Sodium Load Increases Renal Angiotensin Type 1 Receptors and Decreases Bradykinin Type 2 Receptors

Pia STEWEN*,**, Eero MERVAAALA**, Heikki KARPPANEN**, Tuulikki NYMAN*, Outi SAIJONMAA*.,***, Ilkka TIKKANEN*.*,***, and Frej FYHRQUIST*.***

The regulation of both angiotensin receptors and bradykinin receptors during sodium intake is poorly understood. We hypothesized that an altered balance between renal angiotensin type 1 (AT1) receptors and bradykinin type 2 (B2) receptors might contribute to an increase in blood pressure during periods of high-sodium intake. We studied the effects of high-sodium intake on renal AT1 receptors and B2 receptors in 5–6-week-old spontaneously hypertensive rats (SHR) receiving high-sodium chloride (6% NaCl) or mineral salts (10.5%, composition: 57% NaCl, 28% KCl, 12% MgSO4) compared to those receiving a low-sodium (NaCl 0.125%) diet for 10 weeks. Mineral salt intake was included due to its beneficial effects on blood pressure and cardiac hypertrophy. Receptor densities were measured by quantitative autoradiography. AT1 receptors were quantified using incubation with 125I-Sar1-Ile8-angiotensin II and displacement was measured with PD123319 (10⁻⁷ mol/l), whereas B2 receptors were quantified using 125I-HPP-icatibant and displacement was measured with icatibant (3 mol/l). Compared to the SHR controls, a further increase in blood pressure occurred after 2 weeks in the 6% NaCl group and after 6 weeks in the mineral salt group. AT1 receptor density increased in the renal cortex by 41% (p < 0.01) in the 6% NaCl group and by 26% (p < 0.05) in the mineral salt group. B2 receptor density decreased in the renal medulla by 26% (p < 0.01) in the 6% NaCl group, and by 45% (p < 0.001) in the mineral salt group. It was shown that a 6% NaCl or a 10.5% mineral salt loading was capable of increasing renal AT1 receptor density and decreasing renal B2 receptor density. An altered balance between these receptors might be associated with hypertension under conditions of sodium loading. (Hypertens Res 2003; 26: 583–589)

Key Words: sodium intake, mineral salts, spontaneously hypertensive rats, kidney, autoradiography

Introduction

The renin-angiotensin-aldosterone (RAAS) and kallikrein-kinin systems (KKS) play crucial roles in regulating renal and cardiovascular functions. Sodium loading downregulates the RAAS, whereas the KKS is upregulated under the same conditions (1, 2). Activation of these systems has opposing effects, mainly mediated via angiotensin type 1 (AT1) and bradykinin (BK) type 2 (B2) receptors. Angiotensin II (Ang II), a potent vasoconstricting and blood pressure (BP) raising polypeptide, plays an important role in the autoregulation of renal blood flow, glomerular filtration pressure, and tubular functions via AT1 receptors located both in the renal cortex and medulla (3). Ang II regulates electrolyte and fluid balance by directly enhancing salt and water retention or via aldosterone release. Ang II also has growth-promoting effects. In contrast, BK acts mainly via B2 receptors to enhance salt
and water extrusion, causing vasodilatation, reduction in BP, and in general the downregulation of growth (1, 2, 4).

High-sodium intake, which is known to cause hypertension and end-organ damage in susceptible individuals, reduces the activity of RAAS and increases the activity of KKS (1, 5–8). Suppression of the RAAS by high-sodium intake is thought to be involved in a compensatory upregulation of AT₁ receptor expression, which may in turn lead to enhanced vasoconstriction and water retention; however, the mechanisms underlying this type of elevation in BP are poorly understood (1, 3, 4, 7). Activation of the KKS under conditions of high-sodium intake has been reported (9), although its effects on B₂ receptors, as well as its effects on the balance between AT₁ receptors and B₂ receptors during salt loading, remain to a large extent unknown.

We hypothesized that changes in the balance of renal AT₁ and B₂ receptors might contribute to the maintenance of hypertension under conditions of sodium loading. This hypothesis was tested in spontaneously hypertensive rats (SHR) treated for 10 weeks with high-sodium chloride (6% NaCl) or mineral salts (10.5% salt, composed of 57% NaCl, 28% KCl, 12% MgSO₄·7H₂O; in our previous studies, the latter were shown to produce a smaller increase in blood pressure and to induce less cardiac hypertrophy than common salt in SHR (10). Here, we present data in favor of the hypothesis that an altered balance between renal AT₁ and B₂ receptors might be associated with hypertension under conditions of sodium loading.

