Selective Modification of Renal $\alpha_2$-Adrenergic Receptors in Milan Hypertensive Rat Strain

ANGELO PARINI, LAURENT DIOP, PATRIZIA FERRARI, GIUSEPPE P. BONDIOLOTTI, JEAN-PIERRE DAUSSE, AND GIUSEPPE BIANCHI

SUMMARY Cerebral and renal $\alpha$-adrenergic receptors play an important role in the control of blood pressure. We studied $\alpha$-adrenergic receptors in the cerebral and renal cortex of Milan hypertensive strain (MHS) and normotensive strain (MNS) rats, a genetic model of spontaneous hypertension linked to a kidney abnormality. Binding of the selective $\alpha_1$-adrenergic antagonist [3H]prazosin and the $\alpha_2$-adrenergic antagonist [3H]rauwolscine was used for receptor studies in tissues of prehypertensive (24-day-old) and hypertensive (60-day-old) rats. In the cerebral cortex, no between-strain differences in $\alpha_1$-adrenergic and $\alpha_2$-adrenergic receptor density and affinity were observed in prehypertensive and hypertensive periods. The density of these receptors increased similarly with age in MHS and MNS rats. In the renal cortex, the differences between MHS and MNS rats concerned $\alpha_2$-adrenergic receptors only. Compared with their age-matched normotensive controls, MHS rats showed 1) a lower affinity for the antagonist ($p<0.05$) in the prehypertensive period, 2) absence of the normal age-related increase in receptor density, and 3) a lower density of [3H]rauwolscine binding sites ($p<0.001$) in the hypertensive period. In this period, studies of competitive inhibition of [3H]rauwolscine binding showed that $\tau$-epinephrine bound to one class of sites in MHS rats (pseudo-Hill plot, 0.90) and to two classes in MNS rats (pseudo-Hill plot, 0.68). In addition, the lack of any guanylylimidodiphosphate effect on the $\tau$-epinephrine competition curve observed in MHS rats suggests the uncoupling of these receptors from the guanosine 5'-triphosphate binding protein. The modifications of renal $\alpha_2$-adrenergic receptors were more evident in the hypertensive period when the impaired tubular sodium reabsorption observed in the prehypertensive period had almost returned to normal. Taken together, these data suggest that the modification of renal $\alpha_2$-adrenergic receptors in MHS rats could be a compensatory mechanism to counteract the increased renal sodium reabsorption rather than the cause of the development of hypertension in MHS rats. (Hypertension 10: 505-511, 1987)

KEY WORDS • Milan hypertensive rats • cerebral and renal $\alpha$-adrenergic receptors • [3H]rauwolscine • [3H]prazosin

IT is widely recognized that sympathetic activity directly modulates sodium reabsorption in the renal proximal tubule; however, the class of adrenergic receptors involved in this action is unclear. Previous studies performed in rabbit and dog suggested that $\alpha_1$-adrenergic stimulation is responsible for the increase in tubular sodium reabsorption. Stimulation of $\alpha_2$-adrenergic receptors is thought to induce opposite effects (natriuretic or antinatriuretic) through the inhibition of adenylate cyclase. These effects are not evident at basal levels of sodium excretion; they appear only after modification of the natriuresis by other hormones stimulating adenylate cyclase.

These observations led to the proposal that an abnormality of renal $\alpha_2$-adrenergic receptors can induce hypertension in genetically hypertensive rats and in humans through excess reabsorption and retention of sodium. In agreement with this hypothesis, studies performed on spontaneously hypertensive rats (SHR) and salt-sensitive rats (Dahl, Sabra) showed that the density of renal $\alpha_2$-adrenergic receptors in hypertensive substrains was higher than that in the respective normotensive controls. In addition, a peculiar modification of this receptor subclass under high sodium in-

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take was demonstrated, indicating a selective sodium regulation of renal $\alpha_2$-adrenergic receptors.

