Upregulation of Renal $\alpha_2$-Adrenergic Receptors Is Not Indicative of Salt-Related Increases in Blood Pressure in Spontaneously Hypertensive Rats

Michael J. Meldrum, Norman Singletary, and Ralph Dawson Jr.

The aim of the present study was to determine if elevations in salt intake were coupled to increases in renal $\alpha_2$-adrenergic receptors in SHR that differ in their blood pressure response to high salt diets. Salt-resistant spontaneously hypertensive rats (SHR-R), which do not increase their blood pressure in response to high salt intake, and salt-sensitive spontaneously hypertensive rats (SHR-S), which do exhibit significant elevations in blood pressure on high salt diets (3.15% NaCl), were used. Radioligand binding studies using $[^3H]$ruwolscine were performed on 6- and 11-week-old SHR-S and Wistar-Kyoto (WKY) rats to determine the effects of age, strain, and salt intake on $\alpha_2$-adrenergic receptor number and affinity. One week of high salt intake significantly increased blood pressure 22% in 6-week-old SHR-S and increased the blood pressure of 11-week-old SHR-S 12% without altering WKY rat controls. This treatment did not significantly increase renal $\alpha_2$-adrenergic receptors in either SHR-S or WKY rats. SHR-S had significantly higher numbers of renal $\alpha_2$-adrenergic receptors than WKY rats on the high salt diets. One week of high (3.15%) or low (0.05%) salt intake did not significantly alter renal $\alpha_2$-adrenergic receptor number in 11-week-old SHR-S or WKY rats; however, blood pressure was significantly elevated in the SHR-S (175.0±3.5 versus 196.0±3.0 mm Hg). Two weeks of high salt intake did produce significant 37-48% increases in renal $\alpha_2$-adrenergic receptor numbers in both SHR-S and SHR-R; however, this treatment increased blood pressure significantly only in the SHR-S. The results of these studies show that salt-induced increases in blood pressure in the SHR-S occur previous to significant increases in renal $\alpha_2$-adrenergic receptors that are seen in both SHR-S and SHR-R after 2 weeks of high salt treatment. These findings suggest that salt-induced increases in blood pressure are independent of the upregulation of renal $\alpha_2$-adrenergic receptors. (Hypertension 1990;16:49–54)

Increased dietary intake of NaCl has been shown to increase renal $\alpha_2$-adrenergic receptor number in normotensive and hypertensive rats.

Salt-induced increases in blood pressure have also been associated with an upregulation of renal $\alpha_2$-adrenergic receptors. Previously, our laboratory has shown that increased dietary salt intake was accompanied by increases in renal $\alpha_2$-adrenergic receptors and significant reductions in renal norepinephrine stores. Therefore, there appears to be a link between salt-induced alterations in sympathetic nervous system function, elevated blood pressure, and renal $\alpha_2$-adrenergic receptors. Recent studies have clarified the role of the renal nerves and $\alpha_2$-adrenergic receptors in facilitating sodium reabsorption; however, the functional role of renal $\alpha_2$-adrenergic receptors in electrolyte balance is still a matter of contention. Renal $\alpha_2$-adrenergic receptors are thought to be located extrajunctionally and to be responsive to circulating epinephrine. Other actions of renal $\alpha_2$-adrenergic receptor agonists can produce natriuretic and diuretic effects in rodents. Therefore, renal $\alpha_2$-adrenergic receptors may play an important role in water and electrolyte balance.

