Regulation of Primate Angiotensin II Receptors During Altered Sodium Intake

MARIA PIA PLATIA, KEVIN J. CATT, GARY D. HODGEN, AND GRETI AGUILERA

SUMMARY In the rat, angiotensin II receptors of the adrenal glomerulosa and smooth muscle undergo reciprocal regulatory changes that parallel the changes in target cell sensitivity to angiotensin II during altered sodium intake. In primates, the relative importance of angiotensin II receptor regulation during sodium-induced changes in angiotensin II sensitivity is not clear. To evaluate the role of angiotensin II receptor regulation in the primate, we analyzed the changes in angiotensin II receptors of adrenal and bladder membrane-rich particles after 4 to 6 days of high or low sodium intake in the monkey (Macaca fascicularis). Consistent with the decreased pressor response to angiotensin II, smooth muscle angiotensin II receptors were fewer in sodium-restricted monkeys (93 ± 17 fmol/mg) than in sodium-loaded monkeys (171 ± 6 fmol/mg). However, in contrast to the rat, changes in zona glomerulosa angiotensin II receptors in monkey adrenal were similar to those in smooth muscle, decreasing with sodium restriction and increasing with sodium loading (344 ± 64 and 660 ± 68 fmol/mg, respectively). There was no change in angiotensin II receptor affinity in either smooth muscle or adrenal particles during altered sodium intake. Concomitant with the decrease in adrenal angiotensin II receptors, 18-hydroxylase activity was increased twofold in adrenal mitochondria from sodium-restricted monkeys (74 ± 8 fmol/mg/min) compared with sodium-loaded animals (28 ± 11 fmol/mg/min). The increased sensitivity of the primate adrenal to angiotensin II despite a fall in angiotensin II receptors indicates that full activation of steroidogenesis by angiotensin II can be maintained with partial receptor occupancy. These results suggest that postreceptor events including increased 18-hydroxylase activity are important determinants of the aldosterone response to angiotensin II during sodium restriction in primates. (Hypertension 8: 1121-1126, 1986)

KEY WORDS • angiotensin II • adrenal receptors • vascular receptors • sodium balance • steroidogenesis

In several species, altered sodium intake is known to cause reciprocal changes in the sensitivity of the adrenal zona glomerulosa and vascular smooth muscle to angiotensin II (ANG II). During sodium deprivation, aldosterone responses to ANG II are enhanced, whereas the vascular effects of ANG II are attenuated. The opposite changes occur during sodium loading, in which the aldosterone response to ANG II is diminished while vascular reactivity is increased.

In the rat, such changes in sensitivity to ANG II are accompanied by parallel changes in the number of ANG II receptors in several target tissues. Thus, ANG II receptors in smooth muscle are increased during sodium loading and decreased during sodium restriction. These changes in ANG II receptors occur not only in vascular smooth muscle but also in tissues such as the urinary bladder, which provides a model for studies on smooth muscle ANG II receptor regulation. The converse form of regulation occurs in the adrenal glomerulosa, where binding studies in isolated cells and membranes show an increase in ANG II receptors during sodium restriction and a decrease during sodium loading. As in smooth muscle, the changes in ANG II receptors are parallel to those in target cell sensitivity to ANG II and probably contribute to the regulation of adrenal responsiveness to the peptide during altered sodium intake. In addition to ANG II receptor regulation, changes in the enzymatic activity of the aldosterone biosynthetic pathway during altered sodium intake are important in determining the secretory capacity of the adrenal glomerulosa cell.
creases after sodium loading. In the rat, sodium restriction has also been shown to stimulate the activity of the early aldosterone biosynthetic pathway, leading to pregnenolone formation.13,14

Although the effects of sodium intake on ANG II receptors and aldosterone biosynthetic activity have been analyzed extensively in the rat, the nature and importance of ANG II receptor regulation in the control of adrenal and vascular sensitivity to ANG II have not been defined in primates. Studies in humans, based on the effects of ANG II antagonists and converting enzyme inhibitors, demonstrate that increasing circulating ANG II levels during sodium restriction decrease vascular ANG II sensitivity.15 This finding supports the concept that changes in vascular ANG II receptors have a regulatory role in the pressor responses to ANG II. The situation is more complex in the adrenal, where, in contrast to the rat, pharmacological studies suggest that sodium-induced changes in circulating ANG II levels are not involved in the control of ANG II responsiveness.

In this report, the mechanisms through which sodium intake controls ANG II sensitivity in primates were examined by directly measuring the changes in vascular and adrenal ANG II receptors and adrenal 18-hydroxylase activity in monkeys on low and high sodium diets.

