EFFECTS OF DIETARY SODIUM ON BRAIN ANGIOTENSIN II
RECEPTORS IN SPONTANEOUSLY HYPERTENSIVE RATS

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The effects of dietary sodium on the characteristics of angiotensin II (A II) receptor sites in the hypothalamus-thalamus-septum-midbrain (HTSM) region were examined in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Twenty-four SHR and 24 WKY were divided into two groups respectively, which were maintained on high sodium diets or low sodium diets for 4 weeks, respectively. The binding capacity and affinity of the A II receptors were measured by radioreceptor assay.

In WKY, the binding capacity of the A II receptors in the high sodium group was significantly lower than that in the low sodium group. On the other hand, the binding capacity of A II receptors was not significantly different between high and low sodium groups in SHR. The secretion of arginine vasopressin (AVP) increased significantly in SHR with high sodium intake.

The present results suggest that in WKY the decrease of the binding capacity of the A II receptors in the HTSM region in response to a high sodium intake serves to attenuate an osmotical stimulus to AVP secretion. However, in SHR such a regulatory mechanism as adjusting the binding capacity of the A II receptors is lacking, and this seems to be responsible, at least in part, for the enhanced secretion of AVP on the sodium loading.

It has been noted that angiotensin II (A II) controls thirst and a release of arginine vasopressin (AVP, antidiuretic hormone) which are related to water and electrolyte metabolisms.1-4 These A II actions are thought to be mediated through specific A II receptors in the brain, and the high affinity of these binding sites may indicate a physiological role of angiotensin in the brain.5,6

On the other hand, evidence that these A II receptors in their target organs are influenced by changes in sodium balance has been accumulated. We have previously demonstrated the effects of dietary sodium on A II receptors in rabbit adrenal cortex and aorta.7 The binding capacity of the A II receptors in the adrenals of low sodium intake animals, which had higher levels of plasma A II concentrations, was significantly greater than that in high sodium intake animals. However, the binding capacity of the A II receptors of the aorta was not significantly different in these two groups. The aldosterone

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- Spontaneous hypertension
- Brain angiotensin receptor
- Sodium balance
- Arginine vasopressin

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Fig. 1. $^{125}$I-angiotensin II ($^{125}$I-A II) binding to brain membranes as a time dependent reaction at 22°C. Fifty pM of $^{125}$I-A II was incubated for various intervals with 20,000 x g particulate fraction from the brain HTSM region as described under "Materials and Methods" (0.5 mg of protein). Bound radioactivity was determined as described under "Materials and Methods". This figure shows the concentration of bound $^{125}$I-A II from assay mixtures incubated in the presence of $10^{-5}$ M unlabelled A II (non-specific binding: O-----O) and the difference between samples without or with $10^{-5}$ M unlabelled A II (specific binding: •--•).

**TABLE I**

<table>
<thead>
<tr>
<th>Treatment (4 wk)</th>
<th>Weight gain (%)</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Serum sodium (mEq/L)</th>
<th>Serum potassium (mEq/L)</th>
<th>Plasma renin activity (ng/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHR</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>High sodium</td>
<td>20.3 ± 2.9</td>
<td>185 ± 5</td>
<td>146.4 ± 0.7</td>
<td>4.50 ± 0.20</td>
<td>4.6 ± 0.6</td>
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<tr>
<td>(n = 13)</td>
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<tr>
<td>Low sodium</td>
<td>21.3 ± 2.6</td>
<td>183 ± 5</td>
<td>145.2 ± 1.0</td>
<td>4.53 ± 0.12</td>
<td>7.3 ± 0.2**</td>
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<tr>
<td>(n = 14)</td>
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<tr>
<td><strong>WKY</strong></td>
<td></td>
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<tr>
<td>High sodium</td>
<td>40.6 ± 3.4</td>
<td>122 ± 2</td>
<td>144.8 ± 0.4</td>
<td>4.93 ± 0.08</td>
<td>7.4 ± 0.7</td>
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<tr>
<td>(n = 16)</td>
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<tr>
<td>Low sodium</td>
<td>44.3 ± 2.1</td>
<td>135 ± 5</td>
<td>146.2 ± 0.6</td>
<td>4.70 ± 0.13</td>
<td>9.4 ± 0.5*</td>
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<tr>
<td>(n = 19)</td>
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Data is shown as mean ± SE. * = p < 0.05, ** = p < 0.01

The response of the adrenal cortex to A II is potentiated during sodium restriction and decreased by sodium loading. The altered content of the adrenal A II receptor during changes in sodium intake may be responsible for the control of adrenal sensitivity to A II. Recently, we have also reported that binding capacity of the brain A II receptor was markedly increased in deoxycorticosterone acetate (DOCA)-salt hypertensive rats as compared to that in normal Wistar rats.