Recently, spontaneously hypertensive rats (SHR) that differ in their responsiveness to increased
dietary salt intake have been available from commercial sources. The availability of SHR that exhibit a rapid salt-induced exacerbation of their hypertension (Taconic Farms, Germantown, N.Y.) provides a useful model of salt-sensitive hypertension. These salt-sensitive SHR (SHR-S) are distinct from SHR that do not show salt-induced elevations in blood pressure (Charles River Breeding Laboratories, Wilmington, Mass.). The aim of the present study was to determine if the upregulation of renal \( \alpha_2 \)-adrenergic receptors was specifically associated with salt-sensitive hypertension or if elevations in renal \( \alpha_2 \)-adrenergic receptors accompany increased dietary salt intake as a compensatory mechanism. The SHR-S and salt-resistant SHR (SHR-R) differentially respond to dietary salt intake and therefore allow the assessment of salt-induced changes in \( \alpha_2 \)-adrenergic receptors. Therefore, the question of the causal relation between salt-induced increases in blood pressure and increased renal \( \alpha_2 \)-adrenergic receptors can be addressed by using these animals.

### Methods

#### Animals

SHR were obtained from Charles River and IBU 3 Colony, Taconic Farms. Age-matched Wistar-Kyoto (WKY) control rats were also obtained from the same suppliers. The rats were housed in hanging wire cages and fed commercially prepared diets (Purina, Richmond, Ind.) of varying NaCl content. The basal diet contained 0.5% NaCl, high salt diets contained 3.15% NaCl, and low salt diets were 0.05% NaCl. Food and water were available ad libitum. One study used 1.0% NaCl in the drinking water as a means to increase daily NaCl intake, and tap water served as the control. The basal diet was available ad libitum for this group. The rats were on a 12-hour light/dark cycle and were housed in a temperature-controlled animal colony room. Weekly blood pressure determinations were made using the tail-cuff method.

#### Receptor Binding Studies

The rats were killed by decapitation and the kidneys were rapidly removed and frozen on dry ice. The kidneys were stored at \(-80^\circ\)C until assay. The \(^{3}H\)rauwolscine binding studies were done according to the method of Dawson and Wallace. Briefly, the left whole kidneys were weighed and homogenized using a Brinkman Homogenizer, Westbury, N.Y. in 20 volumes (wt/vol) of ice-cold 50 mM Tris buffer (pH = 7.4) containing 1.0 mM Na\(_2\)EDTA. The homogenate was centrifuged for 15 minutes at 40,000g (at 4°C). The supernatant was discarded and the pellet was resuspended in 20 volumes Tris buffer (pH = 7.4) containing 100 mM NaCl. The homogenate was again centrifuged at 40,000g for 15 minutes (at 4°C). The final pellet was resuspended in 50 volumes Tris buffer (pH = 7.4) containing 100 mM NaCl to give a protein concentration of 1.0–1.5 mg protein/ml homogenate. The 100 mM NaCl Tris buffer was used because in previous experiments this buffer was shown to provide optimal conditions for rauwolscine binding. \(^{3}H\)Rauwolscine (77–90 Ci/mmol, New England Nuclear, Boston, Mass.) binding was performed by incubating renal homogenates (250 μl) in triplicate with a range (0.25–10 nM) of rauwolscine concentrations for 45 minutes at 25°C. The total assay volume was 1.0 ml, and 10 μM phentolamine was used to determine nonspecific binding. The assay was terminated by rapid filtration using a tissue harvester (Brandel, Gaithersburg, Md.). \(^{3}H\)Aminoclonidine binding was done according to previously described methods. Radioactivity trapped on GF/B fiberglass filters was counted at 46–50% efficiency in a Beckman LS 3801 scintillation counter (Beckman Instruments, Inc., Irvine, Calif.). Specific binding of \(^{3}H\)rauwolscine in all treatments was 75–85% near the dissociation constant \((K_d)\) value, and the binding was to a single class of noninteracting sites. The data from the saturation analysis using six to seven different concentrations of \(^{3}H\)rauwolscine were analyzed using a computer program to perform least-squares linear regression analysis of the Scatchard plot.

#### Renal Norepinephrine Determination

The right kidneys from two of the groups of the rats evaluated in the radioligand binding assays were homogenized in 10.0 ml of 0.1 M perchloric acid containing 100 mg/l Na\(_2\)EDTA. The homogenates were centrifuged at 15,000g for 15 minutes, and aliquots of the supernatants were alumina extracted. The samples were spiked with an internal standard, and recovery after alumina extraction averaged 60–70%. The extracted samples were assayed using high-performance liquid chromatography and electrochemical detection as previously described.

