Central Actions and Brain Receptor Binding of Angiotensin II: Influence of Sodium Intake

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SUMMARY The effects of dietary sodium on the central actions of angiotensin II (AI) and on 125I-AI binding to brain membranes were investigated in rats fed a low-sodium or control diet and implanted with a permanent cannula into the lateral cerebral ventricle. Blood pressure (BP) responses to AI injections intracerebroventricularly (i.v.t.) were blunted in sodium-deficient rats compared with controls. The BP increases in response to i.v.t.-injected Carbachol were the same in the two groups. In sodium-depleted rats, water intake was lower than in controls after AI given i.v.t.; higher after 1.5% NaCl i.v.t.; and unchanged after Carbachol i.v.t. The pressor response to AI given i.v.t. was higher in spontaneously hypertensive rats (SHR) than Wistar-Kyoto (WKY) control rats. This hyperresponsiveness to central AI was abolished by feeding a low-sodium diet. Specific 125I-AI binding in vitro to brain membranes was consistently lower in sodium-depleted rats. The results suggest that sodium depletion modifies the central actions of AI. This may be related in part to changes in the binding properties of AI receptors in the brain. (Hypertension 2: 437-443, 1980)

KEY WORDS: sodium · angiotensin · rats · brain · angiotensin receptors · blood pressure · thirst

ANGIOTENSIN II (AI) acts on the brain to increase blood pressure (BP) and water intake and to stimulate ACTH and vasopressin secretions.1-4 The central pressor effect may be of pathophysiological significance, since specific AI antagonists injected into the brain lower BP in several types of experimental hypertension, including genetic hypertension of rats.4 Furthermore, the dipsogenic effect of endogenous AI is also diminished by centrally administered 1-Sar, 8-Ala-AI, an AI antagonist.6 This suggests that the central actions of AI depend upon interaction with specific receptors, which have been shown to exist in the brain.7-8

We designed our present study to investigate how central AI receptors adapt to changes in sodium balance. In rats fed diets of different sodium content, we observed the central pressor and dipsogenic effects of AI. We also tested the central actions of carbachol to see whether the effects of sodium were specific for AI. We undertook similar experiments in stroke-prone spontaneously hypertensive rats (SHR), in which the central actions of AI appear to contribute to the elevated BP. To understand whether the changes found were due to an alteration in the number of AI receptors or in the affinity of AI for its receptor, we studied the specific binding of 125I-AI to brain membranes of rats on different sodium intakes.

Material and Methods

Effects of Central AI In Vivo

Experiment 1: Low Sodium Intake

Male Wistar rats (Canadian Breeding Laboratories) weighing 290-340 g were placed on a sodium-deficient diet (< 5 mEq Na/kg)9 (prepared by ICN, Cleveland, Ohio No. 902902) and received a diuretic (furosemide 20 mg/kg, i.p.) on Days 1, 5, and 10. Controls received a regular rat chow (Purina, Richmond, Indiana) containing 150 mEq Na/kg and 0.9% NaCl i.p. on Days 1, 5 and 10. All rats had demineralized water ad libitum. On Days 7 and 8, permanent stainless steel cannulas were implanted into the right lateral cerebral ventricle under pentobarbital anesthesia.4 On Days 14 and 15, the drinking tests and on Days 16 and 17 the BP tests were performed, and 1 ml of blood was obtained from each rat via the arterial catheter for determination of plasma renin activity.
(PRA) and plasma sodium concentration. Three to 4 days later, the rats were decapitated and the brains were analyzed for $^{125}$I-AII binding.

**Experiment 2. High Sodium Intake**

Male Wistar rats weighing 290–340 g were put on a high-sodium diet containing 1500 mEq/kg Na (prepared by ICN, No. 902931). Controls received the control rat chow (Purina) containing 150 mEq/kg sodium. All rats received demineralized water as drinking fluid and were implanted intracerebroventricularly (i.v.t.) with cannulas 6–7 days prior to i.v.t. testing. On Days 14 and 15, drinking tests were performed. Six days later, the rats were decapitated, and blood was collected for determination of PRA and plasma sodium concentration.

