Role of Female Sex Hormones in the Development and Reversal of Dahl Hypertension

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Abstract—Female sex hormones protect against the development of Dahl hypertension mediated by increases in dietary sodium. The role of female sex hormones in the reversal of Dahl hypertension mediated by decreases in dietary sodium is unknown. The goal of this study was to identify sex differences in the reversal of Dahl hypertension and the associated changes in water and electrolyte homeostasis. Male (M, n=8), female (F, n=8), and ovariectomized female (OVX, n=9) Dahl salt-sensitive rats were instrumented with an abdominal radiotelemetry device for 24-hour monitoring of blood pressure (BP) and heart rate. Daily measurements of food intake, water intake, and urine output were recorded as diet was changed from a low-sodium diet (0.15% NaCl) to a diet containing 8% NaCl. The diet was then changed back to 0.15% NaCl. The responses to changes in the salt diet were compared with responses observed in rats (M, n=4; F, n=4; OVX, n=4) that were maintained on 0.15% NaCl during the experiment. Sex differences in BP were observed when M, F, and OVX rats were fed 8% NaCl diet for 2 weeks (152±4, 141±3, and 154±5 mm Hg, respectively). BP was significantly greater (P<0.05) in M and OVX rats than in F rats. Fluid balance (water intake minus urine volume) and sodium balance (sodium intake minus sodium excretion) were similar in all groups on the 8% NaCl diet. BP in time-control M, F, and OVX rats was 121±3, 130±4, and 162±11 mm Hg, respectively. Compared with time-control groups, differences in BP while rats were eating the 8% NaCl diet were observed in M and F rats but not OVX rats. Reinstatement of an NaCl-deficient diet reversed the hypertension in M and F but not OVX rats (124±4, 124±2, and 145±5 mm Hg, respectively). The changes in dietary sodium caused similar changes in renal handling of sodium and water in all groups of rats; therefore, the effect on blood pressure was independent of renal excretory function. The inability to reverse the hypertension by decreasing sodium intake in OVX rats and the development of spontaneous hypertension in OVX females maintained on a low-sodium diet indicates that removal of the female sex hormones predisposes the animal to the development of hypertension that is sodium independent. We conclude that female sex hormones protect Dahl S rats against the development of sodium-dependent and -independent hypertension. (Hypertension. 2000;35[part 2]:484-489.)

Key Words: hypertension, sodium-dependent □ salt sensitivity □ hormones

The relationship between salt intake and blood pressure has been extensively studied in humans and provides clear evidence that a subpopulation of humans are sensitive to alterations in salt intake. Salt sensitivity is usually identified by a reduction in blood pressure (5% to 10%) in response to a decrease in sodium intake or a rise in blood pressure (5% to 10%) in response to an increase in sodium intake. Although the prevalence of hypertension in premenopausal women is lower than in men of the same age, the incidence of hypertension in women doubles after menopause, which suggests that female sex hormones protect against the development of hypertension. The increased incidence of hypertension in males and postmenopausal females appears to be related to a greater sodium retention or blood pressure response to sodium.

Many of the physiological characteristics of salt-sensitive human hypertension have been observed in the Dahl salt-sensitive (Dahl S) rat. Dahl salt-sensitive hypertension is a genetic form of hypertension, which develops when salt-sensitive animals are fed a high-sodium diet. The kidneys of salt-sensitive rats have been implicated in the development of this hypertension; studies have revealed that salt-resistant (Dahl R) rats developed hypertension after they received a transplanted kidney from a salt-sensitive animal. Conversely, when kidneys from Dahl R rats were transplanted into Dahl S rats, hypertension was abolished. Additional studies of renal function in prehypertensive Dahl salt-sensitive rats indicate that although glomerular filtration rate is not different between Dahl S and R rats, Dahl S rats have an impaired ability to excrete sodium. The slope of the pressure-natriuresis relationship is less in Dahl S rats than in Dahl R rats.

