Sexual Dimorphism of Renal $\alpha_2$-Adrenergic Receptor Regulation in Dahl Rats

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Male Dahl salt-sensitive hypertensive (S) rats develop hypertension faster than females. We measured renal $\alpha_2$-adrenergic receptor density of inbred Dahl salt-sensitive (SS/JR) and salt-resistant (SR/JR) rats, using $[^3H]$-rauwolscine saturation binding studies. Male and female SS/JR rats were gonadectomized or sham-operated at 6 weeks of age and fed a high salt diet for 4 weeks. Additional intact SS/JR and SR/JR rats of both sexes were fed the high salt diet for a longer period of time (7 weeks instead of 4 weeks). Both blood pressure and renal $\alpha_2$-adrenergic receptor density were significantly higher in male than female SS/JR rats on high salt diet for 4 weeks. Gonadectomy did not change blood pressure nor did it change renal $\alpha_2$-adrenergic receptor density measured at the 4th week of high salt feeding in either male or female SS/JR rats. When the SS/JR rats were fed high salt diet for a longer period (for 7 weeks), blood pressure of female SS/JR reached the level of males, but the density of renal $\alpha_2$-adrenergic receptors was still significantly lower than that of males. Both renal $\alpha_2$-adrenergic receptor density and blood pressure were higher in male than female SR/JR. We conclude that higher blood pressure in male Dahl SS/JR and SR/JR rats is associated with higher renal $\alpha_2$-adrenergic receptor density compared with their female counterparts. (*Hypertens Res* 1996; 19: 83-89)

Key Words: receptors, adrenergic, alpha, rauwolscine, Dahl rats

Blood pressure is influenced by sex in mice (1), rats (2), and humans (3). Male spontaneously hypertensive rats (SHR) and Dahl salt-sensitive (S) rats develop more severe hypertension than their female counterparts (4-11). Androgen receptors and male hormones potentiate and estrogen attenuates blood pressure in SHR (4-9). In Dahl S rats, however, the sex difference in blood pressure appears to be solely attributed to the ovaries (11). Ely et al. (4) and Turner et al. (5) have demonstrated that a component of SHR hypertension is linked to the Y chromosome, which is partially responsible for the higher blood pressure in males than in females. They have further identified a sex-influenced component of hypertension associated with the autosomes in SHR (6). Thus, blood pressure is both sex-influenced and sex (Y)-linked trait in SHR. Notably, male normotensive control Wistar-Kyoto (WKY) (4) and SR/JR rats (12) also have higher blood pressure than their respective females. However, the exact mechanisms whereby sex affects blood pressure is not clear. Ellison et al. (13) found that renal angiotensinogen mRNA concentration in the male WKY is higher than females. Castration lowered the mRNA level by 60%. Chen et al. (14) recently found that sex difference in the renin-angiotensin system is present in SHR as well, which may be responsible for the sexual dimorphism of blood pressure in that strain.

Several lines of evidence suggest that $\alpha_2$-adrenergic receptors may be involved in the development of the sex difference in blood pressure. For example, infusion of clonidine, an $\alpha_2$-adrenergic agonist, causes a dose-related vasoconstriction in men but not women (15). Further, we have recently found that male SHR and WKY have higher renal $\alpha_2$-adrenergic receptor density in association with higher blood pressure than their female counterparts (9). Castration decreases both blood pressure and renal $\alpha_2$-adrenergic receptor density by 60%. Testosterone treatment restored these two parameters to the level of intact males (9). We have further demonstrated that testosterone regulates renal $\alpha_2$-adrenergic receptor density at the mRNA level in SHR (16). The difference in $\alpha_2$-adrenergic receptor density may potentially result in functional differences between sexes, since $\alpha_2$-adrenergic receptors mediate many important biochemical and physiological effects. They regulate cAMP production, K$^+$ and Ca$^{2+}$ channels (17, 18), Na$^+$-H$^+$ exchangers and Na$^+$-K$^+$ ATPase activity (19, 20). Activation of renal $\alpha_2$-adrenergic receptors inhibits renin release (21) and promotes sodium reabsorption (19, 20).
The physiological effects of α₂-adrenergic receptors correlate with their density (22). Thus, altered density of renal α₂-adrenergic receptors might be expected to affect salt and water balance, and blood pressure.

However, the mechanisms whereby gender influences the expression of renal α₂-adrenergic receptors and blood pressure may be different in Dahl rats compared with SHR. The present study was designed to test the following hypotheses: (1) There is a sexual difference in the density of renal α₂-adrenergic receptors in SS/JR and/or SR/JR rats. (2) Density of renal α₂-adrenergic receptors will parallel blood pressure after gonadectomy in SS/JR. (3) The pattern of sexual dimorphism of renal α₂-adrenergic receptor regulation is different in Dahl rats from that in SHR (estrogen- vs. androgen-dependent).