**Methods**

**Animals and Study Design**

Five to six-week-old male SHR (Harlan Sprague Dawley, Indianapolis, USA) were fed low-sodium chloride (control group) (0.125% NaCl; n = 7–9), high-sodium chloride (6% NaCl; n = 7–10) (Sigma, St. Louis, USA), or mineral salts (Pansalt®; 10.5%; Orion, Espoo, Finland; n = 7–10) diets (Harlan Teklad LM-485 low-sodium rat diet; 0.125% NaCl) for 10 weeks. The sodium (Na⁺) content of the 6% NaCl group diet and that of the 10.5% mineral salt group diet was the same, i.e., 2.5%; in the low-sodium group, the sodium content was 0.08%. The mineral salts composition was as follows: 57% NaCl, 28% KCl, 12%, MgSO₄·7H₂O, 2% l-lysine hydrochloride, and 1% anti-caking agents (SiO₂, MgCO₃).

Systolic BP, heart rate (HR), and the body weight (bw) of the rats were measured every second week. BP and HR were measured with tail-cuff plethysmograph (Apollo-2AB Blood Pressure Analyzer, Model 179-2AB, IITC Life Science, Woodland Hills, USA). On the final day of the experiment, urine was collected for 24 h from the rats, which had been placed in metabolic cages for the measurement of urinary protein according to the method of Lowry et al. (11).

Urinary osmolality was measured with an enzymatic analyzer (Kone Specific, Kone Corp., Espoo, Finland) and urinary sodium, potassium, magnesium, calcium, and phosphorus concentrations were determined using a Baird PS-4 inductively coupled plasma emission spectrometer (Baird Co., Bedford, USA). The urine chloride concentration was measured as described by Schales and Schales (12). Serum and EDTA-plasma samples were collected following decapitation. Serum aldosterone (Coat-A-Count Aldosterone, Diagnostic Products Corp., Los Angeles, USA) and plasma renin activity were determined by radioimmunoassays, as described by Tikkanen et al. (13). At the end of the experiment, the kidneys were excised, frozen, and stored at -70°C for autoradiographic studies.

Animal experiments were approved by the appropriate institutional committee at Helsinki University Central Hospital and were in accordance with institutional guidelines for animal treatment in experimental research.

**Autoradiography**

Frozen kidney sections (20 µm) were cut on a cryostat at -17°C; they were then thaw-mounted on Super Frost® Plus slides (Menzel-Gläser, Braunschweig, Germany), dried, and stored at -70°C until needed for further processing for the autoradiographic studies. Iodination with 125I (Amersham Pharmacia Biotech, Buckinghamshire, UK) of peptides was performed by the chloramine-T method, and the labeled peptides were purified on SP-Sephadex C-25 columns (Pharmacia, Uppsala, Sweden). Only mono-iodinated peptides were used.

For quantitative AT₁ receptor autoradiography, a previously described method suggested by Mendelsohn et al. (14) was applied, with minor modifications. Briefly, renal slices were preincubated for 15 min at room temperature in 10 mmol/l sodium phosphate buffer containing 150 mmol/l NaCl, 5 mmol/l EDTA, 0.2% bovine serum albumin (BSA) (Sigma, St. Louis, USA), at pH 7.4, followed by incubation with 0.3 µCi/ml of 125I-Sar²-Ile³-Ang II at 37°C for 1 h and displacement with 10 µmol/l losartan (an AT₁ receptor antagonist) or 10 µmol/l PD123319 (an AT₂ receptor antagonist). Nonspecific binding was determined in the presence of 1 µmol/l Ang II. Specific AT₁ receptor binding was calculated from the total binding value, subtracting the binding value obtained in the presence of excess losartan. Specific AT₂ receptor binding was calculated from total binding value, subtracting the binding value obtained in the presence of excess PD 123319. The AT₁ receptor binding was minimal or zero, and it was therefore difficult to distinguish AT₁ receptor binding from nonspecific binding.

For the quantitative B₂ receptor autoradiography, a previously described method, reported by Dean, was applied (15). Briefly, renal slices were preincubated for 15 min at room temperature in 170 mmol/l Tris-HCl buffer containing 0.2% BSA and 20 µmol/l captopril (Sigma), followed by incubation overnight at 4°C with 0.3 µCi/ml of 125I-HPP-HOE (an analog of icatibant, a B₂ receptor antagonist, and a gift of Dr.
Juergen Puenter, Hoechst, Frankfurt am Main, Germany. Displacement was carried out with the addition of 3 µmol/l of icatibant (Hoechst, Frankfurt am Main, Germany). Specific binding was calculated from the total binding value, subtracting the nonspecific binding value obtained in the presence of excess icatibant.

