Altered Renal Alpha₂-Adrenergic Receptor Regulation in Genetically Hypertensive Rats

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SUMMARY Renal α₁ and α₂-adrenergic receptors were quantified in Dahl salt-sensitive and salt-resistant rats, in Okamoto-Aoki spontaneously hypertensive rats (SHR), in Wistar Kyoto "normotensive" (WKY), and in Charles River rats made hypertensive by the Grollman ligature technique and by DOC-NaCl administration after unilateral nephrectomy. The effect of high dietary NaCl on renal α receptors was studied in Dahl, SHR, and WKY rats.

Renal α₁ and α₂ receptor densities were higher (p < 0.05) in SHR and in Dahl salt-sensitive rats than in their normotensive controls. High dietary sodium increased renal α₁ receptors and blood pressure in SHR, WKY and Dahl salt-sensitive, but not in resistant Dahl rats. A study of time relationships revealed that the increase in renal α₁ receptors preceded most of the blood pressure elevation due to high dietary sodium. Renal α₁-adrenergic receptor densities of surgical (Grollman) and endocrine (DOC-NaCl) forms of rat hypertension were not different from normotensive controls. Thus, renal α₁ receptor density and increase thereof by dietary sodium may be: 1) a biochemical marker for genetic forms of hypertension in the rat, and 2) closely linked to the basic mechanism of high blood pressure. (Hypertension 4 (suppl II): II-188-II-192, 1982)

KEY WORDS • dietary sodium • genetically hypertensive rats • renal α-adrenergic receptor regulation • spontaneously hypertensive rats • hypertensive mechanism • genetic regulation of α receptors

INTACT renal sympathetic nerves are essential for the development of high blood pressure in genetic and nongenetic forms of rat hypertension. Sectioning of renal nerves increases urinary sodium excretion. Activation of adrenergic receptors in conscious dogs by renal arterial infusion of norepinephrine induces high blood pressure as long as the infusion is sustained. Thus, α₁-adrenergic receptors have some effect on the kidney that causes high blood pressure, possibly through renal retention of sodium.

Yet excess activity of the sympathetic nervous system, as indicated by plasma norepinephrine levels, is not present in many patients with essential hypertension. Thus, we were interested in the possibility that animal models that are supposedly models of essential hypertension might have altered renal α₁-adrenergic receptors in some way that might mediate an exaggerated response to "normal" sympathetic nerve activity. Once an abnormality was found in spontaneously hypertensive rats (SHR) and Dahl rats, the effect of high dietary sodium on blood pressure and renal α₁-adrenergic receptors was examined in these models. We also studied renal α₁ receptors in two nongenetic forms of rat hypertension.

Methods

Rat Studies

All rats were housed four per cage in a room with constant temperature (22°-25° C) and humidity (50%-60%) and were exposed to light by an automated system from 7 a.m. to 7 p.m. Blood pressure was measured with a programmed electrophygmanometer (Model PE-500, Narco Biosystems, Houston, Texas). The animals were sacrificed by decapitation without anesthesia. Kidneys were removed and rapidly frozen on dry ice and stored at -20°C until assayed.

Dahl, SHR, and Wistar Kyoto (WKY) Rat Studies

Male Dahl salt-sensitive (S) and salt-resistant (R) rats were used. Two groups of 16 rats from S and from

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R lines were fed either high sodium chloride (8% dry weight, ICN Pharmaceuticals, Cleveland, Ohio) or low sodium chloride (0.45% dry weight) diet from weaning until 16 weeks of age. Studies involving SHR and WKY rats were similar to the above except that the “low” sodium rat chow contained 0.6% NaCl by dry weight. The chronology of each experiment is described in the results and figures.

**DOC-NaCl Model**

The left kidney of eight male Sprague-Dawley rats was removed, and 4 days later, DOC-NaCl treatment was initiated. DOC was administered daily by subcutaneous injection of 10 mg/kg body weight in sesame oil. Animals were allowed to drink 1% NaCl solution ad libitum. Control groups included eight nephrectomized rats that received daily injections of the vehicle without DOC in order to follow the change in blood pressure due to growth and distilled water as a drinking source throughout the study.