Sex hormones have also been shown to play a role in the development of Dahl S hypertension. When male and female

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Dahl S rats were fed an 8% NaCl diet, female rats became less hypertensive than male rats.\textsuperscript{12} Gonadectomy did not affect the development of hypertension in the male rats;\textsuperscript{12}, however, gonadectomy in female Dahl S rats resulted in an accelerated development of hypertension to a level that was not different from male Dahl S rats.\textsuperscript{12–14} In addition, the increase in salt sensitivity after ovariectomy is associated with a blunted pressure-natriuresis relationship.\textsuperscript{14} These findings suggest that the female sex hormones protect against the development of Dahl hypertension, possibly by augmenting renal excretion of sodium.

Although increasing dietary sodium has been clearly shown to cause hypertension in Dahl S rats, the effects of decreasing dietary sodium on the reversal of hypertension are not well documented. The goal of the present study was to ascertain the blood pressure response to the stepwise increase in salt intake, as well as to determine the effectiveness of a reduced sodium diet in decreasing blood pressure in hypertensive Dahl S rats. The hypothesis was that sodium would cause a reduced hypertension in intact females, whereas males and ovariectomized females would demonstrate an overt hypertensive response. Conversely, a decrease in dietary sodium would cause a more complete reversal of hypertension in intact female Dahl S rats than in male rats. The recovery of blood pressure in the intact females was expected to be mediated by a more effective excretion of sodium.

Methods

All experimental protocols were approved by the University of Texas Health Science Center (UTHSC) Animal Care and Use Committee. Animals were treated in accordance with the American Physiological Society’s “Guiding Principles for the Use of Animals in Research and Teaching.” The UTHSC laboratory animal facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Age-matched (6 to 7 weeks old) male and female Dahl S rats (DS/JR strain) were purchased from Harlan Sprague Dawley, Inc (Indianapolis, Ind). These rats were fed a sodium-deficient diet (0.15% NaCl) on weaning that was maintained after arrival at the animal care facility. Rats were housed in temperature-controlled rooms with a constant light/dark cycle (14/10 hours). Vaginal smears were performed daily to monitor phases of the estrous cycle in female rats. A group of female rats underwent ovariectomy during gaseous anesthesia (Metofane, Mallinckrodt Veterinary). The ovaries were exposed via bilateral flank incisions and excised. Ovariectomy was confirmed by the cessation of the estrous cycle. The animals were allowed to recover for 10 days before radiotelemetry transmitters were implanted.

Under gas anesthesia (Metofane, Mallinckrodt Veterinary), rats were implanted with an abdominal aortic catheter attached to a CA111PA-C40 radiotelemetry transmitter (Data Sciences). The transmitter was secured to the abdominal muscle and remained in the abdominal cavity for the duration of the experiment. Rats were placed in individual metabolic cages equipped with an RLA-3000 radiotelemetry receiver. Systolic, diastolic, and mean arterial pressure (MAP) and heart rate (HR) were monitored continuously. The blood pressure and HR measurements obtained during a 10-second sampling period (500 Hz) were averaged and recorded every 10 minutes.

Two weeks after transmitter implantation, MAP, HR, body weight, food intake, water intake, and urine volume were recorded daily while male (n=8), female (n=8), and ovariectomized (OVX) female (n=9) rats were subjected to a series of increases in dietary sodium content. These parameters were recorded while the animals were fed 0.15% NaCl (7 days), then a standard laboratory rat diet containing 1% NaCl (14 days), then a 4% NaCl diet (14 days), followed by an 8% NaCl diet (14 days), and finally a reduction in dietary sodium back to 0.15% NaCl (14 days). Similar measurements were made in separate groups of male (n=4), female (n=4), and OVX female (n=4) rats that were fed a low-sodium diet (0.15% NaCl) throughout the course of the experiment. Sodium content in food and urine samples was measured with a NOVA-1 sodium/potassium analyzer (NOVA Biomedical).

Data are expressed as mean±SEM. The changes in MAP, HR, body weight, fluid intake, urine volume, fluid balance, sodium intake, urinary sodium excretion, and sodium balance were analyzed with a 3-way ANOVA with repeated measures. The effects of gender, diet, and time on each of these parameters were determined. If significant group effects were observed, then the specific differences between groups were determined with 2- and 1-factor repeated-measures ANOVA and the Student-Newman-Keuls post hoc test. All data were analyzed with StatView software (SAS Institute, Inc).

