Altered Renal \textit{\alpha}_2-Adrenergic Receptor Regulation in Genetically Hypertensive Rats

WILLIAM A. PETTINGER, M.D., ADELA SANCHEZ, PH.D., M.D., JUAN SAAVEDRA, M.D.,
JAMES R. HAYWOOD, PH.D., TAMAR GANDLER, B.S., AND THOMAS RODES, M.S.

SUMMARY Renal \textit{\alpha}_1 and \textit{\alpha}_2-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 \textit{\alpha}_1 receptors was studied in Dahl, SHR, and WKY rats.

Renal \textit{\alpha}_1 and \textit{\alpha}_2 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 \textit{\alpha}_1 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 \textit{\alpha}_1 receptors preceded most of the blood pressure elevation due to high dietary sodium. Renal \textit{\alpha}_1-adrenergic receptor densities of surgical (Grollman) and endocrine (DOC-NaCl) forms of rat hypertension were not different from normotensive controls. Thus, renal \textit{\alpha}_1 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 • spontaneously hypertensive rats • hypertensive mechanism • genetic regulation of \textit{\alpha}_1 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, 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 \textit{\alpha}-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 \textit{\alpha}-adrenergic receptors was examined in these models. We also studied renal \textit{\alpha}_1 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 electrosphygmomanometer (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
RENAL $\alpha_1$ RECEPTORS IN HYPERTENSION/Pettinger et al.  II-189

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.\(^{11}\) with modifications by Schmitz et al.\(^{14}\) using [H]-prazosin (0.1-4 nM) and [H]-yohimbine (0.5-40 nM) for construction of Scatchard plots in duplicate. For each assay, 100 $\mu$l 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 NaKPO$_4$, pH 7.4, in a final volume of 150 $\mu$l. At the end 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 inhibitable by 10 $\mu$m 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 [H]-prazosin and 45%-60% for [H]-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.\(^{18}\) with bovine serum albumin as the standard.

Chemicals

The drugs used and their source of supply were as follows: [H]-prazosin was purchased from Amercham (Arlington, Illinois) and [H]-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.\(^{14}\)

Results

Renal $\alpha_1$ and $\alpha_2$ 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 $\alpha_1$ receptor density was increased in salt-sensitive rats to more than twofold greater ($p < 0.001$) than in resistant rats. No change occurred in $\alpha_2$ receptors nor in the $\alpha_4$ 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 $\alpha_2$ 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 diets.*

Renal $\alpha_1$ and $\alpha_2$-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.\(^{14}\) 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

*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 ligature 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).
alphareceptor number and affinity constants for 2a, (3H)-prazosin and 3H-yohimbine) receptor number (Bmax) and affinity constants (Kd) for binding to renal plasma membranes from grollman wrap and sham operated rats.

<table>
<thead>
<tr>
<th></th>
<th>[3H]-Prazosin</th>
<th>[3H]-Yohimbine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Bmax (fmole/mg protein)</td>
<td>Kd (nM)</td>
</tr>
<tr>
<td>Sham</td>
<td>39 ± 6.0</td>
<td>0.49 ± 0.10</td>
</tr>
<tr>
<td>Wrap</td>
<td>49 ± 6.0</td>
<td>0.37 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± se from 10 rats per group.

Discussion

Alpha-adrenergic receptors are functionally classified into 1a and 2a subtypes. The 1a-adrenergic receptors mediate resistance changes from sympathetic nerve stimulation and from 1a-adrenergic receptor agonists in the isolated perfused kidney. The functional role of renal 1a receptors has not been directly demonstrated.

In general, 1a receptors mediate a suppressive effect on neuroendocrine and other functions by inhibiting adenylyl cyclase 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. The inhibition of adenylyl cyclase by activation of renal 1a 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 1a receptors from an abnormally regulated high number could theoretically induce a subtle excessive retention of sodium.

Norman and Guyton, Guyton et al., and Tobian et al. 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 1a-adrenergic receptor control. Steele and Underwood, 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 1a- or an 2a-adrenergic receptor mediated effect.

In normal animals and humans, the sympathetic nervous system clearly enhances proximal tubular reabsorption of sodium. While there are teleologic inferences as described above, there is no direct pharmacologic evidence that this effect is mediated by 1a receptors in the proximal tubule.

From the results above, we can conclude that renal 2a-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 2a receptor density antedates the increase in blood pressure. Therefore, it is much more likely that the increased renal 2a 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. Presence of increased 2a-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 2a-adrenergic receptor density and blood pressure regulation.

The increase in 2a 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 2a 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. These relationships suggest that sufficient reduction of dietary sodium of the SHR might prevent high blood pressure and high renal 2a 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 2a-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 2a-adrenergic receptors? Is renal sympathetic tone lower in spontaneously hypertensive and Dahl salt-sensitive rats, and does this decrement lead to higher renal 2a receptors?

Judy et al., by direct monitoring of splanchnic sympathetic nerves, found sympathetic tone to be in-
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 α₁ and α₂ receptor densities are higher in two genetic forms of rat hypertension. This α₂ 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 predominates 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 α₂ blocker yohimbine lends further support to this thesis.

High concentrations of renal α₂ 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.

References

1. Liard JF: Renal denervation delays blood pressure increase in the spontaneously hypertensive rat. Expierienza 33: 339, 1977
Altered renal alpha 2-adrenergic receptor regulation in genetically hypertensive rats.

W A Pettinger, A Sanchez, J Saavedra, J R Haywood, T Gandler and T Rodes

Hypertension. 1982;4:188-192
doi: 10.1161/01.HYP.4.3_Pt.2.188

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/4/3_Pt_2/188