**Grollman Model**

Sprague-Dawley rats were subjected to one-kidney, figure-8 renal wrap according to the Grollman procedure, and the opposite kidney removed. The sham operation, carried out in 10 rats, consisted of opening and closing the flank on the side of the remaining kidney. Three weeks later the animals were prepared with femoral artery catheters (PE 50) under ether anesthesia. Blood pressure was measured 3 hours later. The rats had access to 0.6% NaCl diet and water ad libitum.

**Radioligand Binding Studies**

Renal plasma membranes were prepared, and binding studies were performed, as described by Williams et al. with modifications by Schmitz et al. using [3H]-prazosin (0.1-4 nM) and [3H]-yohimbine (0.5-40 nM) for construction of Scatchard plots in duplicate. For each assay, 100 μl of renal plasma membranes (protein concentration 2-4 mg/ml) were incubated with the appropriate radioligand for 30 minutes at 25°C in 50 mM NaKPO4, pH 7.4, in a final volume of 150 μl. The net of the incubation, samples were diluted with 5 ml of the above buffer at 4°C and instantaneously filtered through Whatman GF/C glass fiber filters. The filters were then washed with three additional 5 ml aliquots of buffer (4°C), dried, placed in scintillation vials, and counted in 10 ml of triton-toluene aqueous scintillation mixture at a counting efficiency of 38%. All results are expressed in terms of specific binding, which was defined as the binding that was inhabitable by 10 μM phentolamine. For use in the assay, the radioligands were diluted to the appropriate concentrations in 10% ethanol, 5 mM HCl, and 0.2% bovine serum albumin. This diluent did not alter the pH of the incubation mixture or the binding of the radioligands to the renal membranes, but was effective in decreasing the nonspecific binding of the compounds to the plastic tubes used in the assay.

In the lowest concentrations, specific binding was 70%-80% of the total binding for [3H]-prazosin and 45%-60% for [3H]-yohimbine. All experiments were performed in duplicate, and the results represent the mean values of separate studies performed in membrane preparations from seven to nine rat kidneys studied individually. Membrane protein was determined according to the procedure of Lowry et al. with bovine serum albumin as the standard.

**Chemicals**

The drugs used and their source of supply were as follows: [3H]-prazosin was purchased from Amer sham (Arlington, Illinois) and [3H]-yohimbine from New England Nuclear (Billerica, Massachusetts). Phentolamine was a gift from the Ciba-Geigy Corporation (Summit, New Jersey). Deoxycorticosterone acetate (DOC) and yohimbine were obtained from Sigma Chemical Company (St. Louis, Missouri).

**Analysis of Data**

Three-way analysis of variance was done. Factors included: condition (normotensive vs hypertensive); time (2 vs 5 weeks); and dose (0.6% and 8% sodium diet). If significant interactions were found, appropriate multiple comparisons were made using Newman-Keul’s multiple comparison procedure.

**Results**

Renal α1 and α2 receptor densities were higher (p < 0.05) in Dahl salt-sensitive than salt-resistant rats while ingesting a 0.45% NaCl diet (fig. 1). Renal α1 receptor density was increased in salt-sensitive to more than twofold greater (p < 0.001) than in resistant rats. No change occurred in α1 receptors nor in the α2 receptors of salt-resistant rats when ingesting high salt diets. Systolic blood pressure of the four groups is also shown in figure 1. The higher α2 receptor density in Dahl salt-sensitive rats appeared to be independent of blood pressure since blood pressure was nearly the same for all groups (SH > (p < 0.01) SL = RH > (p < 0.05) RL) except for the salt-sensitive rats ingesting the 8% NaCl diet.∗

Renal α1 and α2-adrenergic receptor density was also higher (p < 0.05) in Okamoto-Aoki SHR than in “normotensive” WKY rats ingesting 0.6% NaCl diets (fig. 2). In contrast to the Dahl rats, however, the SHR developed striking elevation of blood pressure while ingesting a normal salt diet, as previously reported. Blood pressure was increased by condition (SHR vs WKY) (p < 0.001), by time (p < 0.001) at 2 and at 5 weeks, and by high dietary sodium (p < 0.01) except for SHR at 2 weeks relative to ingestion of 0.6% NaCl diet. Interestingly, 2 weeks of ingesting the

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*SH = salt sensitive high; SL = salt sensitive low; RH = salt resistant high; RL = salt resistant low.
8% NaCl diet increased renal $\alpha_2$ receptor density further, and shortly thereafter enhanced the blood pressure elevation which persisted throughout the remainder of the 5-week period. The 8% NaCl diet increased renal $\alpha_2$ receptor density even more at 5 weeks.

