Role of the renal nerves in blood pressure in male and female SHR

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Iliescu, Radu, Licy L. Yanes, William Bell, Terry Dwyer, Ovidiu C. Baltatu, and Jane F. Reckelhoff. Role of the renal nerves in blood pressure in male and female SHR. Am J Physiol Regul Integr Comp Physiol 290: R341–R344, 2006.—Female spontaneously hypertensive rats (SHR) have lower blood pressures than males. The renin-angiotensin system plays an important role in the sexual dimorphism of blood pressure in SHR. The sympathetic nervous system can stimulate renin release, and, therefore, the present study was performed to determine whether the renal sympathetic nerves play a role in the sexual dimorphism of blood pressure in SHR. Male and female SHR underwent bilateral kidney denervation or sham surgery, and, 2 wk later, mean arterial pressure (MAP) and pulse interval were recorded, and baroreflex sensitivity (BRS) was measured by the sequence technique. Left ventricle index (LVI) was also calculated. MAP was higher in sham-operated males than females (182 ± 5 vs. 169 ± 4 mmHg; P < 0.01), but, despite the higher MAP in males, LVI was significantly greater in female rats. BRS was not different between sham-operated male and female SHR. Following bilateral renal denervation, MAP was decreased by a similar percentage (8–10%) in males (169 ± 2 mmHg) and females (152 ± 3 mmHg), whereas LVI was reduced only in female SHR. BRS was not altered by renal denervation in either sex. These data indicate that renal nerves play a role in the control of blood pressure in SHR independent of sex, but do not play a role in mediating the sex differences in blood pressure. Baroreflex sensitivity; mean arterial pressure; sexual dimorphism; renal denervation.

ACCUMULATING EVIDENCE SUGGESTS that men are at greater risk for cardiovascular disease than are age-matched, premenopausal women. Also, the incidence and severity of hypertension, a major risk factor for cardiovascular disease, are lower in women than in men (19). The mechanisms responsible for sex differences in blood pressure in men and women are not clear, but indexes of sympathetic nerve activity appear to be lower in women compared with men (14), and this could contribute to sex differences in blood pressure control (7).

As found in human studies, we have previously shown that, in the spontaneously hypertensive rats (SHR), males have higher blood pressure than females (17). We have also shown that the renin-angiotensin system (RAS) plays a role in the sex differences in blood pressure in SHR (17). Converting enzyme inhibition normalizes blood pressure in all SHR, regardless of sex, but also mediates the 25- to 30-mmHg difference in blood pressure between males and females. In addition, androgens are incapable of increasing blood pressure if the RAS is blocked. Extensive evidence points to the renal nerves as a link between the sympathetic nervous system and long-term blood pressure control by the kidneys (8). Activation of the renal nerves stimulates renin release (9). Other investigators have shown that the male SHR has increased sympathetic outflow (3, 20) and renal sympathetic nerve activity compared with normotensive rats (4). Furthermore, renal denervation attenuates hypertension in male SHR (23). However, there have been no studies in which the role of the renal sympathetic nerves in mediating the sex difference in hypertension in SHR has been studied. It is, therefore, possible that a lower sympathetic outflow to the kidney of females may contribute to their lower blood pressure (7).

Baroreceptor reflexes modulate the sympathetic nervous system and act to buffer changes in arterial pressure. Lohmeier et al. (10) demonstrated that a sustained activation of the baroreflex leads to a decrease in blood pressure by reducing renin release. Therefore, differences in baroreflex sensitivity (BRS) could also mediate the sex differences in blood pressure in SHR, with males having a lower BRS than the females and, consequently, higher renal sympathetic outflow and renin release. The male SHR exhibits impaired BRS (5), and, whereas there have been no studies on the BRS in female SHR, Chen and DiCarlo (2) showed that the gain of baroreflex control of heart rate was greater in female than male normotensive rats, and estrogens have been shown to modulate BRS in normotensive rats (13, 18) and mice (6). Therefore, we also measured BRS in male and female SHR to determine whether there is a sex difference in BRS that could mediate sex differences in renal sympathetic activity and ultimately in blood pressure.

The present studies were undertaken to test the hypothesis that the female SHR have lower renal sympathetic activity and greater BRS, which plays a role in the lower blood pressure in female SHR compared with males. To accomplish this goal, male and female SHR were subjected to sham surgery or bilateral renal denervation, steady-state levels of blood pressure were measured, and BRS was calculated by using the sequence technique, allowing evaluation of spontaneous fluctuations in blood pressure and pulse interval (PI) (16). Plasma renin activity (PRA) was also measured in plasma from conscious rats. We anticipated that, if the renal nerves were involved in the sex differences in blood pressure, females would experience a reduced depressor response to renal denervation compared with males and may also exhibit differences in renin release indicated by sex differences in PRA.