In order to quantify the amount of angiotensin-converting enzyme (ACE), lisinopril analog 351A, a p-hydroxybenzamidine derivative of N-(1-carboxy-3-phenyl-propyl)-L-lysyl-L-proline (Merck, Darmstadt, Germany), was iodinated, as has been described elsewhere (16). For the quantitative ACE autoradiography, a previously described method was used (17). Briefly, renal slices were preincubated for 15 min at room temperature in 10 mmol/l sodium phosphate buffer, pH 7.4, containing 150 mmol/l NaCl and 0.2% BSA (Sigma), followed by incubation for 1 h with 0.3 µCi/ml of 125I-Sar1-Ile8-Ang II at 37°C, 1 h, and displacement with 10 µmol/l of PD 123319 (an AT1 receptor antagonist). AT1 receptor binding is shown in the presence of PD 123319 at left, and nonspecific binding is shown in the presence of Ang II at right. AT1 receptor binding values in control rats, 6% NaCl-fed rats, and 10.5% mineral salt-fed rats are shown in the upper, middle, and lower panels, respectively. B: B2 receptors were revealed by the preincubation of renal slices for 15 min at room temperature in 170 mmol/l Tris-HCl buffer containing 0.2% BSA and 20 µmol/l captopril, followed by incubation overnight at 4°C with 0.3 µCi/ml of 125I-HPP-HOE and displacement with 3 µmol/l of HOE = icatibant (a B2 receptor antagonist). Total binding is shown at left and nonspecific binding at right. B2 receptor binding values in control rats, 6% NaCl-fed rats, and 10.5% mineral salt-fed rats are shown in the upper, middle, and lower panels, respectively.

Statistical Analysis

Statistical analysis was carried out using an analysis of variance (ANOVA) followed by Dunnett’s or Bonferroni’s tests. A difference of \( p < 0.05 \) was considered significant. Results are expressed as mean ± SEM.

Results

Autoradiography

Autoradiography revealed the renal distribution of AT1 receptors and B2 receptors in control rats, 6% NaCl-treated rats, and 10.5% mineral salt-treated rats (Fig. 1). The AT1 receptors were located both in the renal cortex and in the medulla. Cortical AT1 receptor binding was observed in the glomeruli, whereas medullar binding was observed in vasa recta bundles and in the inner stripe of the outer medulla. B2 receptors were located throughout the medulla, in the inner...
stripe of the outer medulla, in the inner medulla, and in the pelvic capsule. Quantitative autoradiography showed a 41% ($p < 0.01$) increase in the density of AT$_1$ receptors in the renal cortex in the 6% NaCl group and similar increase of 26% ($p < 0.05$) in the mineral salt group, although in both groups, no such increase was found in the medulla (Fig. 2). High-salt intake reduced B$_2$ receptor density in the medulla by 26% ($p < 0.01$) in the 6% NaCl group and by 45% ($p < 0.001$) in the rats administered a mineral salt diet. The decrease in B$_2$ receptor density was more pronounced in the mineral salt group compared to that in the 6% NaCl group ($p < 0.05$) (Fig. 3). Salt loading did not lead to changes in renal ACE enzyme density (data not shown).

Fig. 2. Renal AT$_1$ receptor density observed during salt loading, as quantified by autoradiography. Ten randomly placed squares/cortex and six squares/medulla from each kidney were analyzed. Specific AT$_1$ receptor binding was calculated from the total binding value by subtracting the binding value obtained in the presence of excess losartan and Ang II. Results are expressed as mean pixels/square area ± SEM. Significant differences from the control group are indicated by * $p < 0.05$ and ** $p < 0.01$.

Fig. 3. Renal medullary B$_2$ receptor density during salt loading, as quantified by autoradiography. Eight randomly placed squares/medulla from each kidney were analyzed. Specific B$_2$ receptor binding was calculated from the total binding value by subtracting the binding value obtained in the presence of excess HOE. Results are expressed as means ± SEM. Significant differences are indicated by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Fig. 4. Blood pressure increase during salt load in SHR. Values are expressed as means ± SEM from three to five repetitive measurements. Significant differences are indicated by * (compared to the controls) and by # (between treatment groups): * or ** $p < 0.05$, *** or ## $p < 0.01$, and ### $p < 0.001$.