### Results

One week of high salt treatment produced significant increases in blood pressure in both 6- and 11-week-old SHR-S (116.5±2.0 versus 142.0±2.0 mm Hg for 6-week-old, 175.0±3.5 versus 196.0±3.0 mm Hg for 11-week-old SHR-S) (Table 1). High salt intake did not affect the blood pressure of WKY rats. Blood pressure in SHR-S (6 and 11 weeks) was significantly higher than WKY rats on either the basal or high salt diets. High salt intake for 1 week did not significantly increase the number \((B_{max})\) of \(\alpha_2\)-adrenergic receptors labeled by \(^{3}H\)rauwolscine in either SHR-S or WKY rats (Table 1). Renal \(\alpha_2\)-adrenergic receptor numbers were increased in SHR-S relative to WKY rats on high salt diets (Table 1). No strain or diet effects were seen in the affinity \((K_d)\) of renal \(\alpha_2\)-adrenergic receptors. Kidney weights were significantly lower in SHR-S than WKY rats, and there were no significant effects of diet (Table 1). Renal norepinephrine content was also determined in an effort to correlate content with effects on receptor binding in the 11-week-old SHR-S and WKY rats. Norepinephrine content was significantly greater in SHR than WKY rats on both basal and...
TABLE 1. Renal α2-Adrenergic Receptors in Salt-Sensitive Spontaneously Hypertensive Rats on Basal and High Salt Diets

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (wk)</th>
<th>n</th>
<th>(K_d)</th>
<th>(B_{max})</th>
<th>Kidney wt</th>
<th>BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR-Basal (0.5%)</td>
<td>6</td>
<td>5</td>
<td>2.25±0.19</td>
<td>232±14</td>
<td>0.57±0.03*</td>
<td>116.5±2.0*</td>
</tr>
<tr>
<td>SHR-High (3.15%)</td>
<td>6</td>
<td>5</td>
<td>2.19±0.15</td>
<td>240±9*</td>
<td>0.63±0.02*</td>
<td>142.0±2.0*</td>
</tr>
<tr>
<td>WKY-Basal (0.5%)</td>
<td>6</td>
<td>5</td>
<td>2.05±0.14</td>
<td>196±19</td>
<td>0.72±0.04</td>
<td>104.0±2.0</td>
</tr>
<tr>
<td>WKY-High (3.15%)</td>
<td>6</td>
<td>5</td>
<td>2.18±0.07</td>
<td>194±13</td>
<td>0.71±0.07</td>
<td>104.0±2.0</td>
</tr>
<tr>
<td>SHR-Basal (0.5%)</td>
<td>11</td>
<td>5</td>
<td>2.59±0.15</td>
<td>178±16</td>
<td>0.88±0.03*</td>
<td>175.0±3.5*</td>
</tr>
<tr>
<td>SHR-High (3.15%)</td>
<td>11</td>
<td>5</td>
<td>2.62±0.14</td>
<td>200±10*</td>
<td>0.91±0.05*</td>
<td>196.0±3.0*</td>
</tr>
<tr>
<td>WKY-Basal (0.5%)</td>
<td>11</td>
<td>5</td>
<td>2.59±0.34</td>
<td>153±8</td>
<td>1.20±0.06</td>
<td>123.0±2.5</td>
</tr>
<tr>
<td>WKY-High (3.15%)</td>
<td>11</td>
<td>5</td>
<td>2.22±0.07</td>
<td>157±10</td>
<td>1.16±0.09</td>
<td>124.0±4.0</td>
</tr>
</tbody>
</table>

Dissociation constant \( (K_d) \) is expressed as nanomoles/liter and maximal number of binding sites \( (B_{max}) \) as femtomoles bound per milligrams protein±SEM. BP, blood pressure; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

*p<0.05 SHR vs. WKY rats.

higher in SHR-S on low salt diets when compared with WKY rats on the same diet. WKY rats on high salt diets had significantly increased numbers of α2-adrenergic receptors when compared with WKY rats on low salt diets. Kidney weights were significantly lower in SHR-S than WKY rats.