**Experiment 3. Low Sodium Intake in Spontaneously Hypertensive Rats**

Male SHR of the stroke-prone strain, 6–9 months old (280–350 g body weight) were used. Half of the SHR, matched for BP (by tail plethysmography), received the same diet with 100 mEq Na/kg added. In addition, male normotensive sodium-replete, age-matched Wistar Kyoto (WKY) control rats (340–400 g body weight) were tested. Two days before the beginning of the dietary regimen, permanent cannulas were implanted i.v.t. After 8–10 days on the diet, BP tests were performed.

$^{125}$I-AII Binding to Brain Membranes In Vitro

**Experiment 4A and B: Low and High Sodium Intake**

Male Wistar rats (270–310 g body weight) were treated as in Experiments 1 and 2. No cannula was implanted and no test performed. At 14–18 days after the start of the dietary regimen, the rats were killed and the brains were analyzed for $^{125}$I-AII binding. One rat on a low and one on a high sodium intake was always assayed with one control animal in parallel, and binding in the experimental rat calculated as % of the control rat.

Male Wistar rats (290–340 g body weight) were fed a sodium-deficient diet. Half of the rats received demineralized water, half 0.5% NaCl as drinking fluid. The former group was injected i.p. with furosemide (20 mg/kg) on Days 1, 5 and 10, the latter group with 0.9% NaCl. At 14–16 days after the start of the experiment, the rats were killed and the brains were analyzed for $^{125}$I-AII binding.

**Test Procedures**

**Blood Pressure Response**

Rats were anesthetized with ether, and catheters were inserted into the right femoral artery (PE 50) and vein (PE 10). The catheters were brought under the skin to exit through the scruff of the neck. The BP was continuously recorded from the arterial catheter via Statham transducers on a polygraph, and mean arterial pressure (MAP) was calculated (diastolic BP — 1/3 pulse pressure). At 90 minutes after surgery, 1 ml of blood was drawn from the arterial catheter for determination of PRA and plasma sodium concentration. Blood was replaced by the same amount of blood from a rat of the same experimental group. At 2 hours after surgery, All (Hypertensin, Ciba) (1, 10, 100, and 3000 pmol) and Carbachol (carbamylcholine chloride, Sigma) (10, 100, and 1000 pmol) were injected i.v.t. in randomized order. The injection volume was 2 μl. After i.v.t. testing, argininevasopressin (Parke-Davis) (1, 10, 25, 50, and 100 mU/kg) was injected i.v. in a volume of 0.5 ml/kg. Both SHR and WKY rats were tested under α-chloralose anesthesia (35 mg/kg i.v.), and All (1, 10, 100, and 1000 pmol/min in 2 μl/min) was infused for 5–10 minutes per dose, until the BP had reached a plateau.

**Drinking Response**

A stainless steel injector was inserted into the permanent brain cannula. The injector was connected with a piece of PE-10 tubing to a 10-μl microsyringe. Rats were injected with 400 pmol of All or with 1.5% NaCl (i.v.t.), and water intake was measured for the following 30 minutes by weighing the water bottles. The next day, rats were injected i.v.t. with 400 pmol of Carbachol and again with 400 pmol of All. Drugs were dissolved in 0.9% NaCl, injection volume was always 4 μl, and the interval between two tests on each day was 90 minutes. No food was available during test periods.

$^{125}$I-AII Binding

Rats were decapitated, and the brains were immediately removed and rinsed in chilled 0.05 M Tris-HCl buffer (pH 7.4). Brains were dissected; a tissue block consisting of hypothalamus, thalamus, septum and midbrain was removed, weighed, and used to study the binding of $^{125}$I-AII by the method of Bennett and Snyder with some modifications. Briefly, the tissue was homogenized with a Brinkman Polytron setting 5 for 20 seconds in 40 volumes of ice-cold Tris buffer and centrifuged at 30,000 × g for 40 minutes at 4°C. The pellets were washed twice with Tris buffer and recentrifuged at 30,000 × g for 10 minutes.