Results

Body weight was measured daily in all groups. Figure 1 shows that male rats weighed more than both groups of females (P<0.001), whereas OVX rats weighed more than intact females (P<0.001). All animals gained weight during the time course of the study in a similar pattern that was independent of their diets.

The effects of changing dietary NaCl on MAP and HR were recorded in males, females, and OVX rats (Figure 2). These effects were compared with the level of MAP and HR recorded
in similar groups of control animals maintained on a low-NaCl diet during the entire study (Figure 3). Male rats maintained on low-sodium diet (Figure 3) demonstrated a gradual increase in MAP (7±3 mm Hg) during the study that was not statistically significant. When males were fed increasing amounts of dietary sodium (Figure 2), MAP increased significantly (P<0.001). After 2 weeks on the 8% diet, MAP was 152±4 mm Hg compared with 121±3 mm Hg in low-salt–fed controls. HR in the male rats decreased during the experiment in salt-fed and time controls; however, the decrease in HR was significantly greater in the hypertensive rats (P<0.001). When the sodium diet was decreased from 8% to 0.15% NaCl in the males, MAP decreased to 124±2 mm Hg, which was not different from the time-control group.

Blood pressure in female rats maintained on a constant low-NaCl diet (Figure 3) was significantly (P<0.001) increased (by 16±5 mm Hg) by the end of the study. This level of MAP was significantly greater than that observed in males on the low-NaCl diet (133±6 and 122±3 mm Hg, respectively). In response to increases in dietary sodium (Figure 2), female Dahl S rats responded with an increase in MAP and a decrease in HR (P<0.001). After 2 weeks on the 8% diet, MAP was 141±3 mm Hg compared with 130±4 mm Hg in low-salt–fed controls. Compared with males, females demonstrated a significant attenuation in the level of Dahl S hypertension (P<0.001). When the sodium diet was decreased from 8% NaCl to 0.15% NaCl, the females responded similarly to males by decreasing MAP (124±2 mm Hg) and increasing HR to levels that were similar to those measured in low-salt–fed time controls.

The pattern of hemodynamic effects of the changing salt diet in OVX rats (Figure 2) showed an increase in MAP and...
decrease in HR similar to that observed in male and female rats; however, the level of MAP at the end of the 8% NaCl diet (154±5 mm Hg) was not different from that observed in males (152±4 mm Hg) but was significantly greater than in intact females (141±3 mm Hg). When the 8% NaCl diet was replaced with the 0.15% NaCl diet, it caused a transient decrease in blood pressure, but this effect was not statistically different from blood pressure in time-control rats. Interestingly, we observed a significant increase in MAP in low-salt–fed OVX females (Figure 3). At the end of the study, MAP in time-control animals was significantly greater in OVX females than in males and intact females (170±9, 123±4, and 133±6 mm Hg, respectively).

The level of dietary sodium content was reflected in the corresponding measurements of sodium intake and urinary sodium excretion in the groups subjected to changes in NaCl diet (Figure 4). All high-salt–fed animals increased sodium intake and excretion proportionately such that sodium balance did not change during alterations in dietary NaCl content. In addition, there were no differences in sodium balance between males, females, and OVX females. A cyclic pattern was observed in sodium intake and excretion in intact females when fed 8% NaCl that was a result of a significant decrease in food intake during the estrous phase of the estral cycle. Similar results were observed in water intake and urine volume (Figure 5). Male, female, and OVX females increased water intake and urine volume in proportion to the level of dietary sodium. Therefore, fluid balance was maintained in all salt-fed rats during changes in diets, which was not different between groups. In low-NaCl–fed control groups, sodium and water balance also remained unchanged during the study (data not shown).
Discussion

The goal of this study was to evaluate sex differences in the development and reversal of sodium-dependent hypertension in Dahl S rats and the associated responses in water and sodium handling. We observed that the development of sodium-dependent hypertension in Dahl S rats was significantly attenuated in females compared with males (141±3 and 152±4 mm Hg, respectively). OVX females fed a diet high in salt developed hypertension (154±25 mm Hg) that was similar to males. Therefore, ovariectomy abolished this gender difference. When dietary NaCl was decreased from 8% to 0.15%, blood pressure was normalized in male and female rats but not OVX rats. The changes in dietary sodium caused similar changes in renal handling of sodium and water in all groups of rats; therefore, the effect on blood pressure was independent of renal excretory function. These data do not support our hypothesis that the reversal of hypertension in response to a decrease in dietary sodium in female Dahl S rats would be more effective than that observed in male rats. However, the inability to reverse the hypertension by decreasing sodium intake in OVX rats indicates that removal of the female sex hormones predisposes the animal to the development of hypertension, which is sodium independent. The development of hypertension in ovariectomized rats maintained on a low-NaCl diet clearly supports this hypothesis.

