensive rats (a) and in sham-operated rats (b). Closed circles and closed triangles represent sites where an injection of losartan (0.1 μg) caused decreases of more than 10 mmHg and of 5-9 mmHg, respectively, in mean blood pressure. Open circles indicate sites from which no or only a small decrease in mean blood pressure was evoked. In (a), A, C, E, F, G, and H are 0.5 mm apart, respectively. B and D are 0.1 mm behind A and C, respectively. In (b), A, C, and D are 0.5 mm apart, respectively. B is 0.1 mm behind A. ac, anterior commissure; AHA, anterior hypothalamic preoptic area, anterior; AHC, anterior hypothalamic area, central; AMPO, anterior medial preoptic area; DM, dorsomedial hypothalamic nucleus; MnPO, median preoptic nucleus; OX, optic chiasm; PVN, paraventricular hypothalamic nucleus; Sch, suprachiasmatic nucleus; SO, supraoptic hypothalamic nucleus; VMH, ventromedial hypothalamic nucleus.
When angiotensin II (0.1 or 1 ng) was injected into the AHA, it produced a dose-dependent pressor response in sham-operated and DOCA-salt hypertensive rats (Fig. 6A, B). The pressor response to angiotensin II (1 ng) was greater in DOCA-salt hypertensive rats than that in sham-operated rats.

**Angiotensin Release in the Anterior Hypothalamic Preoptic Area of DOCA-Salt Hypertensive and Sham-Operated Rats**

The AHA of DOCA-salt hypertensive and sham-operated rats was perfused with Ringer solution. Perfusates were successively collected every 40 min from 60 min after the start of perfusion. The average angiotensin level of 3 consecutive samples beginning 60 min after the start of perfusion was $7.7 \pm 0.2 \text{ pg}/40 \text{ min}$ ($n = 7$) in DOCA-salt hypertensive rats as compared with $4.6 \pm 0.2 \text{ pg}/40 \text{ min}$ ($n = 7$) in sham-operated rats ($p < 0.05$).

**Discussion**

In the present study, the angiotensin receptor antagonist losartan injected into the lateral ventricle consistently caused a depressor response in DOCA-salt hypertensive rats but not in sham-operated rats. These findings suggest that functions of central angiotensin system are enhanced and this enhancement of angiotensin system is responsible for maintenance of hypertension in DOCA-salt hypertensive rats. The results of the present study are compatible with the previous finding of Itaya et al. (17) showing that

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**Table 3. Maximal Pressor Responses to Angiotensin II Injected into the AHA Before and After, Intravenous Injection of Hexamethonium and a Vasopressin V1 Antagonist, and AHA Microinjection of Losartan and PD123319, in Normotensive Rats**

<table>
<thead>
<tr>
<th>Treatment (intravenous injection)</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BP before</td>
<td>$\Delta$BP induced by</td>
</tr>
<tr>
<td></td>
<td>Ang II</td>
<td>Ang II (1 ng)</td>
</tr>
<tr>
<td>Saline</td>
<td>110±4</td>
<td>+15±1</td>
</tr>
<tr>
<td>Hexamethonium 20 mg/kg</td>
<td>111±5</td>
<td>+17±2</td>
</tr>
<tr>
<td>V1 antagonist 20 μg/kg</td>
<td>105±4</td>
<td>+16±1</td>
</tr>
<tr>
<td>Hexamethonium 20 mg/kg + V1 antagonist 20 μg/kg</td>
<td>108±3</td>
<td>+15±1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment (AHA injection)</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BP before</td>
<td>$\Delta$BP induced by</td>
</tr>
<tr>
<td></td>
<td>Ang II</td>
<td>Ang II (0.1 ng)</td>
</tr>
<tr>
<td>Saline</td>
<td>108±5</td>
<td>+10±1</td>
</tr>
<tr>
<td>Losartan 0.1 μg</td>
<td>110±4</td>
<td>+11±1</td>
</tr>
<tr>
<td>PD123319 0.1 μg</td>
<td>105±5</td>
<td>+10±1</td>
</tr>
</tbody>
</table>

Values are the mean±SEM from 5 animals. *$p<0.05$, compared to values before treatment. **$p<0.05$, compared to values before treatment. BP: blood pressure (mmHg), Ang II: angiotensin II, V1 antagonist: d(CH2)3Tyr(Me)arginine vasopressin.
central administration of captopril, an angiotensin-converting enzyme inhibitor, significantly decreases blood pressure in DOCA-salt hypertensive rats.

Losartan injected into the rostral parts of the third ventricle also caused a depressor response, whereas the antagonist injected into the caudal parts of the third ventricle produced only a small or no depressor response in DOCA-salt hypertensive rats. Pontamine sky blue injected into the rostral parts but not into the caudal parts of the third ventricle diffused to tissues around the rostral parts of the third ventricle. The angiotensin receptor antagonist injected into the fourth ventricle did not affect blood pressure in DOCA-salt hypertensive rats. These findings suggest that sites of action of losartan for the depressor response in DOCA-salt hypertensive rats may exist somewhere around the rostral parts of the third ventricle.