Hypertension develops spontaneously in Milan hypertensive strain (MHS) rats, apparently as a consequence of a primary kidney abnormality. Kidney cross-transplantation experiments clearly showed that hypertension can be induced in normotensive controls, Milan normotensive strain (MNS) rats, by transplanting a hypertensive kidney. Renal sodium retention occurs during the development of hypertension in MHS rats, and since MHS rats have a higher glomerular filtration rate than MNS rats have, this sodium retention could be ascribed to a secondary increase in sodium transport across the tubular cells. All of these findings suggest a correlation between $\alpha$-adrenergic receptors and the modification of tubular sodium reabsorption in MHS rats. The present study was performed to characterize the cerebral and renal $\alpha_2$-adrenergic and $\alpha_3$-adrenergic receptors of MHS and MNS rats at prehypertensive and hypertensive ages.

Materials and Methods

Animals

The MHS and MNS rats are descended from two pairs of Wistar-derived rats. The two strains were established by two-way selection for high and low blood pressure and have been inbred in parallel by sister-brother mating (Farmitalia Carlo Erba, Milan, Italy). Male MHS and MNS rats were housed four per cage and maintained on a standard diet (Altromin MT; A Rieper Spa, Vandois, Italy) with average sodium and potassium contents of 2.9 and 73 g/kg, respectively. Animals were allowed free access to food and water from 2 weeks of age up to the moment of the experiment. Experiments were performed in prehypertensive (24 days of age) and hypertensive (60 days of age) periods. Blood pressure was measured in conscious, unrestrained rats by the tail-cuff method with an electronic sphygmomanometer (physiograph MK III; Narco Bio-Systems, Houston, TX, USA).

Materials

$[^3H]$Rauwolscine (specific activity, 79–88 Ci/mmol) was obtained from New England Nuclear (Boston, MA, USA). $[^3H]$Prazosin (specific activity, 20–25 Ci/mmol) was purchased from the Radiochemical Centre, Amersham, Buckinghamshire, Great Britain. The following compounds were obtained from Sigma Chemical (St. Louis, MO, USA): Tris, MgCl$_2$, EDTA, adenosine 5'-monophosphate, sodium $\beta$-glycerophosphate, ammonium, molybdate, 1-amino-2-naphthol-4-sulfonic acid, sodium sulfite, sodium bisulfite, potassium phosphate, bovine serum albumin, epinephrine, guanyllylimidodiphosphate (Gpp[NH]p), ascorbic acid. Phentolamine was supplied by CIBA-Geigy (Basel, Switzerland).

Membrane Preparation

For binding experiments, animals were killed by decapitation without anesthetic between 0900 and 1000. Cerebral and renal cortex were carefully dissected, and fresh tissues were minced and homogenized in 30 volumes (vol/wt) of ice-cold buffer (0.32 M sucrose, 10 mM Tris HCl, 1 mM potassium EDTA, pH 7.4 at 4°C) with a Brinkmann Polytron PT10 homogenizer at Setting 8 (Stamford, CT, USA). The crude cerebral and renal plasma membrane preparations were obtained by differential centrifugations as previously described.

Pellets of membrane preparations were resuspended in incubation Buffer A (50 mM Tris HCl, 50 µM K EDTA, pH 7.4 at 25°C) for $[^3H]$rauwolscine and Buffer B (50 mM Tris HCl, 10 mM MgCl$_2$, pH 7.4 at 25°C) for $[^3H]$prazosin. Protein concentrations were determined by the Lowry et al. method modified from that of Dixon and Purdom, was used as an index of plasma membrane recovery. No significant differences were found in cerebral and renal cortex for plasma membrane yields between MHS and MNS rats in prehypertensive and hypertensive periods (23.71 ± 0.97 vs 23.52 ± 1.92 µg inorganic phosphate/hr/mg protein).