The tissue was subsequently suspended in ice-cold 0.1 M sodium phosphate buffer (pH 7.0) containing 5 mM Na$_2$EDTA, 10$^{-3}$ phenylmethylsulfonyl fluoride, 5 mM dithiothreitol and 0.4% bovine serum albumin (assay buffer). Tissue concentration was usually 25 mg/ml assay buffer. Then 1 ml aliquots were incubated with $^{125}$I-AII in polyethylene tubes for 25 minutes at 37°C. The Ile$^2$-AII was iodinated as described by the method of Freedlender and Goodfriend to a specific activity of 1.5 Ci/mol. The $^{125}$I-AII was stored at −20°C and thawed only once. Nonspecific binding was determined by addition of 1 μM unlabelled Ile$^2$-AII.

Incubations were performed in trilicate.
water and diluting with ice-cold Tris buffer. Bound and unbound homones were separated by centrifugation at 4°C (in low sodium WKY, Experiment 4A only) or by filtration through Whatman GF/C filters. The tissue pellets were resuspended 5 times in 1.5 ml of ice-cold Tris buffer and recentrifuged in the centrifugation assay; the filters were washed twice with 5 ml of ice-cold Tris buffer in the filtration assay. All procedures of the centrifugation assay were done at 4°C. Radioactivity of the washed pellets or of the filters was counted in a Nuclear-Chicago γ-scintillation counter with 85% counting efficiency. Nonspecific binding of 125I-AII to the filters (0.1%-0.2%) was determined in each assay and subtracted from the total amount of 125I-AII bound. Binding was calculated as fmol/g tissue (wet weight). In Experiment 1, the brain homogenates of several rats per group were pooled, and subsequently aliquots were incubated with 0.02-0.75 nM 125I-AII. In Experiment 4, the incubation concentration ranged from 0.05 to 0.2 nM.

Analytical Methods

The PRA was determined by radioimmunoassay of generated angiotensin I (AI).2 Blood for PRA measurements was collected in chilled tubes containing EDTA and centrifuged at 4°C. Plasma was separated and stored at -20°C until assayed. Plasma sodium concentration was analyzed by flame photometry.

Values are given as mean ± standard error of the mean (SEM). Statistical significance was calculated by Student’s paired and unpaired t tests. Regression lines were calculated by the method of least squares.

Results

Blood Pressure

The central pressor effect of AII was about 30% lower in normotensive sodium deplete rats than in sodium-replete rats (fig. 1). The difference was significant at the doses of 10, 100, 1000, and 3000 pmol, (8.1 ± 1.0 vs 11.3 ± 0.7; 12.2 ± 0.8 vs 16.3 ± 1.0; 14.6 ± 0.9 vs 19.5 ± 1.1; and 17.3 ± 1.8 vs 25 ± 2.2 mm Hg). After carbachol administration i.v.t., however, the BP changes were not significantly different between the two groups (6.6 ± 2.3 vs 6.0 ± 1.8 mm Hg at 10 pmol; 16.4 ± 1.8 vs 18.7 ± 2.7 mm Hg at 100 pmol; and 22.5 ± 1.9 vs 27.7 ± 1.8 mm Hg at 1000 pmol in sodium-depleted (n = 8) and -replete rats (n = 8) respectively). Vasopressin injection i.v. yielded increases of BP which were about the same in the two groups (table 1). Mean arterial pressure (MAP) was the same for sodium-depleted and sodium-replete rats at the beginning of the experiment (118.7 ± 2.5 vs 122.3 ± 2.6 mm Hg). The PRA was 8.8 ± 1.7 ng AI/ml/hr in sodium-depleted and 1.2 ± 0.3 ng AI/ml/hr in controls (p < 0.001, n = 8 per group), and plasma sodium concentrations were 139.6 ± 0.7 and 142.6 ± 1.0 mEq/l (n = 8 per group, p < 0.05).