In the present study, we characterize in greater detail the A II binding properties of the membrane fraction of the diencephalon, which includes the thirst center, and compare the changes in the binding characteristics of the A II
and 65 ml of 1.0% saline as drinking water per day (Na 13 mEq/day); b) low sodium group, which was given 26 g of low sodium rat pellets (Japan Clea Co., Ltd., Tokyo) containing 4 mEq of sodium and 19 mEq of potassium per 100 g and tap water ad libitum (Na 1 mEq/day).

The systolic blood pressure was measured every week by tail plethysmography in the conscious state at a temperature of 30°C for 15 to 20 min. Body weight was also measured every week, and the volume of fluid ingested was recorded daily.

**Preparation of Membrane Fraction**

Animals were killed by decapitation, and the brains were excised immediately and placed into an ice-cold Krebs-Ringer-phosphate buffer solution (pH 7.4) containing 0.2% glucose and 1% bovine serum albumin. Tissues obtained from 4 rats were pooled in each experiment. The following steps were performed at 4°C: The hypothalamus-thalamus-septum-midbrain (HTSM) region was excised as a block from the whole brain using the optic chiasm and mamillary body as markers, as previously described by Sirett et al. The tissue was homogenized with 20 volumes of 20 mM sodium bicarbonate in a glass homogenizer with 10 strokes of a tight teflon pestle. The homogenates were then centrifuged at 1,500 × g for 10 min. The supernatant was centrifuged at 20,000 × g for 30 min. The 20,000 × g pellets were washed once with 20 mM sodium bicarbonate (4 ml per g wet weight of starting material) and resuspended in assay buffer (Medium A) containing 120 mM NaCl, 20 mM Tris HCl (pH 7.4), 5 mM EDTA Na2, 5 mM diithiothreitol and 10⁻⁴ M phenylmethylsulfonyl fluoride to give a protein concentration of 8 to 10 mg/ml. Protein concentrations were measured by the method of Lowry et al.

**Angiotensin Binding Assay**

Angiotensin binding studies were performed according to the method previously described by Glossman et al. with some modification. At 22°C, 0.05 ml aliquots of freshly prepared particulate fractions were incubated in assay medium (Medium A and 0.2% serum albumin) with I-angiotensin II and unlabelled ligands in a final volume of 0.2 ml. After 45 min, each assay tube was filled rapidly with 4 ml of ice-cold assay buffer (120 mM NaCl, 20 mM Tris HCl [pH 7.4]) and the diluted solution was filtered through Whatman GF/B glass fiber filter (2.5 cm in

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**MATERIALS AND METHODS**

**Animal Experiment**

Twenty-four male SHR, aged 8 weeks and weighing 200–250 g, and 24 male WKY, aged 8 weeks and weighing 250–300 g, were maintained on one of the two kinds of diets with different electrolyte composition for 4 weeks and divided as follows: a) high sodium group, which was given 26 g of ordinary rat pellets (CR 2: Japan Clea Co., Ltd., Tokyo) which contained 11 mEq of sodium and 19 mEq of potassium per 100 g and receptor in the brain by manipulating dietary sodium in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Furthermore, we will discuss the high secretion rate of AVP in SHR with special reference to the increased binding capacity of the brain A II receptors.
Fig. 3. Effect of dietary sodium intake on amount of A II bound to the brain HTSM region preparations of WKY. The concentrations of A II bound to brain membranes from WKY maintained on high (○-○) or low (○--○) sodium diets for 4 weeks are expressed per mg of total membrane protein. Fifty pM of $^{125}$I-A II was incubated with unlabelled A II of various concentrations in a final volume of 0.2 ml at 22°C for 45 min. Total membrane protein was 0.5 mg. Bound radioactivity was determined as described under "Materials and Methods".

Each filter disk was washed once with 4 ml of cold assay buffer. Radioactivity on filter disks was measured in an automatic γ spectrometer with a counting efficiency of 50% for $^{125}$I. In the present study, specific binding of $^{125}$I-A II is defined as the portion of total binding which is displaced by the presence of an excess (10$^{-5}$ M) non-radioactive A II. Synthetic 1-Asp-5-Ileu-A II and its analogs were obtained from Protein Research Foundation, Osaka, Japan. Monoiodinated $^{125}$I-A II was obtained from New England Nuclear Corporation, Boston, Mass. The specific activity of each tracer preparation was 1,500 μCi/μg.

**Measurement of Vasopressin**


AVP concentrations were determined using a specific and highly sensitive radio-immunoassay procedure developed by Ishikawa et al. Blood samples were collected in Na$_2$EDTA tubes after decapitation, and plasma was stored at −20°C until the time of an assay. AVP content in the pituitary was also measured. Plasma osmolarities and electrolytes were determined by an osmometer and a flame photometer, respectively.