To verify a possible interference of endogenous catecholamines in the binding assay, membrane preparations were treated with NaCl and Gpp(NH)p, which can remove the retained catecholamines through a decrease in receptor affinity for agonist. Membrane preparations were incubated in 50 mM Tris HCl, 150 mM NaCl, 50 µM K EDTA, 100 µM Gpp(NH)p, pH 7.4, at 20°C for 30 minutes. At the end of the incubation the samples were diluted five times with the same buffer lacking NaCl and Gpp(NH)p and then centrifuged at 37,000 g for 15 minutes. The resulting pellet was washed three more times by resuspension and centrifugation in 50 mM Tris HCl, 50 µM K EDTA, pH 7.4 at 25°C. Aliquots of membrane preparations were taken before and after NaCl and Gpp(NH)p treatment for norepinephrine assay by high performance liquid chromatography, as previously described (limit of detection, 0.2 pmol/mg protein). Pellets of membrane preparations were finally resuspended in buffer A or B for binding assay.

Binding Studies

Experiments were performed in triplicate for $[^3H]$prazosin and $[^3H]$rauwolscine. Cerebral and renal plasma membranes were incubated for 30 minutes at 25°C in a final volume of 1 ml (protein concentration, 0.25 ± 0.03 mg/ml) for $[^3H]$prazosin and 0.3 ml (protein concentration, 0.5 ± 0.05 mg/ml) for $[^3H]$rauwolscine assays, with increasing amounts of $[^3H]$prazosin (0.05–3 nM) or $[^3H]$rauwolscine (0.1–40 nM). At the end of the incubation, samples were diluted with the appropriate cold incubation buffer and rapidly filtered through Whatman GF/C filters (Maidstone, Great Britain). The filters were washed three times with 5 ml of incubation buffer at 4°C. The radioactivity retained on the filters was counted at 49% efficiency in 10 ml of Packard toluene scintillator cocktail (Downers Grove, IL, USA). Specific binding was defined as binding inhibited by 10 µM phentolamine and represented...
80% of the total binding for \([^3H]prazosin\) and 70% for \([^3H]rauwolscine\) at concentrations near their respective \(K_d\) values. The amount of nonspecific binding was similar in both prehypertensive and hypertensive periods. When \(\beta\)-epinephrine was used in competition studies, ascorbic acid (free acid) was included to prevent oxidation.

Norepinephrine Assays

The renal cortex norepinephrine concentration was measured in 24-day-old and 60-day-old MHS and MNS rats. Ten animals for each group were used. The rats were killed by decapitation, and after exsanguination, the kidneys were removed and the renal cortex was dissected, weighed, and immediately frozen in liquid nitrogen. Renal cortical slices were homogenized in ice-cold perchloric acid (0.3 M), 30 \(\mu\)l/mg tissue, containing EGTA (2 mg/ml) and MgCl₂ (0.2 mg/ml). Tissue homogenates were centrifuged for 5 minutes at 1200 g. Norepinephrine was measured in 100 \(\mu\)l of the supernatant by a sensitive and specific radioenzymatic method,²⁰ as previously described.²¹

Data Analysis

Data from equilibrium binding and competition studies were analyzed by a computerized, nonlinear, least-squares, curve-fitting procedure using a generalized model for ligand-receptor interaction.²² Statistical analysis comparing goodness of fit between one or two binding sites was obtained by comparing their residual variance (the ratio of the sum of squares of residuals divided by the degree of freedom) of fit to the data by \(F\) statistics. All computations were performed using a LIgAND program adapted for Apple II Europlus computers (Apple Computer, Cupertino, CA, USA). Results were expressed as means ± SEM. Statistical analysis of the data was carried out using the Student’s unpaired \(t\) test. Changes were reported as significant at \(p<0.05\).

Although the term Scatchard analysis is incorrect (it would be better to use the term Rosenthal analysis for binding of macromolecules of unknown molecular weight and concentration), it has been used in our paper for conventional purposes.²³

Results

Blood Pressure

Systolic blood pressure levels were similar in 24-day-old MHS and MNS rats (118 ± 2 vs 115 ± 3 mm Hg; \(n=10\)). Systolic blood pressure starts to rise in MHS rats at 30 days of age and attains the maximum level of 168 ± 5 mm Hg at 60 days²⁴ in comparison with a maximum level of 130 ± 15 mm Hg in MNS rats; therefore, MHS rats can be considered a model of mild genetic hypertension. No evidence of salt sensitivity in MHS rats has been provided, since a high salt diet did not affect significantly the ontogeny of the rise in blood pressure. In addition, no differences in systolic blood pressure values and hypertension ontogenesis were observed by housing the rats one or more per cage.