MATERIALS AND METHODS

Rats. Male and female SHR (n = 40), 12 wk of age, were obtained from Taconic Farms (Germantown, NY). The rats were maintained on a high-salt diet for 4 wk before the start of experiments. Both male and female SHR have a high-salt diet for 4 wk before the start of experiments. Both male and female SHR have lower blood pressures than their respective age-matched normotensive strains. All rats were housed five to a cage and placed in a temperature-controlled laboratory (22°C) with a 12:12-h light-dark cycle. The rats were fed a diet consisting of 4% fat and 5% protein from Taconic Farms (Germantown, NY). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
standard rat chow (Teklad, Harlan SD, Indianapolis, IN) and tap water in an environment with 12:12-h light-dark cycle. The protocols complied with the Guidelines for the Care and Use of Laboratory Animals by the National Institutes of Health and were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.

**Kidney denervation.** Male and female SHR were used (n = 6 per group). Under 5% isoflurane anesthesia, the right and left renal arteries were exposed via a midline incision, and, with the use of a dissecting microscope, the renal nerves were isolated and cut. To ensure complete removal of nerve fibers, the renal arteries were painted with a 10% phenol in ethanol solution. For the sham operation, renal nerves were identified but left undisturbed.

**Blood pressure measurement and BRS data acquisition and analysis.** The rats were allowed to recover 2 wk after the denervation or sham surgery. In addition, a separate group of male (n = 7) and female (n = 7) SHR did not undergo surgery and was used for the measurement of BRS to eliminate the role of surgical stress on BRS calculations. For measurement of blood pressure chronically, rats were anesthetized at the end of the 2-wk period of recovery from the denervation surgery with isoflurane and instrumented with femoral arterial catheters. After a recovery period of 3 days from the catheter placement, blood pressure was recorded for 2 h in conscious, freely moving rats via a pressure transducer connected to a computerized data-acquisition system (PowerLab, ADInstruments). The blood pressure signal was sampled beat to beat at a frequency of 1,000 Hz.

The baroreceptor heart rate reflex was investigated for the whole 2-h recording, using spontaneous changes in blood pressure and heart rate, according to the method described by Oosting et al. (16). Spontaneously occurring ramps of increasing systolic blood pressure (SBP) of three beats or more were used, and the BRS was calculated as the mean value of the significant slopes Sequences with correlation coefficients of at least 0.85 were used. We used only positive slope values, thereby avoiding contamination of the baroreflex data with nonbaroreflex-mediated changes in PI (21). Sequences with correlation coefficients of at least 0.85 were used. BRS was calculated as the mean value of the significant slopes obtained.

**Measurement of norepinephrine in kidneys and PRA in plasma of SHR.** At the end of the experiment, arterial blood samples were drawn from awake rats for measurement of PRA. The animals were then anesthetized with isoflurane, and kidneys were removed and snap-frozen in liquid nitrogen for measurement of norepinephrine content. Kidneys were homogenized in 0.1-N perchloric acid, and norepinephrine content was measured by HPLC. PRA was measured, as previously described, by radioimmunoassay (10).

**Measurement of left ventricle index.** Because heart hypertrophy could influence the BRS and male SHR have been shown to exhibit cardiac hypertrophy compared with normotensive rats (15), we measured left ventricle (LV) indexes (LVI) (LV weight per 100 g body wt). The heart was removed and blotted dry, and the LV was carefully dissected and weighed.

**Statistics.** Statistical differences were determined with a two-way ANOVA, using GraphPad software. P < 0.05 was considered to be statistically significant. Data are expressed as means ± SE.

**RESULTS**

**Effect of kidney denervation on renal catecholamines, PRA, mean arterial pressure, and PI.** To verify that effective and similar renal denervation was achieved in male and female SHR, renal norepinephrine content was measured by HPLC. Although renal norepinephrine content tended to be higher in sham females than males, the difference was not significant. In both sexes, renal denervation resulted in a 30-fold reduction in renal norepinephrine levels (sham: male, 245.3 ± 16.2; female, 349.6 ± 31.7; denervated: male: 5.1 ± 1.1; female, 10.9 ± 7.3 pg/mg protein; P < 0.0001 denervated compared with sham).