The results were presented as mean ± SE. Statistical analysis was performed using Student’s t-test.

**RESULTS**

*Specificity of Angiotensin II Binding*
Since preliminary experiments showed that specific binding of $^{125}$I-A II occurred linearly up to 12 mg protein/ml, we used brain particle concentrations between 8 and 10 mg protein/ml in all of the following experiments. The relationship between the time of incubation and the specific binding of $^{125}$I-A II is shown in Fig. 1. In brain preparations, the reactions reached their maximum levels within 30 min and then maintained their plateaus for at least 30 min at 22°C. The specificity of the A II binding sites was indicated by the relationship between the binding inhibition potency and the biological activity of angiotensin analogs. In binding experiments on brain membranes (Fig. 2), angiotensin I, which lacks physiological activity, was...
a less potent inhibitor than the intact octapeptide. The effective antagonist, 1-Sar-8-Ileu-A II, displayed a slightly higher binding potency than A II. The inhibition by angiotensin I and 1-Sar-8-Ileu-A II on 123I-A II binding in brain membranes preparations was compatible with their biological activity in other target organs such as adrenal cortex and vascular smooth muscle. These characteristics suggest that the binding sites represent the receptor implicated in the pharmacological responses of the brain.

**Effects of Dietary Sodium on Body Weight, Blood Pressure, Serum Electrolytes and Plasma Renin Activity**

Changes in body weight, systolic blood pressure, serum electrolytes and plasma renin activity (PRA) 4 weeks after the initiation of a high or a low sodium diet are shown in Table I. Body weight increases, systolic blood pressure, and serum sodium and potassium concentrations were not significantly different between the high and low sodium groups in both SHR and WKY (p > 0.05). The mean value of the systolic blood pressure in SHR was 184 mmHg. The mean value of PRA in animals maintained on a low sodium diet for 4 weeks was higher than that in animals on a high sodium diet in both SHR and WKY (p < 0.01 and p < 0.05, respectively). However, PRA levels in SHR were significantly lower as compared to those in WKY in each sodium diet group.

**Effects of Dietary Sodium on A II Binding Sites**

A II binding to brain membranes at various concentrations of A II was compared between the rats on high and low sodium intakes. As shown in Fig. 3, brain membranes from WKY on a low sodium diet bound more A II at each concentration than those from the animals on a high sodium diet. Scatchard plots of brain receptors in high and low sodium intake animals indicated the presence of two binding sites with different affinities in both SHR and WKY (Fig. 4A and B). We calculated the binding constants of high affinity sites by least square analysis. The mean

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**TABLE II**

<table>
<thead>
<tr>
<th>Treatment (4 wk)</th>
<th>Binding affinity (nM⁻¹)</th>
<th>Binding capacity (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High sodium (n = 4)</td>
<td>1.30 ± 0.05</td>
<td>13.4 ± 2.0</td>
</tr>
<tr>
<td>Low sodium (n = 4)</td>
<td>1.63 ± 0.20</td>
<td>12.4 ± 0.9</td>
</tr>
<tr>
<td><strong>WKY</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High sodium (n = 4)</td>
<td>2.90 ± 0.41</td>
<td>8.63 ± 0.91</td>
</tr>
<tr>
<td>Low sodium (n = 4)</td>
<td>2.30 ± 0.39</td>
<td>16.2 ± 2.6*</td>
</tr>
</tbody>
</table>

*Data is shown as mean ± SE. * = p < 0.05

**TABLE III**

<table>
<thead>
<tr>
<th>Treatment (4 wk)</th>
<th>Plasma osmolality (mOsm/kg H₂O)</th>
<th>Plasma concentrations of AVP (pg/ml)</th>
<th>Pituitary AVP content (ng/100g BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High sodium (n = 4)</td>
<td>289.8 ± 1.5</td>
<td>23.8 ± 0.8</td>
<td>101.8 ± 2.4</td>
</tr>
<tr>
<td>Low sodium (n = 4)</td>
<td>281.1 ± 3.3</td>
<td>n.s.</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td><strong>WKY</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High sodium (n = 3)</td>
<td>282.9 ± 0.7</td>
<td>16.6 ± 2.2</td>
<td>117.2 ± 9.0</td>
</tr>
<tr>
<td>Low sodium (n = 4)</td>
<td>274.3 ± 3.5</td>
<td>n.s.</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

*n.s. = not significant
values of the binding affinities and the binding capacities of brain receptors in SHR and WKY calculated from Scatchard plots are listed in Table II.