We expected the WKY normotensive rat to be a good control like the Dahl salt-resistant rat. However, the 8% NaCl diet increased renal $\alpha_2$ receptor density at 2 weeks ($p < 0.001$) and 5 weeks ($p < 0.01$) in these rats (fig. 2). Following the increase in renal $\alpha_2$ receptor density, blood pressure increased impressively ($p < 0.001$) in the WKY rats fed high salt diets. Thus, high blood pressure per se did not cause the dietary salt-related increase in rat renal $\alpha_2$ receptors.

Charles River rats made hypertensive (systolic blood pressure > 170 mm Hg) with the Grollman ligation technique did not have higher renal $\alpha_2$ receptors than sham-operated controls (table 1). Also, rats made hypertensive (systolic blood pressure > 155 mm Hg) using DOC-NaCl had no higher $\alpha_2$ receptor density than their controls (table 2).

![Figure 1](link)

**Figure 1.** Effect of high dietary sodium on blood pressure and renal $\alpha_1$ and $\alpha_2$-adrenergic receptor density of Dahl salt-sensitive (S) and salt-resistant (R) rats. Values are means ± se of eight rats per group.

![Figure 2](link)

**Figure 2.** Effect of high dietary sodium on blood pressure and renal $\alpha_1$ and $\alpha_2$-adrenergic receptor density of spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats (after 2 and 5 weeks on 0.6% and 8% NaCl). Values are means ± se of 10 rats per groups.
TABLE 1. \( \alpha_1 ([^{3}H]-Prazosin) \) and \( \alpha_2 ([^{3}H]-Yohimbine) \) Receptor Number (B<sub>max</sub>) and Affinity Constants (K<sub>D</sub>) for Binding to Renal Plasma Membranes from Grollman WRAP and SHAM Operated Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>( B_{\text{max}} ) (fmole/mg protein)</th>
<th>K&lt;sub&gt;D&lt;/sub&gt; (nM)</th>
<th>( B_{\text{max}} ) (fmole/mg protein)</th>
<th>K&lt;sub&gt;D&lt;/sub&gt; (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>39 ± 6.0</td>
<td>0.49 ± 0.10</td>
<td>186 ± 14.0</td>
<td>22 ± 1.1</td>
</tr>
<tr>
<td>Wrap</td>
<td>49 ± 6.0</td>
<td>0.37 ± 0.04</td>
<td>104 ± 8.0</td>
<td>16 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE from 10 rats per group.

### Discussion

Alpha-adrenergic receptors are functionally classified into \( \alpha_1 \) and \( \alpha_2 \) subtypes.\(^{14}\) The \( \alpha_1 \)-adrenergic receptors mediate resistance changes from sympathetic nerve stimulation and from \( \alpha_2 \)-adrenergic receptor agonists in the isolated perfused kidney.\(^{14}\) The functional role of renal \( \alpha_2 \) receptors has not been directly demonstrated.

In general, \( \alpha_1 \) receptors mediate a suppressive effect on neuroendocrine and other functions by inhibiting adenylate cyclase\(^{21,22}\) and thus reducing cyclic AMP formation. Cyclic AMP in the kidney enhances the flux of sodium and water through the wall of the proximal tubule to the lumen.\(^{24}\) The inhibition of adenylate cyclase by activation of renal \( \alpha_2 \) receptors, if one assumes that this renal nucleotide system is similar to many other organs, may thus increase renal retention of sodium. An excessive activity of renal \( \alpha_2 \) receptors from an abnormally regulated high number could theoretically induce a subtle excessive retention of sodium.

Norman and Guyton,\(^{23}\) Guyton et al.,\(^{2,26}\) and Tobian et al.\(^{27}\) found excess retention of sodium relative to the renal perfusion pressure in hypertensive models. Their theses are that in essential hypertension and hypertensive models blood pressure increases to maintain urinary excretion of sodium. This excess retention of sodium may be under \( \alpha_2 \)-adrenergic receptor control. Steele and Underwood,\(^{28}\) using isolated perfused kidneys from SH and WKY rats, found that norepinephrine was required in the perfusate to demonstrate enhanced renal tubular reabsorption of sodium in SHR kidneys. This observation is consistent with either an \( \alpha_1 \)- or an \( \alpha_2 \)-adrenergic receptor mediated effect.