The effects of 2 weeks of high salt treatment (1.0% NaCl in the drinking water) on renal α2-adrenergic receptors and blood pressure are presented in Table 3. There was no difference in blood pressure or renal α2-adrenergic receptors in 12-week-old SHR-S or SHR-R on normal salt diets. However, 2 weeks of high salt treatment significantly elevated blood pressure in SHR-S but not SHR-R (177.5±1.1 versus 197.5±2.1 mm Hg SHR-S; 175.8±0.8 versus 176.5±2.1 mm Hg SHR-R). The increase in blood pressure in the SHR-S was similar between the 7 days of high salt diet and the 2 weeks of 1% NaCl in the drinking water. In contrast to 7 days of high salt treatment, renal α2-adrenergic receptors were significantly elevated by high salt treatment in both SHR-S and SHR-R, even though blood pressure changes were seen only in SHR-S. The affinity for \([3H]p\)-aminoclonidine binding was significantly decreased by high salt intake in SHR-R, whereas the increase in \(K_d\) in SHR-S was not statistically significant. Kidney weights were not altered by high salt treatment, and there were no differences between SHR-S and SHR-R in kidney weights.

Experiments were also conducted to determine the effects of 5'-quanylyl-imidodiphosphate (GIMP) on renal α2-adrenergic receptor binding of \([3H]p\)-rauwolscine. \([3H]p\)-Rauwolscine (2 nM) binding in the absence of GIMP was not different between SHR-S \((n=5, 195±12 fmol/mg protein)\) and SHR-R \((n=5, 213±9 fmol/mg protein)\) on high salt diets (3.15% NaCl) for 2 weeks. The presence of 100 μM GIMP during the 45-minute incubation period did not significantly lower the specific binding of 2 nM \([3H]p\)-rauwolscine (SHR-S 184±12 and SHR-R 204±10 fmol/mg protein). GIMP regulation of agonist binding was also examined using \([3H]p\)-aminoclonidine (2 nM). \([3H]p\)-Aminoclonidine binding was not different between SHR-S and SHR-R. The presence of GIMP reduced \([3H]p\)-aminoclonidine—specific binding by 65-

**Figure 1.** Bar graph showing renal norepinephrine (NE) content in kidney of 11-week-old salt-sensitive spontaneously hypertensive rats (SHR-S) and Wistar-Kyoto (WKY) rats on basal or high salt diets. SHR-S on both basal and high salt diets had significantly (*p<0.05) higher renal NE content than WKY rats on same diets. High salt diet significantly (*p<0.05) decreased renal NE content in WKY rats when compared with basal diet.
TABLE 2. Effects of Salt Intake on Renal α₂-Adrenergic Receptors in Salt-Sensitive Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (wk)</th>
<th>n</th>
<th>Kd</th>
<th>Bmax</th>
<th>Kidney wt</th>
<th>BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR-Low (0.05%)</td>
<td>11</td>
<td>5</td>
<td>2.61±0.18</td>
<td>230±27*</td>
<td>0.79±0.02</td>
<td>177.0±4.6*</td>
</tr>
<tr>
<td>SHR-Basal (0.5%)</td>
<td>11</td>
<td>5</td>
<td>2.64±0.20</td>
<td>234±8</td>
<td>0.78±0.02</td>
<td>171.6±3.2*</td>
</tr>
<tr>
<td>SHR-High (3.15%)</td>
<td>11</td>
<td>5</td>
<td>2.64±0.23</td>
<td>241±22</td>
<td>0.83±0.02</td>
<td>199.0±6.2*</td>
</tr>
<tr>
<td>WKY-Low (0.05%)</td>
<td>11</td>
<td>4</td>
<td>2.39±0.13</td>
<td>146±11</td>
<td>0.96±0.04</td>
<td>118.0±2.6</td>
</tr>
<tr>
<td>WKY-Basal (0.5%)</td>
<td>11</td>
<td>5</td>
<td>2.77±0.30</td>
<td>186±30</td>
<td>1.02±0.06</td>
<td>118.0±4.1</td>
</tr>
<tr>
<td>WKY-High (3.15%)</td>
<td>11</td>
<td>5</td>
<td>2.58±0.28</td>
<td>189±12t</td>
<td>1.14±0.09</td>
<td>118.0±5.1</td>
</tr>
</tbody>
</table>

