Sex Influence on Renal $\alpha_2$-Adrenergic Receptor Density in the Spontaneously Hypertensive Rat

Guodong Gong, Asya Dobin, Shari McArdle, Lu Sun, Mark L. Johnson, William A. Pettinger

Abstract Male spontaneously hypertensive rats (SHR) have higher blood pressure than females. We compared renal $\alpha_2$-adrenergic receptor density among intact SHR and Wistar-Kyoto (WKY) rats of both sexes, male and female SHR gonadectomized at 4 weeks of age, and gonadectomized SHR supplemented with testosterone. Additional groups of SHR were treated with enalapril (30 mg/kg per day), an angiotensin-converting enzyme inhibitor, from 5 to 14 weeks of age. Renal $\alpha_2$-adrenergic receptor density was higher in males than females in both SHR and WKY rats. Female SHR and WKY rats had identical low renal $\alpha_2$-adrenergic receptor density. Castration of male SHR reduced the male-female differences in blood pressure and renal $\alpha_2$-adrenergic receptor density by 60%. Treatment with testosterone raised blood pressure and renal $\alpha_2$-adrenergic receptor density to the intact male levels in both gonadectomized males and females. Treatment with enalapril decreased blood pressure but not renal $\alpha_2$-adrenergic receptor density in both male and female SHR. We conclude that (1) both renal $\alpha_2$-adrenergic receptor density and blood pressure are influenced by sex in SHR and WKY, (2) renal $\alpha_2$-adrenergic receptor density like blood pressure is regulated by androgens, and (3) increased renal $\alpha_2$-adrenergic receptor density is not a consequence of high blood pressure in male SHR.

Key Words: sex characteristics • angiotensin-converting enzyme inhibitor • enalapril • receptors, adrenergic, alpha • rauwolscine • rats, inbred SHR

The $\alpha_2$-adrenergic receptors exert important functions, such as regulation of cyclic AMP production, $K^+$ channel opening, and voltage-sensitive Ca$^{2+}$ channel closing. Activation of renal $\alpha_2$-adrenergic receptors regulates renin release, Na$^+$-H$^+$ exchange, and sodium reabsorption through G-protein-mediated inhibition of hormone-stimulated cyclic AMP production. Physiological effects of $\alpha_2$-adrenergic receptors are a function of their density. Thus, altered density of renal $\alpha_2$-adrenergic receptors might be expected to affect salt and water balance and blood pressure. Renal $\alpha_2$-adrenergic receptors are overexpressed in several animal models of genetic hypertension, including the spontaneously hypertensive rat (SHR), salt-sensitive hypertensive rat, Dahl salt-sensitive hypertensive rat, Sabra hypertensive rat, and New Zealand genetically hypertensive rat, when compared with their respective normotensive control strains. The overexpression of renal $\alpha_2$-adrenergic receptors occurs as early as 3 weeks of age in SHR compared with Wistar-Kyoto (WKY) rats, an age when blood pressure is not yet different. High dietary salt further elevates blood pressure, which parallels an increase in the density of these receptors in SHR. This overexpression of renal $\alpha_2$-adrenergic receptors in genetically hypertensive rats probably represents a genetic abnormality because renal $\alpha_2$-adrenergic receptor density is not increased in nongenetic forms of hypertension, such as two-kidney, one clip hypertension; one-kidney, one clip hypertension; and deoxycorticosterone acetate–salt hypertension. However, it remains unresolved whether the increased renal $\alpha_2$-adrenergic receptor density is a cause or a consequence of high blood pressure in genetically hypertensive rats.

Interestingly, not one of the aforementioned studies has investigated the role of a potential difference in renal $\alpha_2$-adrenergic receptor density between male and female SHR. It is known that blood pressure is higher in males than females in rats and humans. Men have higher blood pressure than women before the age of 45 years. This sexual difference in blood pressure is present in genetically hypertensive rats, including SHR and the Dahl salt-sensitive rat as well as their normotensive control strains, WKY rats and the Dahl salt-resistant rat, respectively. Although the sexual dimorphism of hypertension in Dahl salt-sensitive rats is influenced by the presence of the ovaries, it is androgen dependent in SHR. Recently, Ely and Turner and Hilbert et al have demonstrated that high blood pressure is linked to certain genes located on the sex chromosomes (the Y and X chromosomes). The Y chromosome is responsible in part for both hypertension and the sexual dimorphism of hypertension in SHR, with males having more severe hypertension. The hypertensinogenic X chromosome from WKY rats may be responsible for the relatively higher blood pressure of this strain when compared with other normotensive control strains. Ely et al have further shown recently that the testes and androgen receptor play a major role in mediating the effects of the hypertensinogenic Y chromosome.