**Blood Pressure, Renin and Aldosterone, Organ and Body Weight Measurements**

Systolic BP increased in the 6% NaCl group by 40% ($p < 0.001$) and by 26% ($p < 0.001$) in the mineral salt group during 10 weeks of salt treatment. The BP increase was lower in the mineral salt group than in the 6% NaCl group throughout this 10-week period ($p < 0.01$) (Fig. 4). High-salt intake reduced plasma renin activity by 50% ($p < 0.01$) and by 49% ($p < 0.01$) in the 6% NaCl and the mineral salt groups, respectively. Aldosterone concentration was 60% ($p < 0.01$) lower in the 6% NaCl group and 68% ($p < 0.001$) lower in the mineral salt group than in the control group. Left ventricular weight increased 13% in the 6% NaCl group ($p < 0.05$). Renal weight increased in the 6% NaCl group by
Values are expressed as mean ± SEM, n = 7–9. * , ** , *** indicates p < 0.05, p < 0.01, p < 0.001, respectively, as compared to the controls.

Table 2. One-day-Proteinuria, Urine Volume, Urine Electrolytes (Na⁺, K⁺, Cl⁻, Mg²⁺, Ca²⁺, PO₄⁻), and One-day-Osmolality Were Measured at the End of the Experiment

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>6% NaCl</th>
<th>10.5% Mineral salts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria (mg/day)</td>
<td>49.2 ± 2.7</td>
<td>65.3 ± 5.7 *</td>
<td>70.2 ± 4.6 **</td>
</tr>
<tr>
<td>Urine volume (ml/day)</td>
<td>13.9 ± 1.0</td>
<td>59.4 ± 4.8 ***</td>
<td>59.3 ± 4.0 ***</td>
</tr>
<tr>
<td>Na⁺ (mmol/day)</td>
<td>0.2 ± 0.04</td>
<td>16.8 ± 0.7 ***</td>
<td>14.7 ± 0.8 ***</td>
</tr>
<tr>
<td>K⁺ (mmol/day)</td>
<td>2.8 ± 0.09</td>
<td>3.2 ± 0.1</td>
<td>8.8 ± 0.4 ***</td>
</tr>
<tr>
<td>Cl⁻ (mmol/day)</td>
<td>0.05 ± 0.004</td>
<td>1.14 ± 0.05 ***</td>
<td>1.32 ± 0.07 ***</td>
</tr>
<tr>
<td>Mg²⁺ (mmol/day)</td>
<td>0.06 ± 0.004</td>
<td>0.08 ± 0.01</td>
<td>0.23 ± 0.02 ***</td>
</tr>
<tr>
<td>Ca²⁺ (mmol/day)</td>
<td>0.02 ± 0.002</td>
<td>0.17 ± 0.02 ***</td>
<td>0.14 ± 0.02 ***</td>
</tr>
<tr>
<td>PO₄⁻ (mmol/day)</td>
<td>0.070 ± 0.005</td>
<td>0.33 ± 0.03 **</td>
<td>0.45 ± 0.08 ***</td>
</tr>
<tr>
<td>Osmolality (mosmol/day)</td>
<td>16.2 ± 0.4</td>
<td>51.2 ± 1.9 ***</td>
<td>56.3 ± 2.7 ***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n = 7–9. * , ** , *** indicates p < 0.05, p < 0.01, p < 0.001, respectively, as compared to the controls. 5 and 222 indicate p < 0.05 and p < 0.001, respectively, compared to the 6% NaCl group.

13% (p < 0.001). Body weight gain during the salt loading period was 6% lower in the 6% NaCl group (p < 0.01) and 9% lower in the mineral salt group (p < 0.001) than in the controls (Table 1).

**Urine Volume, Electrolytes, Osmolality, and Proteinuria Measurements**

During the period of salt loading, proteinuria increased significantly in the 6% NaCl group, i.e., by 33% (p < 0.05), whereas during the same period, proteinuria increased in the mineral salt group by 43% (p < 0.01). During salt loading, the urine volume increased by 327% (p < 0.001) in the 6% NaCl group and by 326% (p < 0.001) in the mineral salt group. As expected, urinary sodium and chloride excretion increased markedly in both salt groups (p < 0.001), while potassium and magnesium excretion increased only in the mineral salt group (p < 0.001). High-salt intake was associated with an increase in calcium and phosphorus excretion and urine osmolality in both salt groups (p < 0.01) (Table 2).