As we have shown previously (17), mean arterial pressure (MAP) was higher in male SHR than females (see Fig. 1), and renal denervation resulted in a similar reduction in blood pressure (8–10%) in both sexes. The PI was not significantly altered by renal denervation in either male or female rats (157.2 ± 7.8 vs. 142.8 ± 2.8 and 141.7 ± 2 vs. 138.2 ± 4.4 ms, sham-operated vs. denervated, male and female SHR, respectively, P > 0.1).

PRA was also similar in sham rats (male: 4.4 ± 0.9; females: 5.4 ± 1.0 ng ANG I·h⁻¹·ml⁻¹), and, whereas denervation tended to decrease PRA in both sexes, the differences did not reach statistical significance (male denervated: 2.6 ± 0.3 ng ANG I·h⁻¹·ml⁻¹, P = 0.06; female denervated: 2.8 ± 0.4 ng ANG I·h⁻¹·ml⁻¹, P = 0.09).

**BRS measurement in male and female SHR.** Just as there were no sex differences in the depressor response to renal denervation or PRA, there were also no significant differences in the BRS found between sexes or sham vs. denervation, as calculated from the average of upslopes of SBP for a delay of three, four, and five beats (male sham: 1.16 ± 0.16; male denervated: 1.11 ± 0.06; female sham: 1.24 ± 0.12; female denervated: 1.14 ± 0.1 ms/mmHg). BRS was also measured in a separate group of male and female SHR (n = 6) that did not undergo renal denervation surgery (male: 1.14 ± 0.11; female: 1.104 ± 0.08 ms/mmHg). There were no differences in the BRS between untreated and sham-operated rats, excluding the possibility that the extensive surgical procedure might have altered the blood pressure control mechanisms.

**Measurement of LVI in sham and denervated rats.** As shown in Fig. 2, female SHR had significantly higher LVI than did males. With renal denervation, LVI decreased in female SHR, but not in males.

Fig. 1. Effect of renal denervation on mean arterial pressure (MAP) in male and female spontaneously hypertensive rats (SHR) (n = 8/group). Sham, sham operation; den, renal denervation. *P < 0.05, female sham vs. male sham; †P < 0.05, renal denervation vs. sham.
DISCUSSION

The main findings of the present study are as follows. 1) Removal of renal nerves reduces blood pressure by similar percentages in male and female SHR. This finding was contrary to our hypothesis that females would have an attenuated depressor response to renal denervation, suggesting they have lower blood pressure than males due to reduced sympathetic nerve activity. Therefore, differences in renal sympathetic outflow are not likely to play a role in the sex difference in blood pressure found in SHR. 2) Despite lower blood pressure, LVI is higher in sham females than males, and, although the percentage reduction in blood pressure between males and females was similar, renal denervation reduced LVI in females only. 3) BRS is similar in male and female SHR.

In the past few years, our laboratory has been studying the mechanisms that are responsible for the sex differences in the control of blood pressure by using the SHR as a model. We have shown previously that the RAS plays a major role in the sex differences in blood pressure in SHR, as converting enzyme inhibition removes the sex difference and also prevents androgens from being able to increase blood pressure (17). These data suggest that androgens in the male increase blood pressure by stimulating the RAS and that, whereas the RAS activates renin release response to decreases in blood pressure. LVI is higher in sham females than males, and, although the percentage reduction in blood pressure between males and females was similar, renal denervation reduced LVI in females only. 3) BRS is similar in male and female SHR.

Estrogens have been shown to modulate BRS in normotensive rats and mice. For example, Chen and DiCarlo (2) showed that the gain of baroreflex control of heart rate was greater in female than male normotensive rats. Furthermore, estrogens have been shown to stimulate BRS in normotensive rats (13, 18), and ovariectomy impairs BRS in mice (6). However, because BRS was similar between male and female SHR, estrogen may not have the same effect on BRS in female SHR as in normotensive rats. The lack of an estrogen effect is also reflected in the lack of an effect on blood pressure with ovariectomy in female SHR (17). In contrast, castration of male SHR attenuates the hypertension in males. Since BRS was not different between male and female SHR, the data suggest that sex steroids do not play a role in control of BRS in these hypertensive rats.

In summary, in the present study, we found that the renal nerves contribute similarly to the hypertension in male and female SHR, but do not mediate the sex differences in blood pressure. LVI was higher in female SHR than males, and renal denervation resulted in a reduction in LVI only in females. BRS was similar in male and female SHR and was not affected by renal denervation. These findings play an important role in our studies into the mechanisms responsible for the sex differences in control of blood pressure in SHR and rule out a causative role of the renal nerves in mediating the sex difference.

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