The exact mechanisms that mediate the effects of sex hormones on blood pressure are not yet clear. Recent studies by Chen and colleagues suggest that the renin-angiotensin system is responsible for the sexual dimorphism of blood pressure in SHR. In addition, $\alpha_2$-adrenergic receptors may potentially play a role. For example, a dose-related vasodilatation to $\alpha_2$-adrenergic (phenylephrine) or $\alpha_2$-adrenergic (clonidine) agonist infusion...
is present in men but not women. Thus, the sexual dimorphism of α2-adrenergic receptor function is also present in humans.

The present studies were designed to test the following: (1) Do male SHR and WKY rats have higher renal α2-adrenergic receptor density in association with higher blood pressure than their respective female counterparts? (2) What influence do the testes and androgens have on renal α2-adrenergic receptor density? (3) Will angiotensin-converting enzyme (ACE) inhibition decrease renal α2-adrenergic receptor density in SHR?

Methods

Animals

Male and female SHR and WKY rats at 4 weeks of age were purchased from Charles River Laboratories. Rats were maintained in 0.63×0.25×0.2 m3 cages (seven to eight rats per cage) at 22°C, 30% to 50% relative humidity, and constant 12-hour light/dark cycle. Rats were provided with tap water and Purina Rat Chow containing 0.71% NaCl or otherwise as specified below. All animal protocols were approved by the University Animal Use and Care Committee.

Experiment 1

This experiment was designed to determine whether a sex difference in renal α2-adrenergic receptor density exists in SHR and/or WKY rats. Twenty rats, 5 males and 5 females of both strains, were studied. Blood pressure was measured indirectly at 9, 13, and 15 weeks of age by use of a tail-cuff sphygmomanometer with a pneumatic pulse transducer connected to a DMP-4B physiograph (Narco Biosystems). The rats were killed at 15 weeks of age. Both kidneys were removed, quickly frozen with dry ice–methanol, and stored at −80°C for later [3H]rauwolscine binding assays as previously described.

Experiment 2

This experiment was designed to determine the role of the testes and/or their products in the expression of blood pressure and renal α2-adrenergic receptor density in male SHR. Ten male SHR were castrated at 4 weeks of age under pentobarbital (50 mg/kg) anesthesia. Sham operations were performed under the same anesthesia on 9 age-matched male SHR as control. Ten female SHR were used as a reference for sex differences. Blood pressure was measured at 8, 12, and 15 weeks of age. The rats were killed at 15 weeks of age. Both kidneys were removed, quickly frozen with dry ice–methanol, and stored at −80°C for later radioligand binding assays.

Experiment 3

This experiment was designed to study the effects of testosterone on blood pressure and renal α2-adrenergic receptor density after gonadectomy in SHR males and females. Seven male and seven female SHR were gonadectomized under pentobarbital anesthesia at 4 weeks of age. Seven male and eight female SHR at 4 weeks of age were gonadectomized and implanted subcutaneously with 20 mm of silicone elastomer (Silastic) tubing (1.57-mm internal diameter, 3.18-mm outer diameter, Dow Corning Co) packed with crystalline testosterone propionate (Sigma Chemical Co) and sealed with Silastic medical adhesive (type A, Dow Corning). Seven male and eight female SHR of the same age were sham-operated and implanted with empty Silastic tubing at the same time as control rats. All capsules were preincubated in phosphate-buffered saline (5% bovine serum albumin, 10 mmol/L Na2HPO4, 0.9% NaCl, 0.001% Merthiolate, pH 7.0) for 24 hours before implantation. Capsules were changed every 4 weeks.

The rats were housed under the same conditions as mentioned above until death at 20 weeks of age.