Methods

Animals
Inbred Dahl SS/JR and SR/JR rats were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN) before genetic contamination (23) or from our own breeding colony originally provided by Dr. John Rapp.

Experiment 1
This experiment was designed to study the development of sex differences in blood pressure and renal α₂-adrenergic receptor density in SS/JR rats. After weaning at 4 weeks of age, 7 male and 8 female inbred Dahl SS/JR rats were provided with tap water and a rat chow (Purina) containing 1% NaCl for 7 weeks after weaning at 4 weeks of age. They were then gonadectomized under pentobarbital (50 mg/kg) anesthesia. Sham operations were performed on 7 male and 8 female SS/JR rats as control. After operation, these SS/JR rats, both gonadectomized and sham-operated controls, were then provided with normal salt diet (normal salt diet) for 2 weeks. They were then fed high salt diet (high salt diet) for 4 weeks. The resulting supernatant was centrifuged at 19,500 rpm with an SS34 rotor (Du Pont) for 30 min. The pellet was washed in 20 ml of 50 mmol/l Tris buffer containing 5 mmol/l EDTA, and again in 20 ml of EDTA-free 50 mmol/l Tris-HCl buffer by repeating centrifugation at 19,500 rpm with SS34 rotor for 30 min. The pellet was resuspended in 27.2 ml of 50 mmol/l Tris-HCl buffer containing 1 mmol/l EGTA, pH 7.7, per gram kidney wet weight.

Saturation Studies with [³H]Rauwolscine
Renal membranes were incubated in duplicate with 0.4 to 25 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 min. Non-specific binding was determined in the presence of 100 nmol/l norepinephrine. The incubations were stopped by addition of 5 ml of ice-cold 50 mmol/l Tris buffer followed by rapid filtration through Whatman GF/C glass fiber filters. 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. Protein concentration was measured by the method of Lowry et al. (25) using bovine serum albumin as a standard.

Data Analysis
Data from saturation binding studies were analyzed by non-linear least squares curve-fitting of the LIGAND program (26) to determine the best fit and to calculate KD and Bmax. All Bmax values were normalized for protein concentration. Differences among group means of density of renal α₂-adrenergic receptors and blood pressure were evaluated using the Pharm/PCS software (27) to perform the Newman-Kuels’ test and Student’s t-tests.

Results

Experiment 1
Figure 1 shows the time course of blood pressure and body weight in intact and gonadectomized SS/JR rats. Blood pressure was not different after 1 to 2 weeks of high salt feeding between male and female SS/JR rats whether they were gonadectomized or not. Three to four weeks after high salt feeding, the blood pressure of intact male SS/JR rats was significantly higher than that of intact female SS/JR rats (p<0.01). Gonadectomy did not significantly change the blood pressure of male SS/JR rats throughout the study (p>0.05) as compared with intact males. An elevation of blood pressure
Fig. 1. Development of blood pressure and body weight in intact (SS/JR₁) and gonadectomized (SS/JR₀) males and females on high salt diet (1% NaCl in drinking water and Purina chow containing 1% NaCl) for 4 weeks. *p<0.01.

Both intact and gonadectomized male groups had higher renal α₂-adrenergic receptor density than both female groups (p<0.01) (Fig. 2). Renal α₂-adrenergic receptor density was not different between gonadectomized and intact male SS/JR rats, or between gonadectomized and intact female SS/JR rats (p>0.05). KD values were not different among groups (Table 1).

was observed in females on the third week after gonadectomy as compared with intact females (p<0.05). However, gonadectomy of females did not significantly change their blood pressure when compared with intact females at the fourth week of high salt feeding.

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Both body weight and kidney weight were significantly higher in males compared with females in both intact and gonadectomized SS/JR rats (Fig. 1

Table 1. Dissociation Constant (KD) of Dahl SS/JR Rats on High Salt for 4 Weeks

<table>
<thead>
<tr>
<th>Strain and treatment</th>
<th>Sex</th>
<th>n</th>
<th>KD (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS/JR₁</td>
<td>M</td>
<td>7</td>
<td>3.8±0.3</td>
</tr>
<tr>
<td>SS/JR₁</td>
<td>F</td>
<td>8</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td>SS/JR₀</td>
<td>M</td>
<td>7</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td>SS/JR₀</td>
<td>F</td>
<td>8</td>
<td>3.5±0.1</td>
</tr>
</tbody>
</table>

SS/JR₁ and SS/JR₀ = Intact and Gonadectomized Dahl salt-sensitive rats, respectively.
Gonadectomy significantly reduced body weight and kidney weight of males but not females. When expressed as a ratio to body weight, kidney weight was not different between intact males and females or between gonadectomized males and females. Because of increased body weight after ovariectomy, the ratio of kidney weight to body weight was decreased as compared with that of intact females.