In SHR, AII injected into the cerebral ventricle elicited a more marked pressor response than in normotensive WKY rats. The difference was significant at a dose of 1000 pmol (46.8 ± 4.5 vs 27.5 ± 4.6 mm Hg; p < 0.02). When SHR were sodium-depleted, however, AII given i.v.t. produced BP responses similar to those in WKY rats, i.e., about 50% smaller than in sodium-replete SHR rats (fig. 2). The difference in response between sodium-depleted and sodium-replete SHR was significant at the dose of 100 and 1000 pmol (17.4 ± 3.4 vs 34.0 ± 5.8; and 24.0 ± 4.8 vs 46.9 ± 4.5 mm Hg respectively, p < 0.05). When vasopressin was injected i.v., no significant difference of BP increases was observed between sodium-depleted and sodium-replete SHR (table 2). In sodium-replete SHR, MAP was 172.0 ± 8.8 mm Hg; in sodium-depleted SHR rats 186.1 ± 5.6 mm Hg; and in WKY rats 97.0 ± 3.6 mm Hg.

Drinking

When challenged with AII i.v.t., sodium-depleted rats drank about 50% less water than sodium-replete rats. When challenged with AII i.v.t., sodium-depleted rats drank about 50% less water than sodium-replete rats.

Table 1. Blood Pressure (BP) Increases Following Vasopressin Injections i.v. in Sodium-Depleted and Sodium-Replete Rats

<table>
<thead>
<tr>
<th>Doses injected (m U/kg)</th>
<th>1</th>
<th>10</th>
<th>25</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium-depleted rate</td>
<td>0 = 0 15.6 ± 1.6 29.1 ± 1.8 36.2 ± 2.5</td>
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<td></td>
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<tr>
<td>(n = 8)</td>
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<tr>
<td>Sodium-replete rate</td>
<td>0.7 ± 0.7 16.9 ± 1.4 32.0 ± 2.5 40.2 ± 3.1</td>
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<td>(n = 7)</td>
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</table>

Values are given as mean ± SEM. Differences were not significant.
controls (fig. 3). This difference ($p < 0.001$) was the same on the first (6.3 ± 1.4 vs 13.3 ± 1.0 ml) and on the second test days (4.8 ± 1.2 vs 13.1 ± 1.0 ml). When tested with 1.5% NaCl i.v.t., however, sodium-depleted rats drank more water than controls (2.0 ± 0.4 vs 0.7 ± 0.2 ml, $p < 0.001$). Carbachol administration had identical effects on the two groups (9.7 ± 0.9 vs 9.2 ± 1.5 ml).

In rats on a high sodium diet ($n = 8$), the drinking response to All, 1.5% NaCl, and carbachol was not different from that of controls ($n = 8$) (11.8 ± 2.1 vs 14.0 ± 2.0, 1.3 ± 0.5 vs 1.2 ± 0.4, and 10.6 ± 2.5 vs 10.3 ± 1.7 ml in sodium-loaded and control rats respectively). In rats on a high and normal sodium diet respectively, PRA was 0.25 ± 0.1 and 2.2 ± 0.3 ng AI/ml/hr ($p < 0.01$), and plasma sodium concentration was 142.0 ± 0.8 and 139.7 ± 0.3 mEq/l ($p < 0.01$) ($n = 10$ each group).

**3H-I-AII Binding**

Brain homogenates of sodium-depleted rats showed a 27.2% ± 2.0% ($p < 0.01$) lower $^{3}$H-I-AII binding than in sodium-replete controls ($n = 9$ per group) (fig. 4). This was evident in each individual assay, whether bound and unbound hormone was separated by centrifugation or by filtration (30% ± 2% and 23% ± 3% reduction in binding respectively).