Experiment 4

This experiment was designed to determine whether a decrease in blood pressure by ACE inhibition would result in a decrease in renal α2-adrenergic receptor density in SHR. Ten male and 6 female SHR were given enalapril (kindly provided by Merck Sharp & Dohme Ltd) in tap water from 5 to 14 weeks of age. The concentration of enalapril solution was adjusted every 2 to 4 days (from 5 to 10 weeks of age) according to body weight and water intake such that the rats would ingest 30 milligrams enalapril per kilogram per day. From 10 to 14 weeks of age they were given 200 mg enalapril per liter of drinking water ad libitum. At 14 weeks of age the rats were provided with tap water instead of enalapril solution for 4 weeks before death. Eleven male and 6 female SHR were given tap water instead of enalapril from 4 to 18 weeks of age as controls. Some of the male rats (5 enalapril-treated and 6 control SHR) were kept for measurement of food and water intake, urinary excretion, and weight of feces. Blood pressure was measured at 9, 14, and 18 weeks of age. Both kidneys were removed, quickly frozen with dry ice–methanol, and stored at −80°C for later radioligand binding assays. Urine Na+ concentration was measured by flame photometry.

Membrane Preparation for α2-Adrenergic Receptor Measurement

Renal plasma membranes were prepared as described previously. Each frozen kidney was thawed, decapsulated, weighed, and homogenized in 20 mL ice-cold 50 mmol/L Tris-HCl buffer containing 5 mmol/L EDTA, pH 7.7. The homogenate was centrifuged at 1700 rpm for 10 minutes (A500 rotor, Du Pont). The pellet was discarded and the supernatant centrifuged at 19 500 rpm for 30 minutes (SS34 Sorvall rotor, Du Pont). The pellet was washed and recentrifuged twice, once in 20 mL of 50 mmol/L Tris buffer (pH 7.7) containing 5 mmol/L EDTA (pH 7.7) and once in 20 mL of 50 mmol/L Tris buffer without EDTA. The final pellet was resuspended in 27.2 mL of 50 mmol/L Tris-HCl containing 1 mmol/L EGTA (pH 7.7) per gram wet kidney weight, resulting in a protein concentration of approximately 2 mg/mL. All steps were conducted at approximately 4°C. Protein concentrations were determined according to the method of Lowry et al using bovine serum albumin as a standard.

Saturation Binding Assay With [3H]Rauwolscine

Renal membranes were incubated in duplicate with 0.5 to 20 nmol/L (six concentrations) [3H]rauwolscine, 50 mmol/L Tris-HCl, 1 mmol/L EGTA, and 0.001% ascorbic acid in a final volume of 0.15 mL at 25°C for 30 minutes. Nonspecific binding was determined in the presence of 100 μmol/L norepinephrine. The incubations were stopped by addition of 5 mL ice-cold 50 mmol/L Tris buffer followed by rapid filtration through Whatman GF/C glass fiber filters with a Brandel Cell Harvester. The filters were then washed with three 5-mL aliquots of ice-cold 50 mmol/L Tris buffer, and the radioactivity retained on the filters was determined with a Beckman 5000TD scintillation counter.

Data Analysis

Data from saturation binding studies were analyzed by generating Rosenthal plots with a nonlinear least-squares curve-fitting procedure using the LIGAND program. Each curve was analyzed with affinity, receptor concentration (Bmax), and non-specific binding ("background") as fitted parameters. Both one- and two-site models were considered for all curves, and the best fit was then determined using F tests. All Bmax values were normalized for protein concentration. Differences among group
means were evaluated using t tests, ANOVAs, and Newman-Keuls tests, whichever was appropriate.