**Experiment 2**

One male SS/JR rat died after 6 weeks of high salt diet when its systolic blood pressure was 250 mmHg. Both male and female SS/JR rats had higher blood pressure than male or female SR/JR rats (Fig. 4). Blood pressure of female SS/JR rats reached the level of males after seven weeks of high salt feeding. However, SR/JR males still had higher blood pressure than female SR/JR rats on high salt for the same duration (Fig. 4). Males had higher renal $\alpha_2$-adrenergic receptor density than in females in both SS/JR and SR/JR rats (Fig. 4). Both male or female SS/JR rats have significantly higher renal $\alpha_2$-adrenergic receptor density than both male and female SR/JR rats. $K_D$ was not different among groups (data not shown).

Both body weight and kidney weight were greater in males than in females in either SS/JR or SR/JR rats. Both body weight and kidney weight of SS/JR rats were greater than those of SR/JR rats when rats of the same sex were compared (Fig. 5). However, kidney weight to body weight ratio was not different between sexes in either SS/JR or SR/JR rats. When the same sex was compared, kidney weight was consistently higher in Dahl SS/JR rats than Dahl SR/JR whether expressed as the absolute value or as the ratio to body weight.

**Discussion**

Classic genetic studies by Knudson et al. (28) from Dahl’s group first established the multigenetic nature of hypertension in Dahl salt-sensitive hypertensive rats. They proposed that high blood pressure in this strain is caused by two nonlinked, autosomal, diallelic loci with sex-modified (or influenced) genes. In the present studies we found that renal $\alpha_2$-adrenergic receptor density, like blood pressure, is significantly higher in males versus females in both SS/JR and SR/JR rats fed high salt diet. Thus, renal $\alpha_2$-adrenergic receptor density is a sex-influenced trait in these strains.

Our results show that the sex-influenced component of blood pressure in intact SS/JR rats is manifested 3 to 4 weeks after high salt feeding. A sex difference in renal $\alpha_2$-adrenergic receptor density was also present at the fourth week of high salt diet. Blood pressure of female SS/JR rats reached the level of males after 7 weeks of high salt feeding. However, their renal $\alpha_2$-adrenergic receptor density was still significantly lower than that of males. These findings have several implications. First, it seems that the sex difference in renal $\alpha_2$-adrenergic...
receptor density, seen at the fourth week of high salt diet, is not a consequence of the sex difference in blood pressure levels, since the sex difference in renal \(\alpha_2\)-adrenergic receptor density was not eliminated by the equivalent blood pressure levels between male and female SS/JR rats at the seventh week of high salt diet. Second, the sex difference in renal \(\alpha_2\)-adrenergic receptor density seems to initiate the sex difference in blood pressure but fails to maintain the sex difference in blood pressure. The failure may reflect the fact that there is a upper limit of blood pressure for both sexes, which females will eventually reach. However, renal \(\alpha_2\)-adrenergic receptor density does not seem to have a similar limit. Another possibility is that the sex difference in renal \(\alpha_2\)-adrenergic receptor density is only responsible for the initiation, but not for the maintenance of the blood pressure difference between males and females. At the late stage of hypertension, many target organs, especially the kidney, are damaged (I2) to the extent that the sex difference in blood pressure may be overwhelmed.

Dahl et al. (11) first demonstrated that ovariectomy raised the blood pressure of the Dahl S females to the level of males while orchidectomy did not change blood pressure in male Dahl S rats. We made similar observations in the present study. However, the increase in blood pressure in ovariectomized female SS/JR rats became insignificant as compared with intact females after 4 weeks of high salt diet. In addition, we observed no change in blood pressure of male SS/JR rats after orchidectomy. Similarly, renal \(\alpha_2\)-adrenergic receptor density was also unchanged by gonadectomy in both sexes.

These results suggest that testosterone and/or testes do not play a significant role in the sexual dimorphism of blood pressure and renal \(\alpha_2\)-adrenergic receptor density in SS/JR rats. This is in sharp contrast to SHR, in which sex difference in blood pressure is androgen-dependent (9). Another difference between the two animal models is that both male and female SS/JR had higher blood pressure and renal \(\alpha_2\)-adrenergic receptor density than SR/JR rats of either sex, while only blood pressure, but not renal \(\alpha_2\)-adrenergic receptor density, is higher in female SHR than female WKY (9). This suggest that there are at least two components of hypertension in SHR. Genetic factors other than those regulating renal \(\alpha_2\)-adrenergic receptor density are responsible for the high blood pressure in female SHR vs. female WKY. However, this is not the case for Dahl rats because female SS/JR have a higher renal \(\alpha_2\)-adrenergic receptor density than both male and female SR/JR.