The binding affinities of brain receptors were not significantly different between the high and low sodium intake groups in both SHR and WKY. In WKY, the binding capacity was significantly lower in the high sodium intake group than in the low sodium intake group (8.63 ± 0.91 vs 16.2 ± 2.6 fmol/mg protein) (p < 0.05). In contrast, the binding capacity of A II receptors was not significantly decreased in the high sodium intake group in SHR as compared to the low sodium intake group (13.4 ± 2.0 vs 12.4 ± 0.9 fmol/mg protein). In addition, we found an increase in binding capacity in SHR as compared to that in WKY in the high sodium groups.

Arginine Vasopressin Concentration (Table III)

Plasma osmolality was not significantly increased by sodium loading in both SHR and WKY. Plasma AVP concentration was significantly higher in SHR with high salt intake than in SHR with low salt intake (23.8 ± 0.8 vs 17.6 ± 3.4 pg/ml, p < 0.05), whereas pituitary AVP content was lower in SHR with high salt intake than in SHR with low salt intake (101.8 ± 2.4 vs 244.4 ± 6.0 ng/100 g BW, p < 0.01).

DISCUSSION

A number of binding5,10,11 and physiological studies6,14 have suggested that angiotensin peptides have a physiological role in the central nervous system. A recent report by Sladek and Joynt15 suggests that A II mediates or modulates an osmotically stimulated AVP release from neurohypophysis through the specific receptor in the brain. On the other hand, studies on SHR16–18 revealed a high AVP secretion rate in these animals and that antiserum or antagonist of AVP lowers the blood pressure19,20. These reports suggest that AVP plays a role, at least in part, in maintaining hypertension in SHR.

In the present study we examined in SHR and WKY the effects of dietary sodium on the characteristics of the A II receptors of the HTSM region, which are considered to mediate AVP secretion, in order to further clarify the role of AVP in the maintenance of high blood pressure levels in this type of hypertension.

In WKY, the binding capacity of the A II receptor of the HTSM region was significantly lower in the high sodium group than that in the low sodium group, while the binding affinity remained at the same level in both groups. The decrease in the binding capacity of the brain A II receptors indicates a decrease in the number of receptors per cell because the change of the cell numbers in accordance with sodium balance could not take place in the brain tissue. In the case of the adrenal cortex, we could not exclude the possibility of a decrease in the number of the adrenal zona glomerulosa cells 4 weeks after the high sodium diet, instead of a decrease of the receptors per cell.21 These results suggest that the decrease of brain receptor binding of A II inhibits the osmotical stimulus for an increase of AVP secretion induced by salt intake.

In the present study, we also observed that plasma levels of AVP were elevated and that pituitary AVP content was decreased in SHR with high salt intake. This result indicates an increased releasing rate of this hormone from the neurohypophysis into the blood stream. The initial stimulus for such an increase of AVP secretion appears to be a consequence of increased salt intake, i.e., an increased plasma osmolality. However, an unproportional increase in plasma AVP concentrations as compared to the increase in plasma osmolality that occurred in SHR with high salt intake suggests the presence of other factors responsible for the further increase in AVP secretion.

The increased salt intake resulted in a reduction of the binding capacity of the brain A II receptors in WKY. It can be proposed that the decrease of brain receptor binding of A II inhibited the osmotical stimulus for an increase of AVP release induced by salt intake and prevented a further increase in the secretion of the hormone. On the other hand, high salt intake failed to decrease the binding capacity of brain A II receptors in SHR. This indicates that the further increase in AVP secretion which occurred in SHR with high salt intake may have been, at least in part, due to the lack of an adjusting control system in brain A II receptors for sodium balance. This particular relationship between sodium balance and brain A II receptors was also noted in DOCA-salt hypertensive rats in which the enhanced secretion rate of AVP and increased binding capacity of the brain A II receptor were observed as we reported previously.8

Cole et al22 have reported that there was no significant difference in A II receptor affinity or
concentration in the HTSM region between SHR and WKY. They have also reported that there was no significant difference in A II receptor capacity between SHR and WKY with normal or high sodium intake for 2 weeks. Mann et al. have reported that specific A II binding to brain membrane was lower in male Wistar rats with sodium depletion than those with sodium repletion for 14–18 days. The discrepancies between angiotensin receptor binding data in our experiments and those of Cole et al. or Mann et al. may possibly be attributed to the difference of the period of sodium loading or sodium restriction.

In the case of the adrenal cortex, we have reported that plasma A II levels regulate the binding capacity of A II receptor in the tissue. In the present study, we did not determine the A II concentration in the brain. Thus, it is unknown how the brain A II concentration changes by sodium balance. The relationship between brain A II levels and the binding capacity of A II receptors must be clarified. Further study using SHR in the early stage should be also performed in the future to clarify the mechanism of hypertension in SHR.

In conclusion, an increased salt intake in SHR fails to lower brain receptor binding of A II, which appears to modify AVP release from neurohypophysis. This may be related to the substantial increase in AVP secretion in response to the sodium intake in SHR.

Acknowledgement

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