Dissociation constant (Kd) is expressed in nanomoles/liter and maximal number of binding sites as femtomoles bound per milligrams protein±SEM. BP, blood pressure; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

* p<0.05 SHR vs. WKY on the same diet.
† p<0.05 basal (0.5% NaCl) vs. high (3.15% NaCl) salt diet.
‡ p<0.05 low (0.05%) vs. high (3.15%) salt diet.

TABLE 3. Effect of 2 Weeks of High Salt Intake on Renal α₂-Adrenergic Receptors in Salt-Sensitive and Salt-Resistant Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (wk)</th>
<th>Kidney wt</th>
<th>BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap H2O</td>
<td>12</td>
<td>1.42±0.05</td>
<td>176±5</td>
</tr>
<tr>
<td>SHR-R</td>
<td>12</td>
<td>1.43±0.08</td>
<td>179±6</td>
</tr>
<tr>
<td>1.0% NaCl</td>
<td>12</td>
<td>1.76±0.14*</td>
<td>261±21*</td>
</tr>
<tr>
<td>SHR-S</td>
<td>12</td>
<td>1.63±0.07</td>
<td>245±11*</td>
</tr>
</tbody>
</table>

Dissociation constant (Kd) is expressed as nanomoles/liter and maximal number of binding sites (Bmax) as femtomoles bound per milligram protein±SEM. BP, blood pressure; SHR-R, salt-resistant spontaneously hypertensive rats; SHR-S, salt-sensitive SHR.

* p<0.05 tap H2O vs. 1.0% NaCl drinking solution.
† p<0.05 SHR-R vs. SHR-S.

68% (n=3) in SHR-S and SHR-R maintained on the high salt diet for 2 weeks. The blood pressure of the SHR-S maintained on the 3.15% salt diet for 2 weeks was 221.0±14 mm Hg (n=5), whereas that of the SHR-R maintained on the same diet was 202.6±1.0 mm Hg.

Renal norepinephrine content was significantly (p<0.05) greater in the SHR-R when compared with SHR-S when both groups were consuming tap water. Drinking saline for 2 weeks resulted in a significant decline in renal norepinephrine stores (p<0.05) in SHR-S that was comparable with the norepinephrine content of SHR-R drinking either saline or tap water. The decrease in renal norepinephrine in SHR-R was also similar to the decline seen in WKY rats after 7 days of high salt treatment.

Discussion

The present data clearly suggest that in SHR-S the salt-induced rise in blood pressure is dissociated from the salt-induced upregulation of renal α₂-adrenergic receptors. Sripairojthikoon and Wyss have also reported significant elevations of renal α₂-adrenergic receptors in SHR-S after 2 weeks of high salt treatment. Using [3H]p-aminoclonidine binding, they showed renal α₂-adrenergic receptors were increased significantly in both cortex and medulla of SHR-S after 8% NaCl treatment. Blood pressure was also significantly elevated in SHR-S, but neither blood pressure nor renal α₂-adrenergic receptors were elevated in WKY-S, but both blood pressure and α₂-adrenergic receptors were significantly elevated at 2 and 5 weeks of 8% salt treatment. However, the α₂-adrenergic receptor difference was lost after 10 weeks of salt treatment, even though the blood pressure remained elevated compared with SHR-S on basal diets. These data are very interesting in terms of the elevation of α₂-adrenergic receptors in SHR-R that occurred in the

