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. First published September 15, 2005; doi:10.1152/ajpregu.00035.2005.—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.

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 mediate 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

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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 slope of the linear regression between the SBP and the subsequent PI. Measurements were made at a delay of three, four, and five beats, because baroreflex-related changes in PI become apparent after a delay of three to five beats following a pressure change (16). 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. We used only positive slope values, thereby avoiding contamination of the baroreflex data with nonbaroreflex-mediated changes in PI (21). Measurements were made at a delay of three, four, and five beats, because baroreflex-related changes in PI become apparent after a delay of three to five beats following a pressure change (16). We used only positive slope values, thereby avoiding contamination of the baroreflex data with nonbaroreflex-mediated changes in PI (21).

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 1·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 1·h⁻¹·ml⁻¹, P = 0.06; female denervated: 2.8 ± 0.4 ng ANG 1·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 plays a role in the hypertension in all SHR independent of sex, the difference in blood pressure between males and females (25–30 mmHg) is also mediated by the RAS. The present study was a natural extension of these studies, because the activation of the sympathetic nervous system has been shown to stimulate renin release (9). It is also known that the pattern of sympathetic outflow to various target organs is not uniform (11) and might display sex differences (7). We hypothesized then that the female SHR might be protected from hypertension by an attenuated renal sympathoexcitation compared with male SHR, in part by reduced renin release. We reasoned that, if the renal sympathetic nerve activity is lower in female than in male SHR, the decrease in blood pressure after renal denervation should be less pronounced in female rats. As expected (23), we found that renal denervation decreased blood pressure in male SHR. However, the decrease in blood pressure after renal denervation was similar in SHR, independent of the sex of the animals, and PRA was similar between the groups before and after denervation. The data suggest that the renal nerves contribute equally to the hypertension in male and female SHR and thus probably do not contribute to the sex differences in blood pressure. Our data do not provide a mechanism by which renal denervation decreases blood pressure in SHR, as this was not the aim of the study. A reduction in PRA with denervation could have played a role, but we were not able to show statistically significant reductions in PRA in sham animals compared with denervated rats. However, PRA levels tended to be lower in denervated animals, which might indicate a blunted renin release response to decreases in blood pressure.

Cardiac hypertrophy can cause reduced BRS in hypertension (6) and is a commonly recognized feature in the male SHR compared with normotensive males (15). However, despite their lower blood pressure levels, we found that female SHR displayed more pronounced LV hypertrophy than did male SHR, as has been previously described (22). In addition, renal denervation resulted in a significant reduction of LVI only in female SHR, although the blood pressure was similarly decreased by the maneuver in both sexes. This effect occurred without alterations in BRS and thus does not support previous studies indicating that cardiac hypertrophy is associated with an alteration in BRS (1, 18). The mechanism then for the reduction in LVI with renal denervation in female SHR is not clear from the present study. We have found previously that a reduction in blood pressure by other means (i.e., antioxidants) reduces LV heart weight in female SHR, but not in males (Yanes LL, Iliescu R, and Rechelhoff JF, unpublished observations). We hypothesize then that the reduction in LVI in female SHR in the present study is mediated by the reduction in blood pressure, although an effect of the renal denervation itself cannot be ruled out.

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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