Materials and Methods
Seven female cynomolgus monkeys (Macaca fascicularis), initially maintained on a normal diet (0.5% NaCl), were sequentially separated into groups of two, in which one animal received a high salt diet (2.15% NaCl) and the other a low salt diet (0.23% NaCl; Ziegler Brothers, Gardeners, PA, USA). An additional animal on a high sodium diet was included only for blood and urine collection. Animals were maintained and all experimental procedures performed in accordance with National Institutes of Health guidelines. Blood samples for measurement of aldosterone and plasma renin activity (PRA) were obtained from conscious monkeys through indwelling femora catheters, inserted 48 hours before the initial collections.16 In addition, 24-hour urine collections were measured while the monkeys were on normal diets and after 5 and 6 days of altered sodium diets. Plasma aldosterone levels were measured at 100 g for 10 minutes, and the resulting pellet resuspended in 50 mM tris(hydroxymethyl)aminomethane (Tris) HCl buffer, pH 7.4, containing 5 mM MgCl₂ and 2 mM ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) to give a protein concentration of 1 to 2 mg/ml.

Adrenal zona glomerulosa was assayed for 18-hydroxylase activity and ANG II binding. The adrenals were dissected free of fat, and approximately 0.5-mm thick peripheral slices containing the zona glomerulosa were obtained with a microtome blade while the adrenal was pressed between two petri dishes. For ANG II binding studies, tissue was minced and homogenized in ice-cold 20 mM NaHCO₃ with 10 strokes in an all-glass homogenizer, and the 100 to 30,000 g membrane fraction was prepared as already described. The ANG II receptors were measured by binding of [¹²⁵I]ANG II to adrenal capsular or smooth muscle particles. For urinary bladder and mesenteric artery particles, 100 μl of protein suspension was incubated with 0.2 nM labeled ANG II (200,000 cpm) in a total volume of 250 μl of 50 mM Tris HCl buffer, pH 7.4, containing 5 mM MgCl₂, 2 mM EGTA, 0.1 mM dithiothreitol, and 0.5% bovine serum albumin in the presence and in the absence of increasing concentrations (0.2–10 nM) of unlabeled ANG II. After a 45-minute incubation at 22°C, bound radioactivity was separated by filtration through glass fiber filters (Whatman GFC, Clifton, NJ, USA) and counted in a spectrometer. Nonspecific binding was determined in the presence of 1 μM unlabeled ANG II and was less than 0.5% of the radioactivity added. A similar procedure was used for the adrenal particles with the exception that the assay buffer contained 100 mM NaCl and 2 mM dithiothreitol.19 Protein concentrations were measured by the technique of Lowry et al.,18 employing bovine serum albumin as standard. Receptor concentration and binding affinities were calculated by computer analysis of the radioligand binding data.20

For measurement of 18-hydroxylase activity, adrenal capsular tissue was minced and homogenized in 20 mM Tris HCl, pH 7.4, containing 1 mM ethylenediaminetetraacetic acid, 250 mM sucrose, and 1% bovine serum albumin. Crude mitochondrial fractions (2000–10,000 g) were separated by differential centrifugation, and enzyme activity in the isolated mitochondria was assayed by measuring the conversion of corticosterone to aldosterone. Aldosterone formation was quantitated by radioimmunoassay after separation by LH-20 chromatography.21
Statistical analysis of the experimental data was performed by Student's *t* test or by analysis of variance followed by the Newman-Keuls test.

**Results**

The effects of sodium intake on urinary sodium/creatinine ratio, PRA, and plasma aldosterone concentration are shown in Table 1. The changes in sodium intake are reflected in the decreases and increases in sodium excretion after 4 days of low and high sodium diet, respectively. Also, as expected, the PRA and plasma aldosterone concentration were significantly decreased after high sodium diet and were increased after low sodium diet.

**Effect of Sodium Intake on Angiotensin II Receptors in Smooth Muscle**

Since it has been shown in the rat that urinary bladder ANG II receptors are regulated in a manner parallel to vascular ANG II receptors,6 we used vesicular membrane-rich fractions to study smooth muscle receptor regulation. Similar to previous findings in the rat, vesicular ANG II receptors were associated with a single class of binding sites and were significantly reduced after sodium restriction (Table 2, Figure 1). In three experiments, Scatchard analysis of the binding data revealed a decrease in receptor concentration from 163 ± 6 fmol/mg after high sodium diet to 93 ± 17 fmol/mg after low sodium diet (*p < 0.025*). No significant difference in receptor affinity was observed, with dissociation constants of 1.6 ± 0.2 and 1.6 ± 0.4 x 10^-9 M for high and low sodium diets, respectively. Similarly, in each of three experiments using mesenteric artery membrane-rich particles, ANG II receptor concentration was significantly reduced in the sodium-restricted monkey (Table 3). Scatchard analysis of the data in one experiment indicated one class of high affinity sites in the monkeys on low and high sodium diets.