Binding Assays in Cerebral Cortex

In plasma membranes from cerebral cortex, Scatchard plots of \([^3H]rauwolscine\) binding were curvilinear, suggesting a heterogeneity of binding sites. In all experiments, binding data were more compatible with a model for two affinity binding sites than with a model for one (\(p<0.001\)). Computer analysis of data led to identification of high affinity-low density and low affinity-high density binding sites in both MHS and MNS rats (Table 1). Competitive inhibition studies showed a pharmacological profile typical for \(\alpha_2\)-adrenergic receptors for both the high and low affinity sites, as previously reported.¹⁹ ²⁴

In contrast, the Scatchard plot of \([^3H]prazosin\) binding suggested the presence of only one class of sites in both rat substrains. The binding sites occupied by \([^3H]prazosin\) represented \(\alpha_1\)-adrenergic receptors, as shown by the appropriate rank order of potency of the following antagonists and agonists, respectively: prazosin \(K_d = 0.3 \text{nM}\) exceeded AR-C 239 \(K_d = 11.5 \text{nM}\), which exceeded phentolamine \(K_d = 304 \text{nM}\), which exceeded yohimbine \(K_d = 9000 \text{nM}\); epinephrine \(K_d = 1400 \text{nM}\) exceeded dopamine \(K_d = 132,000 \text{nM}\), which exceeded serotonin \(K_d = 300,000 \text{nM}\).

No differences for density and affinity of \(\alpha_2\)-adrenergic and \(\alpha_1\)-adrenergic receptors were observed between the MHS and MNS rats in the prehypertensive period (see Table 1; Table 2). Since aging induced a

<table>
<thead>
<tr>
<th>Group</th>
<th>(B_1) (fmol/mg)</th>
<th>(B_2) (fmol/mg)</th>
<th>(K_d) (25°C) (nM)</th>
<th>(K_d) (25°C) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prehypertensive 24-day-old MHS (n = 5)</td>
<td>17 ± 4</td>
<td>100 ± 29</td>
<td>1.24 ± 0.13</td>
<td>12.5 ± 1.6</td>
</tr>
<tr>
<td>Prehypertensive 24-day-old MNS (n = 5)</td>
<td>24 ± 6</td>
<td>106 ± 12</td>
<td>1.23 ± 0.08</td>
<td>11.0 ± 1.0</td>
</tr>
<tr>
<td>Hypertensive 60-day-old MHS (n = 5)</td>
<td>42 ± 1*</td>
<td>171 ± 13</td>
<td>1.12 ± 0.10</td>
<td>10.7 ± 0.7</td>
</tr>
<tr>
<td>Hypertensive 60-day-old MNS (n = 5)</td>
<td>45 ± 5*</td>
<td>171 ± 12</td>
<td>1.18 ± 0.08</td>
<td>10.9 ± 0.1</td>
</tr>
</tbody>
</table>

Results are means ± SEM. \(B_1\) = high affinity binding capacity; \(B_2\) = low affinity binding capacity.

<table>
<thead>
<tr>
<th>Group</th>
<th>(B_{\text{max}}) (fmol/mg)</th>
<th>(K_d) (25°C) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prehypertensive 24-day-old MHS (n = 5)</td>
<td>102 ± 4</td>
<td>0.110 ± 0.008</td>
</tr>
<tr>
<td>Prehypertensive 24-day-old MNS (n = 5)</td>
<td>100 ± 10</td>
<td>0.120 ± 0.008</td>
</tr>
<tr>
<td>Hypertensive 60-day-old MHS (n = 5)</td>
<td>161 ± 3*</td>
<td>0.110 ± 0.006</td>
</tr>
<tr>
<td>Hypertensive 60-day-old MNS (n = 5)</td>
<td>151 ± 6*</td>
<td>0.110 ± 0.008</td>
</tr>
</tbody>
</table>

Results are means ± SEM. \(B_{\text{max}}\) = maximum binding capacity.