Saturation of brain receptor sites with $^{3}$H-I-AII was reached at concentrations between 0.5 and 0.75 nM (fig. 5). Analysis of the data by Scatchard plots (fig. 6) revealed a lower maximal number of binding sites in sodium-depleted rat brains (233 vs 194 fmol/g tissue). The apparent $K_D$ calculated from the slope of the regression lines changed from 0.16 to 0.24 nM.

The $^{3}$H-I-AII binding to brain membranes of rats on a high sodium diet was the same as in controls (~6.3 ± 4.4%, $n = 9$ per group).

**Discussion**

**Blood Pressure**

The present study shows that the state of sodium balance may modify the biological effects of All on the central nervous system. The central pressor effect of All is blunted in sodium-depleted rats. To assess the specificity of the sodium effect on All, central cholinergic neurons, which may also be implicated in central BP regulation, were stimulated. Carbachol, administered centrally, elicited BP increases that were about equal to those observed with All, but which were not affected by low sodium intake. The central pressor effect of All is mediated peripherally by vasopressin and by the sympathetic nervous system. Changes in pressor responsiveness of both systems might account for the effects of sodium depletion on the central action of All. Our results do not, however, support the hypothesis that there are changes in the response to vasopressin. On the other hand, norepinephrine and tyramine have repeatedly been shown to be even more effective as pressor agents during sodium depletion, although conflicting results have been reported in isolated preparations.
The changes observed in the central pressor effects of All may be relevant to its hypertensive action. It has been reported that delivery of All directly to the brain via the blood leads to a larger BP increase than peripheral administration. Furthermore, blockade of angiotensin receptors in the brain reduces BP in some types of experimental hypertension, and All receptors in the brain may be implicated in the maintenance of high BP in SHR. It has been shown that the central pressor effect of All is increased in these rats. We now confirm this finding and show, in addition, that the augmented responsiveness to central All in SHR may be related to sodium retention, perhaps due to a renal defect of sodium excretion. The low sodium diet reduces the central pressor effects of All to the level of the normotensive WK control rats, a reduction which is more pronounced than in normotensive rats. The peripheral pressor responsiveness of vasopressin was unaltered by sodium status. Interestingly, MAP was not altered by feeding a low sodium diet for 1 week, despite the evidence for a decrease of the central pressor response to All. If central All receptors contribute to spontaneous hypertension, the levels of the endogenous ligand should be elevated during sodium depletion to maintain BP.

![Image of Figure 4](http://hyper.ahajournals.org/)

**FIGURE 4.** Specific $^{125}$I-All binding to brain membranes of sodium-replete (stippled bars) and depleted (open bars) rats. Brains of one rat of each group were always assayed in parallel, and bound was separated from unbound hormone by centrifugation ($n = 5$; Experiment 4A) or filtration ($n = 4$; Experiment 4B), as described in text. Specific binding was calculated from the difference between total and non-specific binding (see also fig. 5).

![Image of Figure 5](http://hyper.ahajournals.org/)

**FIGURE 5.** Saturation of brain membranes with $^{125}$I-All. Bound from unbound hormone was separated by filtration. Total and nonspecific binding (fmol/g tissue) was determined in the absence and presence of 1 μM Ile$^8$-All respectively. Specific binding was calculated from the difference of total and nonspecific binding. Black circle = sodium-replete rats. Open circle = sodium-depleted rats. Brain tissue of rats (Experiment 1, $n = 8$ per group) was pooled and incubated in triplicate on two different days.

**TABLE 2.** Blood Pressure (BP) Increases Following Vasopressin Injections i.v. in Sodium-Depleted and Sodium-Replete SHR Rats

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<tbody>
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<tr>
<td>SH rats ($n = 9$)</td>
<td>$3.8 \pm 2.1$</td>
<td>$8.5 \pm 2.3$</td>
<td>$20.0 \pm 5.8$</td>
<td>$33.7 \pm 4.7$</td>
<td>$43.5 \pm 4.3$</td>
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<tr>
<td>Sodium-replete</td>
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<td></td>
</tr>
<tr>
<td>SH rats ($n = 8$)</td>
<td>$7.9 \pm 4.3$</td>
<td>$14.3 \pm 5.0$</td>
<td>$23.4 \pm 5.3$</td>
<td>$42.2 \pm 7.9$</td>
<td>$54.0 \pm 4.6$</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM. Differences were not significant.
Drinking