![Figure 2](http://hyper.ahajournals.org/Downloadedfrom)

**Figure 2.** Bar graph showing renal norepinephrine (NE) content in 12-week-old salt-resistant (SHR-R) and salt-sensitive (SHR-S) spontaneously hypertensive rats on either basal diet or basal diet plus 1.0% NaCl in drinking water. High salt intake significantly (p<0.05) decreased NE in SHR-R but not SHR-S. Renal NE content was significantly (p<0.05) lower in SHR-S than in SHR-R on basal diet.
present study after 2 weeks of high salt treatment without any increases in blood pressure. Therefore, high salt intake appears to induce an upregulation of renal α2-adrenergic receptors that results in a significant increase in α2-adrenergic receptor number sometime between 7 and 14 days during the chronic ingestion of high salt diets. This is in agreement with previous data from our laboratory that has demonstrated significant increases in low affinity [3H]p-aminoclonidine binding sites in normotensive Sprague-Dawley rats and SHR after 10 days of high salt treatment without any changes in blood pressure.2 Thus, salt-induced changes in renal α2-adrenergic receptors most likely represent a physiological adaptation to high salt diets and may be involved in facilitating sodium and water excretion induced by catecholamines released from the adrenal medulla.12,15,18 Renal α2-adrenergic receptors are also upregulated by adrenomedullary demedullation, which suggests that they are regulated by circulating catecholamines.19 The effects of high salt diets on renal α2-adrenergic receptors may then represent direct actions of salt on the kidney or indirect actions mediated by changes in circulating catecholamines. The effects of increased salt intake on blood pressure in SHR-S do not appear to be mediated by an increase in renal α2-adrenergic receptors.

Sanchez and Pettinger1 reported significant elevations in renal α2-adrenergic receptors in SHR when compared with WKY rats on normal salt diets. High salt (8%) intake for 2 weeks significantly elevated renal α2-adrenergic receptor number and blood pressure in both the SHR and WKY rats used by Sanchez and Pettinger. SHR-S also had consistently higher (15–20%) numbers of α2-adrenergic receptors than WKY rats at both ages (6 and 11 weeks) examined in this study. In the present study, 1 week of high salt (3.15%) intake was not sufficient to significantly increase renal α2-adrenergic receptor number in either SHR-S or WKY rats. One week of low salt (0.05%) intake also did not alter renal α2-adrenergic receptor number in either SHR-S or WKY rats. There was also no difference in renal α2-adrenergic receptor number between SHR-S and SHR-R on either basal or high salt diets. The increase in α2-adrenergic receptor numbers was seen regardless of whether the animals were given the 3.15% salt diet or WKY rats because their kidneys respond to basal dietary levels of sodium chloride such that renal α2-adrenergic receptors are elevated in SHR compared with normotensive rats remains unanswered; however, the SHR may exhibit aberrant responsiveness to sodium chloride such that renal α2-adrenergic receptors are upregulated even when dietary salt intake is within normal limits.

In summary, renal α2-adrenergic receptors do not seem to play a major role in salt-induced elevations in blood pressure that are seen in SHR-S. Furthermore, in the SHR-S, salt-induced upregulation of renal α2-adrenergic receptors does occur without a concomitant rise in blood pressure. Renal α2-adrenergic receptors can mediate an increase in salt and free water excretion, so their upregulation in response to high salt diets would appear to be a normal physiological adaptation. The question of why renal α2-adrenergic receptors are elevated in SHR compared with normotensive rats remains unanswered; however, the SHR may exhibit aberrant responsiveness to sodium chloride such that renal α2-adrenergic receptors are upregulated even when dietary salt intake is within normal limits.

References


**KEY WORDS** • adrenergic receptors • sodium-dependent hypertension • norepinephrine • spontaneously hypertensive rats
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_Hypertension_. 1990;16:49-54
doi: 10.1161/01.HYP.16.1.49

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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