\(p<0.001\), \(p<0.01\), compared with respective prehypertensive value.
similar increase in receptor density in both rat strains, the absence of receptor differences was maintained in 60-day-old rats.

**Binding Assay in Renal Cortex**

In renal plasma membranes, the Scatchard plots of [3H]rauwolscine (Figure 1) and [3H]prazosin were monophasic, fitting better with a model for one high affinity class of sites than for two. The [3H]rauwolscine binding site appears to be the α2-adrenergic receptor since it was inhibited by several agents in the following order of potency: rauwolscine (Kd = 3 nM), which exceeded prazosin (Kd = 370 nM), which exceeded alprenolol (Kd = 200,000 nM), and clonidine (Kd = 40 nM), which exceeded epinephrine (Kd = 300 nM), which exceeded dopamine (Kd = 80,000 nM). On the other hand, [3H]prazosin binding was inhibited with increasing efficiency by prazosin (Kd = 0.3 nM), phenotolamine (Kd = 25 nM), yohimbine (Kd = 600 nM), alprenolol (Kd = 200,000 nM), epinephrine (Kd = 200 nM), clonidine (Kd = 1500 nM), and dopamine (Kd = 100,000 nM), suggesting that the binding site is the α2-adrenergic receptor. In 24-day-old rats, no significant differences for the binding parameters of [3H]rauwolscine and [3H]prazosin were observed between the two strains, except for a small but significant lower affinity of α2-adrenergic receptors for the ligand in MHS than in MNS rats (p<0.05; Table 3).

In the hypertensive period (60-day-old rats), [3H]prazosin binding capacity was increased in both rat substrains (see Table 3) and any differences concerning α2-adrenergic receptors were observed at this age between MHS and MNS rats. In contrast, the age-related increase in density of α2-adrenergic receptors was observed in MNS (p<0.001) but not in MHS rats (see Table 3). Consequently, in the hypertensive period, the density of renal [3H]rauwolscine binding sites was lower in MHS than in MNS rats (p<0.001).

Compared with the binding data obtained by other authors, the density of renal α2-adrenergic receptors in MHS and MNS rats appeared much lower and the previously described ratio of α2-adrenergic receptors to α1-adrenergic receptors (approximately equal to 2–321,26) was not found. To avoid the possibility that the lower density of α2-adrenergic receptors was related to the techniques used for the membrane preparation and receptor assay, we performed binding studies under the same experimental conditions on Sprague-Dawley rat strains.

Binding capacities of [3H]rauwolscine and [3H]prazosin were 200 ± 10 and 70 ± 5 fmol/mg protein, respectively, with an α2-adrenergic to α1-adrenergic receptor ratio equal to 2.85. These results suggest that the low density of α2-adrenergic receptors in MHS and MNS rats is not artificial but represents a strain characteristic.

Since in our membrane preparations we observed a norepinephrine retention approximately equivalent to 1 pmol/mg protein, we investigated whether the low density of α2-adrenergic receptors in the Milan strain and the differences observed between MHS and MNS rats was an artifact resulting from the retention of tissue catecholamines. This does not seem to be the case because, although the NaCl and Gpp(NH)p treatment decreased the tissue norepinephrine (<0.2 pmol/mg protein) and increased the receptor density in both rat strains, the differences observed in nontreated membranes remained unchanged (MHS, 70 ± 5 fmol/mg protein; MNS, 115 ± 4 fmol/mg protein; p<0.001).

To further characterize abnormalities of renal α2-adrenergic receptors in MHS rats, we performed studies of competitive inhibition of [3H]rauwolscine binding by l-epinephrine in the presence and absence of Gpp(NH)p, a nonhydrolyzable analogue of guanosine 5'-triphosphate. As previously described, guanine nucleotides decrease the affinity for the agonists of receptors linked to the adenylyl cyclase27 through interaction with an inhibitory protein (Gi) coupling the receptor to the cyclase.