Next, to more precisely locate the sites responsible for the depressor response to losartan, we microinjected losartan into tissues around the rostral parts of the third ventricle in DOCA-salt hypertensive rats. When losartan (0.1 μg) was microinjected into the anterior hypothalamic preoptic area, anterior (AHA), it caused a depressor response in DOCA-salt hypertensive rats, whereas the antagonist did not affect blood pressure in sham-operated rats. A losartan dose of 0.1 μg did not affect blood pressure when injected intracerebroventricularly. These findings suggest that angiotensin-system enhancement occurs in the AHA of DOCA-salt hypertensive rats.

There are several lines of evidence which indicated that central angiotensin-system activities are enhanced in DOCA-salt hypertensive rats. Matsuguchi et al. (15) have demonstrated that DOCA-salt hypertensive rats show increased pressor reactivity to central administration of angiotensin II. Gutkind et al. (26) and Wilson et al. (27) have shown that angiotensin II receptors are increased in various nuclei of DOCA-salt hypertensive rats. Furthermore, Wilson et al. (27) have demonstrated that the number of angiotensin II binding sites is increased following treatment with mineralocorticoids, even in neuronal cell preparations from brain tissues. In addition, it has been reported that DOCA-salt hypertensive rats show elevated central renin activity (28).

In the present study, the pressor response to angiotensin II injection into the AHA was enhanced in DOCA-salt hypertensive rats as compared with that in sham-operated rats. In addition, release of angiotensin peptides in the AHA was increased in DOCA-salt hypertensive rats as compared with that of sham-operated rats. Thus, both increased pressor reactivity to angiotensin II and increased release of angiotensin peptides appear to be related to the enhanced function of angiotensin system in the AHA of DOCA-salt hypertensive rats.

In sham-operated rats, angiotensin II injection into the AHA produced a pressor response. Angiotensin peptides were released in the AHA of sham-operated rats. These findings suggest that the AHA of sham-operated rats also have angiotensin-system activity capable of inducing pressor responses. In the present study, however, the amount of angiotensin released and the pressor reactivity to angiotensin II in the AHA were smaller in sham-operated rats than in DOCA-salt hypertensive rats. In addition, losartan injected into the AHA of sham-operated rats did not decrease blood pressure. Thus, it seems likely

Fig. 6. Effects of microinjection of angiotensin II 0.1 ng (A) and 1 ng (B) into the AHA on mean blood pressure (BP) in sham-operated (Sham) and DOCA-salt hypertensive rats (DOCA). Values are the mean±S.E.M. from six animals. *p<0.05, compared to sham-operated rats.
that the angiotensin-system activities in the AHA of sham-operated rats are too slight to cause an increase in blood pressure.

The pressor response to angiotensin II injected into the AHA was antagonized by the AT₁ receptor antagonist losartan but not by the angiotensin AT₂ receptor antagonist PD123319 similarly injected, suggesting that angiotensin AT₁ receptors are the main receptors involved in mediation of the pressor response to angiotensin II in the AHA. The pressor response to angiotensin II was blocked either by intraarterial injection of either the ganglion blocking agent hexamethonium or the vasopressin V₁ antagonist, d(CH₂)₅Tyr(Me)arginine vasopressin. Although intraarterial hexamethonium produced a decrease in blood pressure, pressor responses to intravenous noradrenaline after the hexamethonium treatment were greater than those before the hexamethonium treatment, indicating that the decrease in blood pressure after hexamethonium does not cause any reduction of pressor reactivity to noradrenaline. These results suggest that the pressor response to angiotensin II is peripherally mediated via both sympathetic activation and vasopressin release.

In the present study, we observed both enhanced angiotensin release and enhanced reactivity to angiotensin II in the AHA of DOCA-salt hypertensive rats. It might be argued that enhanced angiotensin release usually down-regulates angiotensin receptors. However, it has been reported that DOCA can directly up-regulate angiotensin receptors in brain neuronal cell cultures (27). Thus, it is probable that the DOCA-induced up-regulation of angiotensin receptors surpasses the angiotensin-induced down-regulation of the receptors in the brain of DOCA-salt hypertensive rats, thereby producing both enhanced angiotensin release and enhanced reactivity to angiotensin II.

In the present study, we microinjected only a small amount of losartan (0.1 μg) into the AHA in order to localize sites of action of losartan. It seems unlikely that the depressor response to losartan injected into the AHA is mainly caused by the agent diffused throughout the brain or into the peripheral circulation, since either losartan (0.1 μg) injected into the lateral ventricle or losartan (1 μg) injected intraarterially did not affect blood pressure.

Two primary neural pathways originating in the medial preoptic area, which is located within the AHA, have been demonstrated using tritiated amino acid labeling (29, 30). A medially running pathway descends through the periventricular region and posterior hypothalamus to the periaqueductual gray area, and another pathway courses laterally and descends through the lateral hypothalamus, ventromedial nucleus and ventral tegmental area (30). It has also been suggested that the medial pathway may be related to the pressor response to angiotensin II, since medial cuts disrupted the pressor effects of angiotensin II. In contrast, it has also been suggested that the lateral pathway may be related to angiotensin II-induced drinking responses, since electrolytic destruction of the mid-lateral hypothalamus had been shown to reduce drinking responses to medial preoptic area injections of angiotensin II (31).

In summary, this study provides evidence that the angiotensin-system in the AHA of DOCA-salt hypertensive rats is enhanced, and this enhancement may be involved in the maintenance of hypertension in this animal model. It appears that both increased pressor reactivity to angiotensin II and increased release of angiotensin peptides in the AHA are related to this enhanced function of the angiotensin-system in DOCA-salt hypertensive rats.

References

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