In a previous study we proposed to divide hypertension of Dahl rats into two components, the salt-sensitive and the salt-insensitive increments in blood pressure (24). These two components may represent distinct phenotypes controlled by different genes. In that study, we found that overexpression of renal \(\alpha_2\)-adrenergic receptor in SS/JR was paralleled by the salt-sensitive, but not the salt-insensitive, increments in blood pressure. Both male Dahl SS/JR and SR/JR had higher blood pressure and renal \(\alpha_2\)-adrenergic receptor density than their respective females even on a low salt (0.15% NaCl in the chow plus tap water for drinking) diet (unpublished data), suggesting the sex differences are not salt-dependent in Dahl rats.

Our results of present studies suggest that the inbred Dahl SS/JR rats are different from outbred Dahl salt-sensitive rats in two aspects. First, blood pressure of inbred Dahl SS/JR females reaches the level of males much earlier than that of outbred Dahl salt-sensitive rats (7 weeks vs. 20 weeks on high salt diet). Second, sex influence on blood pressure development also appears to be established earlier in inbred Dahl SS/JR rats, since ovariectomy at six weeks of age failed to eliminate the sex difference of blood pressure, in contrast to outbred Dahl S rats (I2). These characteristics may be consistent with the overall scheme of blood pressure development which is faster in the inbred than the outbred Dahl S rats (I2).

Interestingly, sex differences in renal \(\alpha_2\)-adrenergic receptor density also appear to be established before six weeks of age for a similar reason. The timing of gonadectomy is critical in determining later blood pressure. For example, gonadectomy of SHR performed at 4 weeks of age significantly reduced blood pressure (9, 14), but when performed on older animals it had no effect (29). This suggests that once the sex influence on blood pressure is established, it cannot be reversed by gonadectomy or other procedures such as antiandrogen treatment (30) in SHR. The same is true for renal \(\alpha_2\)-adrenergic receptor density as shown in the present studies. Thus, there appear to be a narrow window of time with which blood pressure and renal \(\alpha_2\)-adrenergic receptor density are influenced by sex in SS/JR rats.

Our results that male SS/JR rats have higher blood pressure than females are not consistent with those of Rapp and Dene who found no sex difference in blood pressure in inbred Dahl SS/JR rats (I2). This may be due to a difference in dietary NaCl content (8% NaCl in the chow in their studies vs. 1% NaCl in the chow plus 1% NaCl in drinking water in our studies), or due to different methods of taking blood pressure (taken under anesthesia in their studies vs. conscious in our studies). A recent study (31) shows that inbred male SS/JR rats have higher blood pressure than female SS/JR rats, supporting our findings.

Clearly, renal \(\alpha_2\)-adrenergic receptor density and blood pressure are similarly regulated in Dahl rats. However, the cause-effect relationship between the two parameters is currently unknown. The physiological and pathophysiological significance of the sex difference in renal \(\alpha_2\)-adrenergic receptor density in Dahl rats is also unclear. However, a growing body of data show that \(\alpha_2\)-adrenergic receptors promote Na\(^+\)-H\(^+\) exchange in proximal and distal tubules (18, 19, 32) in favor of Na\(^+\) reabsorption. Thus, the higher renal \(\alpha_2\)-adrenergic receptor density may be expected to cause higher Na\(^+\) reabsorption, and thus higher circulation volume and higher blood...
pressure in SS/JR vs. SR/JR and Dahl male vs. female rats.

Recently, Simon et al. showed that renal α2-adrenergic receptor class III is linked to systolic blood pressure after salt loading in genetic hypertension (33). Their results have provided strong evidence for a causal relationship between the salt-induced hypertension and the overexpression of renal α2-adrenergic receptors. Interestingly, their results have confirmed our original observation (24) that the increase in renal α2-adrenergic receptor overexpression is linked to the salt-sensitive components of hypertension. Therefore it is reasonable to speculate that higher renal α2-adrenergic receptor density actually causes higher blood pressure in males than in females in Dahl rats.

In conclusion, male inbred Dahl SS/JR and SR/JR rats have higher renal α2-adrenergic receptor density than their female counterparts. The sex difference in renal α2-adrenergic receptor density is not a consequence of sex difference in blood pressure. The sex influence on regulation of both blood pressure development and renal α2-adrenergic receptor density appear to be established before 6 weeks of age in the inbred SS/JR rats.

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References

29. Aoki K: Experimental studies on the relationship between endocrine organs and hypertension in spon-