Prior to the binding assay, the membrane preparations were treated with NaCl and Gpp(NH)p to remove the tissue catecholamines. Indeed, a previous study showed that concentrations of tissue norepinephrine higher than 0.2 pmol/mg protein most likely affect the antagonist binding parameters by occupying the high affinity site for the agonist.18 This interference should be more evident in experiments of agonist competition for antagonist binding.

In studies of competitive inhibition of [3H]rauwolscine binding by l-epinephrine, computer analysis of the binding data revealed only one class of binding sites for the agonist (Kd = 48 ± 3 nM; n = 4) in MHS rats (pseudo-Hill plot, 0.90) and two classes (high affinity, Kd = 17.9 ± 5 nM; low affinity, Kd = 1503 ± 205 nM; n = 4) in MNS rats (pseudo-Hill plot, 0.68; Figures 2 and 3). Moreover, when competition studies were performed in the presence of Gpp(NH)p, the l-epinephrine curve was shifted to the right only in MNS rats. In this case, experimental data was better fitted.

![Figure 1. Scatchard analysis of [3H]rauwolscine binding to membrane preparations of renal cortex from 60-day-old MHS (○) and MNS (*) rats. Membranes were incubated with various concentrations of [3H]rauwolscine, and specific binding was determined as described in Materials and Methods. The plot represents one of five experiments. B = [3H]rauwolscine bound (in fmol/mg protein); F = [3H]rauwolscine concentration (in nM).](image-url)
Table 3. \(^{3}H\)Prazosin and \(^{3}H\)Rauwolscine Binding Capacities and Affinities in Renal Cortex of MHS and MNS Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>(^{3}H)Prazosin</th>
<th>(^{3}H)Rauwolscine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prehypertensive 24-day-old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHS (n = 5)</td>
<td>29 ± 2</td>
<td>62 ± 3</td>
</tr>
<tr>
<td>MNS (n = 5)</td>
<td>29 ± 4</td>
<td>64 ± 1</td>
</tr>
<tr>
<td>Hypertensive 60-day-old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHS (n = 5)</td>
<td>70 ± 3†</td>
<td>54 ± 3</td>
</tr>
<tr>
<td>MNS (n = 5)</td>
<td>78 ± 10§</td>
<td>85 ± 4†</td>
</tr>
</tbody>
</table>

Results are means ± SEM. \(B_{\text{max}}\) = maximum binding capacity.

\(^*p<0.05, \|^p<0.001\), compared with values in MHS rats.
\(\dagger p<0.001, \|p<0.002\), compared with respective prehypertensive values.

Discussion

The present study demonstrates that \(^{3}H\)Rauwolscine (a selective \(\alpha_2\)-adrenergic antagonist) binds to two distinct classes of binding sites and that \(^{3}H\)Prazosin (a specific \(\alpha_1\)-adrenergic antagonist) binds to one class of sites in the cerebral cortex of MHS and MNS rats. Similar high and low affinity \(^{3}H\)Rauwolscine binding sites, both showing the characteristics of \(\alpha_2\)-adrenergic receptors, have been described in the cerebral cortex of Wistar\(^{24}\) and Sabra\(^9\) rats. Recently, it was reported that \(\alpha_2\)-adrenergic receptors in the cerebral cortex might be involved in the control of blood pressure and in the resistance to sodium-induced hypertension.\(^{11}\) In the present study, the lack of differences in cerebral \(\alpha\)-adrenergic receptors between MHS and MNS rats suggests that these receptors probably are not involved in the development of hypertension in the Milan strain.