**Effect of Sodium Intake on Adrenal Glomerulosa Angiotensin II Receptors**

The binding of [125I]ANG II to adrenal glomerulosa membrane-rich fractions from monkeys on high and low sodium diets in a representative experiment is shown in Figure 2. In all experiments, Scatchard analysis of the data revealed one class of high affinity sites. In three experiments, ANG II receptors were markedly reduced after sodium restriction compared with the values after sodium loading (344 ± 64 and 660 ± 64 fmol/mg in low and high sodium diet, respectively; *p < 0.05*). Adrenal glomerulosa ANG II receptor affinity was similar in both groups, with dissociation constants of 1.3 ± 0.6 and 1.8 ± 0.8 x 10^-9 M for high and low sodium diets, respectively (Table 4).

**Sodium Intake and 18-Hydroxylase Activity**

Consistent with previous findings in other species,9-11 18-hydroxylase activity was increased by two-

**Table 1. Effect of Sodium Intake on Urinary Sodium/Cre-atinine Ratio, Plasma Renin Activity, and Plasma Aldosterone in the Monkey**

<table>
<thead>
<tr>
<th>Sodium intake</th>
<th>Urinary Na/Cr ratio (mEq/mg)</th>
<th>Plasma renin activity (ng ANG 1/ml/hr)</th>
<th>Plasma aldosterone (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.106 ± 0.022</td>
<td>2.6 ± 0.20</td>
<td>17.96 ± 2.18</td>
</tr>
<tr>
<td></td>
<td>(n = 9)</td>
<td>(n = 14)</td>
<td>(n = 14)</td>
</tr>
<tr>
<td>High</td>
<td>0.274 ± 0.072*</td>
<td>0.82 ± 0.18†</td>
<td>5.67 ± 0.77†</td>
</tr>
<tr>
<td></td>
<td>(n = 6)</td>
<td>(n = 6)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Low</td>
<td>0.013 ± 0.04†</td>
<td>6.92 ± 0.50†</td>
<td>72.84 ± 7.7†</td>
</tr>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. Figures in parentheses indicate the number of determinations. ANG I = angiotensin I.

*p < 0.025, †p < 0.005, compared with normal sodium intake (Newman-Keuls test).

**Table 2. Effect of Sodium Diet on Angiotensin II Receptors in Urinary Bladder Smooth Muscle Membrane-Rich Fractions**

<table>
<thead>
<tr>
<th></th>
<th>High sodium diet</th>
<th>Low sodium diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor concentration (fmol/mg)</td>
<td>Kd (nM)</td>
<td>Kd (nM)</td>
</tr>
<tr>
<td>163</td>
<td>2.0</td>
<td>91</td>
</tr>
<tr>
<td>183</td>
<td>1.4</td>
<td>123</td>
</tr>
<tr>
<td>167</td>
<td>1.4</td>
<td>65</td>
</tr>
</tbody>
</table>

Mean ± SE = 171 ± 6.1, 1.6 ± 0.2, 93 ± 16.8*, 1.6 ± 0.4

*Kd = dissociation constant*. *p < 0.025, compared with high sodium diet values.*

**Figure 1. Scatchard analysis of angiotensin II binding to urinary bladder smooth muscle particles of monkeys on high and low sodium diets. Data are representative of those obtained in three similar experiments.**
TABLE 3. Effect of Sodium Diet on Angiotensin II Receptors in Mesenteric Artery Smooth Muscle Membrane-Rich Particles

<table>
<thead>
<tr>
<th></th>
<th>High sodium diet</th>
<th>Low sodium diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor concentration (fmol/mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_d$ (nM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>87*</td>
<td>2.5</td>
<td>32</td>
</tr>
<tr>
<td>57†</td>
<td>—</td>
<td>35</td>
</tr>
<tr>
<td>61†</td>
<td>—</td>
<td>42</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>68 ± 9</td>
<td>36 ± 3†</td>
</tr>
</tbody>
</table>

$K_d = $ dissociation constant.

*Receptor concentration and affinity calculated by Scatchard analysis.

†Receptor concentration based on the binding data in the presence of 600 pM of angiotensin II.

Xp < 0.05, compared with high sodium diet values.