Angiotensin II increases water intake in sodium-depleted rats far less effectively than in sodium-replete animals. Since the day-to-day water intake is also reduced on a low sodium diet (unpublished observations), this could be due to a general change of ingestion behavior. Carbachol, however, was equally effective in both experimental groups, which does suggest that sodium specifically affects the dipsogenic response to AII. This is further supported by the increased drinking in response to another dipsogen, hyperosmotic saline, in sodium-depleted rats. Such changes have not been found when only slight changes of sodium balance were induced.19

Present knowledge indicates that blood-borne AII stimulates water intake by acting on brain sites where there is a deficient blood-brain barrier. The significance of this dipsogenic action is, however, debated.20 Thirst states are characterized by parallel changes of sodium and AII levels in plasma, and AII elicits water intake at plasma concentrations below those obtained by stimulation of the endogenous renin-angiotensin system.21 Our data demonstrate that AII-induced water intake under varying physiological conditions is probably modulated by the current state of sodium balance.

When rats were fed a diet with a high sodium content, no clearcut change in the dipsogenic effects of AII, carbachol, and hyperosmotic saline was found. This seems to be in contradiction to the results observed during sodium depletion. The control diet, a usual laboratory chow containing 150 mEq sodium/kg, probably provides sodium in excess of physiological need. In fact, the rat eating this diet has a daily sodium intake amounting to about 30% of its total body sodium content.22 Therefore, the control diet may already impose a maximal sodium load with respect to central actions of AII. It should be pointed out, however, that systemic AII effects on the adrenal gland are affected by increasing the dietary sodium to a level comparable to the high sodium diet we used.23 24

125I-AII Binding

Like other hormones, AII interacts with a specific receptor on its target cell. There is evidence for the existence of these receptors in the adrenal gland,25 in smooth muscle, and in brain.26 27 Our investigations confirm the existence of specific, saturable high-affinity binding sites for AII in the rat brain. Analysis of the data show an apparent dissociation constant (Kd) of 0.16 nM and a maximum number of binding sites of about 230 fmol/g tissue in sodium-replete rats. These results are in close agreement with previous reports.7 8

In addition, we demonstrate that changes of sodium status in an animal will elicit changes of 125I-AII binding to brain tissue in vitro. Decreased binding is probably secondary to a reduced number of AII receptors and a slight change of affinity during sodium depletion, although we cannot completely rule out the possibility of prior receptor occupation by high levels of endogenous AII. The modified receptor binding characteristics may be responsible for the change in the same direction of the biological response to AII. This suggests that at least part of the receptor population studied may be of physiological relevance in the central regulation of BP and body fluid homeostasis.

We have no conclusive evidence on whether the regulation of brain AII receptors found is due to direct effects of sodium or of AII on the receptors. Both sodium and circulating AII have been implicated in AII receptor regulation in the adrenal gland and in smooth muscle.25 28

The source of AII that binds to the brain receptor is unknown. Some parts of the brain, especially the circumventricular organs, may be reached by blood-borne AII.29 Angiotensin II may be produced by a renin-angiotensin system within the brain, too.29 The increase of circulating levels of AII during sodium deficiency is well documented,29 but the levels of brain AII are not known under these conditions.

Our study provides evidence that the AII receptors found in brain tissue by means of binding studies with radioactive ligands may be of physiological significance. The central effects of AII on BP and water intake are blunted during sodium depletion. Changes in the same direction are observed for brain AII receptor binding. Further studies should provide information on the in vivo mechanism of action of sodium on central AII receptors.

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References

Central actions and brain receptor binding of angiotensin II: Influence of sodium intake.

J F Mann, E L Schiffrin, P W Schiller, W Rascher, R Boucher and J Genest

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