In normal animals and humans, the sympathetic nervous system clearly enhances proximal tubular reabsorption of sodium.\(^{29-34}\) While there are teleologic inferences as described above, there is no direct pharmacologic evidence that this effect is mediated by \( \alpha_2 \) receptors in the proximal tubule.

From the results above, we can conclude that renal \( \alpha_2 \)-adrenergic receptor density is increased by high dietary sodium in SHR, in Dahl salt-sensitive, and in WKY rats. While chronicologic studies are still required in the Dahl rats, it appears that the increase in \( \alpha_2 \) receptor density antedates the increase in blood pressure. Therefore, it is much more likely that the increased renal \( \alpha_2 \) receptor density causes the high blood pressure than vice versa. Of course, the two may simply be an associated phenomena and not causally related.

Hypertension in the Dahl rat is genetically determined.\(^{35}\) Presence of increased \( \alpha_2 \)-adrenergic receptor density in these strains of rats while they were ingesting normal sodium diets and the additional increase from a high sodium diet suggest that this biochemical abnormality is genetically determined as well. Further studies are required to determine the genetic relationships of these alterations in \( \alpha_2 \)-adrenergic receptor density and blood pressure regulation.

The increase in \( \alpha_2 \) receptor density and blood pressure of WKY rats ingesting a high salt diet raises the question of an appropriate control for SHR. Thus, further studies are indicated in inbred strains of "normal" rats as well as other strains of genetically hypertensive rats.

The increment in the blood pressure of SHR caused by the high sodium diet appears to be proportional to the increase in \( \alpha_2 \) receptor density. This receptor density increment was considerably less, however, than in the Dahl sensitive rat whose hypertension is largely sodium dependent when sodium feeding is started soon after weaning. These correlations are consistent with the previous observations showing that hypertension in the SHR is less salt dependent but not entirely independent of dietary sodium.\(^{36}\) These relationships suggest that sufficient reduction of dietary sodium of the SHR might prevent high blood pressure and high renal \( \alpha_2 \) receptor density in these animals. Alternatively, the differences between genetically hypertensive rat strains may simply be in the position of the dietary sodium dose-response curve for renal \( \alpha_2 \)-adrenergic receptors and blood pressure.

The observations above beg the questions: Does high dietary sodium, by reducing sympathetic tone, suppress plasma norepinephrine and thus permit an increase in renal \( \alpha_2 \)-adrenergic receptors? Is renal sympathetic tone lower in spontaneously hypertensive and Dahl salt-sensitive rats, and does this decrement lead to higher renal \( \alpha_2 \) receptors? Judy et al.\(^{37}\) by direct monitoring of splanchnic sympathetic nerves, found sympathetic tone to be in-

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creased in SHR rather than decreased. In other Dahl male rat studies, we have found no significant differences in plasma norepinephrine in salt-sensitive (high 620 ± 110 and low salt 700 ± 85 pg/ml) and salt-resistant (590 ± 105 and low salt 750 ± 98 pg/ml) rats. Interestingly, we have found significantly (p < 0.05) higher plasma norepinephrine levels in SHR than WKY rats ingesting a normal salt diet, whereas the renal α2-adrenergic receptor density was also elevated (data not shown). Thus, in considering these three observations together, we have no evidence for a simple reciprocal relationship between the sympathetic nervous system activity and renal α2 receptors in these genetically determined hypertensive rat models. In fact, the preponderant data suggest that there is some defect in down regulation of renal α2-adrenergic receptors in the SHR and Dahl salt-sensitive rats with and without high salt diets.

In conclusion, renal α2 and α1 receptor densities are higher in two genetic forms of rat hypertension. This α1 receptor density increases still more with high dietary sodium. These two abnormalities are not present in two other forms of rat hypertension. This effect of high dietary sodium predates most of the blood pressure increase, thus providing more credence to a mechanistic role of α2 receptors in high blood pressure. Prevention of high blood pressure in Dahl salt-sensitive rats by the selective α1 blocker yohimbine lends further support to this thesis.

High concentrations of renal α1 receptors in the Dahl S rat may represent a genetically mediated change, responsible for sodium retention and hypertension. Genetic studies are needed to test this hypothesis.

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