TABLE 4. Effect of Sodium Intake on Angiotensin II Receptors in the Monkey

<table>
<thead>
<tr>
<th></th>
<th>High sodium diet</th>
<th>Low sodium diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor concentration (fmol/mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_d$ (nM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>539</td>
<td>0.5</td>
<td>273</td>
</tr>
<tr>
<td>750</td>
<td>2.5</td>
<td>290</td>
</tr>
<tr>
<td>693</td>
<td>1.0</td>
<td>470</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>661 ± 63</td>
<td>1.3 ± 0.6</td>
</tr>
</tbody>
</table>

$K_d = $ dissociation constant.

*p < 0.025, compared with high sodium diet values.

TABLE 5. 18-Hydroxylase Activity in Adrenal Glomerulosa Mitochondrial Fractions from Monkeys on Low and High Sodium Diet

<table>
<thead>
<tr>
<th>Conversion of corticosterone to aldosterone (pmol/mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High sodium diet</td>
</tr>
<tr>
<td>17.2</td>
</tr>
<tr>
<td>39.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
</tr>
</tbody>
</table>

*p < 0.08, compared with high sodium diet values (two-tailed unpaired t test).

Discussion

Dietary sodium intake is known to modulate adrenal and vascular smooth muscle sensitivity to ANG II in all species studied, with enhancement of adrenal and reduction of vascular responses to angiotensin during salt restriction. In the rat, ANG II receptor regulation is an important mechanism through which sodium intake controls the responsiveness of target tissues. In vitro binding studies have shown that sodium restriction in the rat decreases vascular smooth muscle ANG II receptor concentration while it increases receptors in the adrenal glomerulosa.*'¨ These changes parallel the changes in vascular and adrenal sensitivity to ANG II observed during sodium depletion, suggesting a causal or contributory relationship between ANG II receptor concentration and target cell responsiveness.

However, in primates the role of receptor changes in the regulation of ANG II sensitivity is unclear. In contrast to the rat, pharmacological studies in humans do not support a sensitizing effect of ANG II or up-regulation of adrenal ANG II receptors after sodium restriction.23 For this reason, the present study was performed to directly examine the regulation of ANG II receptors during altered sodium intake in the primate.

In monkey smooth muscle, ANG II receptor concentration decreased after sodium restriction and increased after sodium loading. These regulatory changes in receptors are similar to those observed in the rat and would contribute to the parallel changes in vascular sensitivity to ANG II that occur during altered sodium intake. The urinary bladder was used as a model to study smooth muscle ANG II receptors. This tissue is abundant in ANG II receptors, which in the rat are identical to those of the mesenteric artery in terms of binding properties and regulation during altered so-

FIGURE 2. Scatchard analysis of angiotensin II binding to adrenal glomerulosa particles of monkeys on high and low sodium diets in one of three similar experiments.
Angiotensin II receptor regulation was also found to mimic that of vascular smooth muscle, as demonstrated by the parallel changes observed in ANG II receptor concentration in mesenteric artery particles.

Circulating ANG II appears to be the mediator of the receptor changes that occur during altered sodium intake. In the rat, ANG II infusions that cause small increases in blood ANG II, in the range of those observed during sodium restriction, decrease smooth muscle ANG II receptors and increase adrenal receptors. The PRA was increased in the sodium-restricted monkey; the consequent increases in circulating ANG II are probably responsible for the decrease in ANG II receptors. Down-regulation of smooth muscle ANG II receptors during increases in the circulatory peptide has also been proposed by pharmacological studies in sodium-restricted humans, in whom blockade of endogenous ANG II formation by converting enzyme inhibition resulted in an increase in sensitivity of the pressor response to ANG II.

Regulation of ANG II receptors in the monkey adrenal does not appear to be a mechanism through which sodium intake modulates ANG II sensitivity. As in other species including humans, monkey plasma aldosterone, 18-hydroxylase activity, and, presumably, adrenal sensitivity to ANG II are increased during sodium restriction. This increased adrenal responsiveness is maintained despite a decrease in ANG II receptors in the zona glomerulosa. In contrast, adrenal glomerulosa ANG II receptors in the rat are increased during sodium restriction and after infusion of ANG II. Although the opposite change has been claimed on the basis of pharmacological studies in isolated rat adrenal cells, increases in adrenal ANG II receptors after sodium restriction have been clearly demonstrated in the rat by binding of radiolabeled ANG II in vivo and by direct measurement of the receptors in membrane-rich fractions and isolated adrenal glomerulosa cells. This difference between species may reflect adaptional changes during evolution, in which receptor up-regulation may facilitate the sensitization of the adrenal in species in which rapid aldosterone-stimulating activity on angiotensin II in dogs. Proc Soc Exp Biol Med 1967;124:1230-1231

References
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