Results

Experiment 1

As shown in Fig 1, males had significantly higher blood pressure than females in both SHR and WKY rats (P<.01). Blood pressure was significantly higher in male or female SHR compared with either male or female WKY rats (P<.01). Renal α₂-adrenergic receptor density was also higher in males than females in both SHR and WKY rats (P<.01) (Fig 1). α₂-Adrenergic receptor density was significantly higher in male SHR than in male WKY rats (P<.01). However, renal α₂-adrenergic receptor density in female SHR was not different than that of female WKY rats (P>.05) and was lower than that of male WKY rats (P<.01). The dissociation constant \( K_d \) of [³H]rauwolscine binding to renal α₂-adrenergic receptors was not significantly different among groups in this and all following experiments (Table).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>n</th>
<th>( K_d ), nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>M</td>
<td>5</td>
<td>3.3±0.33</td>
</tr>
<tr>
<td>SHR</td>
<td>F</td>
<td>5</td>
<td>3.1±0.16</td>
</tr>
<tr>
<td>WKY</td>
<td>M</td>
<td>5</td>
<td>3.3±0.30</td>
</tr>
<tr>
<td>WKY</td>
<td>F</td>
<td>5</td>
<td>2.9±0.20</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rats; and WKY, Wistar-Kyoto rats. Data are mean±SEM; P>.05 for all groups.

Experiment 2

Intact male SHR had a significantly higher blood pressure than castrated male SHR (P<.01), which in turn had a higher blood pressure than female SHR (P<.01) at 12 weeks of age (Fig 2). Castration of male SHR resulted in a 60% decrease in the male-female difference in blood pressure at 12 weeks of age. At 15 weeks of age blood pressure of female SHR rose significantly and was equivalent to that of castrated male SHR. However, intact males still had a higher blood pressure than castrated males or intact females at 15 weeks of age. Renal α₂-adrenergic receptor density was significantly higher in intact male SHR than in castrated male SHR, which had a significantly higher renal α₂-adrenergic receptor density than that of female SHR (Fig 3). Castration of male SHR reduced the male-female difference in renal α₂-adrenergic receptor density by 60%.

Experiment 3

Castration again significantly (P<.01) decreased both blood pressure and renal α₂-adrenergic receptor density in male SHR (Fig 4). Although ovariectomy seemed to increase both blood pressure and renal α₂-adrenergic receptor density, these two parameters were still significantly lower than those of intact males and testoster-
FIG 4. Bar graphs show blood pressure and density of renal \(\alpha_2\)-adrenergic receptors (Bmax) measured at 20 weeks of age in sham-operated female (Fs) and male (Ms) spontaneously hypertensive rats (SHR), gonadectomized (performed at 4 weeks of age) female (Fo) and male (Me) SHR, and gonadectomized female (Ft) and male (Mt) SHR treated with testosterone propionate. **P<.01. Numbers of animals are described in the text. Data represent mean±SEM.

one-treated SHR (P<.01). The levels of blood pressure and renal \(\alpha_2\)-adrenergic receptor density were identical in male and female SHR after gonadectomy. Testosterone treatment in gonadectomized males and females raised both blood pressure and renal \(\alpha_2\)-adrenergic receptor density to the intact male levels (P>.05). Regression analysis of data pooled from the six groups in this experiment showed that blood pressure (BP) correlated well with renal \(\alpha_2\)-adrenergic receptor density (Bmax), giving the following equation:

\[
BP = 142 + 0.18 \times B_{\text{max}} 
\]

\(r = .827, P<10^{-6} \).

Experiment 4

Blood pressure was significantly lowered by enalapril treatment in both male and female SHR compared with their respective control rats (P<.01) as shown in Fig 5. The antihypertensive effect of enalapril was maintained 4 weeks after drug withdrawal through 18 weeks of age in both males and females compared with controls (P<.01). As expected, the untreated control male SHR had significantly higher blood pressure than the untreated control female SHR from 9 to 18 weeks of age. Enalapril treatment did not change the density or affinity of renal \(\alpha_2\)-adrenergic receptors for [\(^3\)H]rauwolscine in either males or females (Fig 6). Renal \(\alpha_2\)-adrenergic receptor density was significantly higher in males compared with females whether treated or untreated with enalapril (P<.01).

Body weight was significantly affected by sex (P<.01), enalapril treatment (P<.05), and age (P<.01, two-way ANOVA) (Fig 7). Although the kidneys were significantly heavier in male than female rats, there was no difference when expressed as a ratio to body weight. Enalapril treatment did not change kidney weight significantly in either males or females (data not shown).