We did find differences between MHS and MNS rats in renal \(\alpha_2\)-adrenergic receptors and renal norepinephrine content. Concomitant modification of neurotransmitter and receptors suggests that the \(\alpha_2\)-adrenergic receptor abnormalities might be secondary to the altered tissue levels of norepinephrine (down-regulation). We did not observe any differences between MHS and MNS rats in \(\alpha_1\)-adrenergic receptors, another catecholamine target. In view of the recent proposals that there exists an extrajunctional and a junc-
tion of normal ontogenesis. This phenomenon, specific for α₂-adrenergic receptors and not evident in cerebral cortex, led to a lower receptor density in MHS rats when compared with their normotensive controls in the hypertensive period. In addition, adult MHS and MNS rats differed in their affinity for the agonist and the sensitivity to Gpp(NH)p of α₂-adrenergic receptors. In MNS rats, the competition curve for l-epinephrine was shallow and best modeled to two binding sites with high and low affinity. In the presence of Gpp(NH)p, only a single class of low affinity sites could be detected. This effect of Gpp(NH)p on agonist binding is typical of receptors coupled in an inhibitory or stimulatory manner to the adenylate cyclase and appears to result from a dissociation of the ternary complex of agonist, receptor, and G protein. These data and the previous studies showing an α₂-adrenergic receptor mediated inhibition of cyclic adenosine 3',5'-monophosphate generation in the kidney suggest that these receptors are coupled to adenylate cyclase.

A possible explanation for the absence of the high affinity state for agonist and the lack of Gpp(NH)p effect in MHS rats could be the uncoupling of the α₂-adrenergic receptor from a G protein. In this case, the inhibitory effect of adenylate cyclase activity following the stimulation of α₂-adrenergic receptors could be absent or attenuated. However, further biochemical and functional studies are needed to confirm this hypothesis.

To clarify a possible correlation between the alterations of renal α₂-adrenergic receptors and the abnormalities in sodium handling by the kidney in MHS rats, the following observations are relevant. First, an increased renal tubular sodium reabsorption has been described in MHS rats before and during the development of hypertension in both isolated and perfused kidney and in vivo. This altered tubular sodium handling is likely to be due to a faster sodium transport across the luminal membrane of the proximal tubule and seems to occur across the Na⁺-H⁺ antiport. The functional alteration of MHS proximal tubule is likely to be primary and to be genetically determined according to the results that we have obtained in MHS red blood cells, taken as a model for the renal cells. In fact, MHS red blood cells showed the same morphological and functional abnormalities of tubular cells: smaller volume, lower sodium content, and faster sodium transport across the cell membrane. Moreover, these characteristics are primarily determined in the stem cell of MHS rats and genetically associated with hypertension.

Second, the impairment of tubular sodium reabsorption is most evident in young prehypertensive (24-day-old) rats. On the contrary, abnormalities of α₂-adrenergic receptors in MHS rats have been observed in adult rats, when sodium handling is normalized.

Third, stimulation of α₂-adrenergic receptors in isolated tubular cells has been reported to activate Na⁺-H⁺ exchange. Moreover, α₂-adrenergic receptor activation seems to modulate the action of natriuretic or antinatriuretic factors through the inhibition of adenylate cyclase.

From these findings it appears probable that the modifications of renal α₂-adrenergic receptors are not responsible for the impairment of sodium reabsorption in MHS rats. The lack of normal ontogenesis, leading to a low density of receptors uncoupled from the guanosine 5'-triphosphate–binding protein, could be a compensatory mechanism to counteract the acceleration of the Na⁺-H⁺ exchange in MHS rats.

Previous studies reported that adult SHR and stroke-prone SHR and salt-sensitive rats (Dahl, Sabra) have a higher density of renal α₂-adrenergic receptors than their respective normotensive controls. When compared with the other hypertensive strains, the MHS is the only one showing a lower α₂-adrenergic receptor density. Thus, this rat strain represents an original model for the study of adrenergic involvement in tubular sodium reabsorption and the renal mechanisms involved in the development of hypertension.

In conclusion, our study demonstrates a selective modification of α₂-adrenergic receptors in MHS rats. Although this alteration does not appear to be primarily involved in the development of hypertension in this strain, it could participate in the normalization of renal sodium reabsorption in adult rats. Further biochemical and functional studies in this rat strain are required to clarify the meaning of our results and, perhaps, the mechanisms by which catecholamines modulate tubular sodium reabsorption.

Acknowledgments

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