**Discussion**

Our results show, for the first time, that high-salt intake altered the balance between renal AT₁ and B₂ receptors. Thus, during a period of high-salt intake, there was an increase in AT₁ receptor density in the renal cortex, whereas B₂ receptor density in the renal medulla was decreased. Regulation of and balance between angiotensin and BK receptors during salt loading has not been examined in depth, although sodium loading is known to influence both the RAAS and the KKS (1, 5, 7, 9). In view of the opposing effects of Ang II, favoring hypertension, and those of BK, favoring a reduction in BP, the present demonstration of an altered balance between renal AT₁ and B₂ receptors during a period of high-salt intake suggests that this change in the receptor density balance could account for the hypertension-influencing capacity of salt. On the other hand, such effects may partly be only a consequence of elevated BP occurring during salt loading.

The mechanism of renal AT₁ receptor upregulation during periods of high-salt intake remains unclear. A plausible explanation for our model would be pressure-induced upregulation of AT₁ receptors in the renal cortex, in the high-pressure area. The AT₁ receptor upregulation in this area could be induced by pronounced BP elevation during high-salt intake. However, no AT₁ receptor upregulation was detected in the medullary low-pressure area. Shear stress is known to induce AT₁ receptor-mediated hypertrophy (1). Another explanation for the observed renal AT₁ receptor upregulation
would be the compensatory upregulation of AT$_1$ receptors due to the suppression of the renal Ang II concentration during a period of high-salt intake; however, a direct salt effect might also be responsible for this result (1, 4, 5, 7). However, the renal Ang II concentration has been reported to be higher in SHR than in Wistar-Kyoto or Sprague-Dawley rats, independent of sodium intake (18). Thus, the pressure-induced mechanism, a negative feedback regulation of Ang II on its receptor or a salt effect might be responsible for the upregulation of renal AT$_1$ receptors.

Upregulation of AT$_1$ receptor mRNA during periods of high-sodium intake has been reported in renal resistance vessels, in particular in afferent arterioles, mesenteric arteries, the aorta, and in the cultured vascular smooth muscle cells of normotensive rats (1, 3, 4). However, such upregulation was not associated with BP elevation, as it was in our SHR. Compensatory upregulation or a direct sodium effect was suggested as possible mechanisms in the previous reports (1, 3, 4). It therefore remains to be determined whether or not our results are applicable to other rat strains, and more importantly, to humans.

High-salt intake downregulates B$_2$ receptors in the renal medulla. The KKS is upregulated during sodium loading; thus, the production of vasodilating BK is increased under such conditions (9). The present results offer a negative feedback explanation for the downregulation of medullary B$_2$ receptors by an increase in renal BK concentration. B$_2$ receptor downregulation occurs in medullary low pressure areas, and may thus be reciprocally regulated by its agonist, BK.

The observed downregulation of B$_2$ receptors in the renal medulla was even more pronounced in rats on a mineral salt diet than in those on a 6% NaCl diet. Potassium has been reported to stimulate renal kallikrein and kininogenase, and thus to increase BK (19, 20). Accordingly, further downregulation of B$_2$ receptors associated with mineral salt intake might be due to a synergistic effect induced by combined sodium and potassium intake. The synergistic upregulatory effect of both sodium and potassium on the KKS, followed by further B$_2$ receptor downregulation, might account for the favorable effects of mineral salts on hypertension.

SHR used in our experiments developed hypertension after 2 weeks of receiving a 6% NaCl diet, whereas an additional hypertensive effect exceeding that of the controls developed after 6 weeks of rats on a mineral salt diet. Throughout the experiment, the increase in BP was more pronounced in the 6% NaCl group. The potassium/magnesium supplementation of mineral salt seemed to delay the increase in BP, especially during the first weeks of high-salt intake. Potassium and magnesium supplementation has been reported to decrease BP in humans and rats (10, 21).

We suggest that the respective mechanisms for the regulation of AT$_1$ and B$_2$ receptor density might differ. Elevated BP during periods of high-salt intake may be influenced by important BP-regulating receptors (e.g., via their agonists, shear stress and/or salt, and altered receptor density balance).

We concluded that renal AT$_1$ and B$_2$ receptors in SHR were observed to react differently to both salt loading and hypertension. Thus, whereas an altered balance between AT$_1$ and B$_2$ receptors (i.e., the upregulation of AT$_1$; and the downregulation of B$_2$ receptors) may be a consequence of hypertension during periods of high-salt intake, it cannot be ruled out that a suppression of renal Ang II and enhanced BK formation play roles in the altered receptor balance.

References

1987; 31 (Suppl 20): S40–S44.