Discussion

We have demonstrated for the first time that higher blood pressure is associated with higher renal \(\alpha_2\)-adrenergic receptor density in male than female SHR and WKY rats. In addition, renal \(\alpha_2\)-adrenergic receptor density is not different between female SHR and WKY rats.
receptors, α₂-adrenergic receptors are not downregulated by high circulating norepinephrine levels in SHR. Thus it is possible that the lower renal α₂-adrenergic receptor density seen in these animals may be due to downregulation of these receptors by high concentrations of epinephrine but not by α₂-adrenergic receptor density. 

Moreover, regression analysis shows that blood pressure correlates well with renal α₂-adrenergic receptor density among intact male, female, and gonadectomized SHR supplemented with or without testosterone. It should be noted that good correlation does not establish a cause-effect relation. However, further studies showed that at least the increase in renal α₂-adrenergic receptor density in SHR is not a consequence of high blood pressure because blood pressure is significantly decreased by ACE inhibition, but renal α₂-adrenergic receptor density is unchanged in both males and females.

An unexpected result in the present studies is that blood pressure was still higher in male than female SHR after 10 weeks of enalapril treatment. This result does not agree with the observation that lifetime treatment was different.16 An early study by Chen and colleagues showed that plasma epinephrine levels were not different among intact, gonadectomized males and females and testosterone-replaced male SHR. Thus it is unlikely that the differences in renal α₂-adrenergic receptor density between sexes are due to a potential difference in sympathetic outflow. A more likely mechanism whereby renal α₂-adrenergic receptor density is increased in male or testosterone-treated animals is that the gene transcription is increased by testosterone, as is the case in adipocytes. Testosterone-replaced male SHR have a higher blood pressure than female SHR with a male SHR kidney. Although they interpret these findings to mean that the sexual dimorphism of hypertension in SHR is not the result of intrinsic renal differences between SHR males and females, we have a different interpretation for their findings as well as ours, as described below.

Sex hormones, especially testosterone, influence blood pressure through the kidney without “intrinsic” renal differences between sexes. For example, renal angiotensinogen, angiotensin,19 and cytochrome P-45032 are expressed as a function of testosterone levels (and androgen receptors).30 Furthermore, renal α₂-adrenergic receptors can be upregulated or downregulated by hormones,25 denervation,33 and dietary salt intake.9 Thus, an SHR female kidney may become functionally a male kidney in terms of blood pressure regulation and renal α₂-adrenergic receptor expression when put in a male environment. During the 10-day period after transplantation, the female kidneys have enough time to functionally become male kidneys in the studies of Harrap et al.31 α₂-Adrenergic receptors can be regenerated in only 3 days after being destroyed.34 On the other hand, in a female hormonal environment, genes responsible for hypertension (such as the SHR Y chromosome) and increased renal α₂-adrenergic receptors may not be expressed when the male kidneys were transplanted into female SHR. Thus, increased renal α₂-adrenergic receptors may still be at least in part responsible for hypertension in SHR in the presence of testosterone.

Ganten and colleagues showed that antiandrogen treatment performed at 25 weeks of age fails to eliminate the sexual dimorphism of blood pressure. Thus, sex hormones may be necessary to initiate the process of sexual differentiation of the kidney in regulating renal α₂-adrenergic receptor density and blood pressure during early life but may not necessarily be responsible for
the maintenance of sexual dimorphism of blood pressure. Once the sex difference in blood pressure is established it may not be readily reversed when hypertension is sustained and its cardiovascular consequences well established in old SHR.26

In conclusion, both renal \textit{\alpha} \textsubscript{2}-adrenergic receptor density and blood pressure are influenced by sex in SHR and WKY rats. Androgens are needed for the full expression of hypertension and increased renal \textit{\alpha} \textsubscript{2}-adrenergic receptor density in SHR. Increased renal \textit{\alpha} \textsubscript{2}-adrenergic receptor density is not a consequence of high blood pressure in male SHR.

Acknowledgments

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References

5. Sanches A, Pettinger WA. Dietary sodium regulation of blood pressure and renal \textit{\alpha} \textsubscript{1}- and \textit{\alpha} \textsubscript{2}-receptors in WKY and SH rats. Life Sci. 1981;29:2797-2802.
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