This study provides evidence that hypertension in Dahl S rats is a result of both sodium-dependent and -independent mechanisms. As with sodium-dependent hypertension, sex differences were observed in the development of sodium-independent hypertension. We observed a significant increase in blood pressure in Dahl S females rats (16±5 mm Hg) maintained on a low-NaCl diet, but not in male rats (7±3 mm Hg). OVX rats maintained on a low-NaCl diet demonstrated a greater increase in blood pressure (46±9 mm Hg) than intact female or male rats. Thus, ovariectomy exaggerated the gender difference in the development of sodium-independent hypertension. We conclude that female Dahl S rats are less sensitive to sodium-dependent hypertension but more sensitive to sodium-independent hypertension than males. This is supported by the observation that compared with male rats, female Dahl S rats developed a lower level of hypertension in response to increases in dietary NaCl but a higher level of hypertension when maintained on a low-NaCl diet. Importantly, the female sex hormones protected animals against the hypertensive effects of both sodium-dependent and -independent mechanisms. We speculate that removal of sex hormones selectively activates the mechanism(s) responsible for sodium-independent hypertension in female Dahl S rats.

Hypertension in Dahl S rats appears to be a result of a combination of neural and humoral mechanisms, which can affect vascular resistance and/or renal excretory function. Three mechanisms, which have been proposed to contribute to the development of Dahl hypertension and can also be affected by female sex hormones, are the sympathetic nervous system, the renin-angiotensin system, and nitric oxide. The contribution of sympathetic neural mechanisms has been demonstrated clearly in Dahl S hypertension during elevated sodium intake. Studies have shown that the prehypertensive Dahl S rat on a low-sodium diet had impaired baroreflex-mediated inhibition of sympathetic nerve activity resulting in an abnormal buffering response to hypertensive stimuli. However, with increased sodium intake, the sympathetic nerves contribute to the elevated vascular resistance in the skeletal muscle of Dahl S rats and to the elevation of total peripheral resistance. Intact females appear to be resistant to these mechanisms, which suggests a link between female hormones and salt sensitivity.

The renin-angiotensin system most likely contributes to Dahl S hypertension independently of sodium intake by acting intrarenally to promote glomerular injury. Prehypertensive Dahl S rats have albuminuric glomerular disease, which is not prevented by maintenance on a low-sodium diet. In separate studies, Otsuka et al and Yoneda et al recently demonstrated that blockade of the renin-angiotensin system by inhibition of the angiotensin converting enzyme or blockade of angiotensin II receptors resulted in marked attenuation of glomerular injury in Dahl S rats independent of the antihypertensive effect of the drug treatment. A possible action of female sex hormones is to protect against the development of spontaneous hypertension in Dahl S rats by limiting the effects of angiotensin II. Female sex hormones can diminish the vasoconstrictor effects of angiotensin II and downregulate vascular angiotensin II receptors. Recent studies by Brosnihan et al have led to the proposal that female sex hormones can shift the direction of the vasoconstrictor-vasodilator balance of the renin-angiotensin system by reducing the formation of the vasoconstrictor (angiotensin II) while increasing the formation of the vasodilator (angiotensin I). The development of hypertension in OVX females maintained on a low-sodium diet may be related to activation of the renin-angiotensin system. The absence of female sex hormones and dietary salt in these animals prevents suppression of this pressor mechanism.

A role for nitric oxide in Dahl S hypertension has been proposed based on studies showing that hypertension can be prevented when animals are treated with the precursor for endothelium-derived nitric oxide, L-arginine. Studies indicate that Dahl S hypertension may be associated with a diminished capacity to produce nitric oxide, particularly in the kidney. Interestingly, the vasculature of females has a greater capacity to produce nitric oxide than that of males. This gender difference in nitric oxide production has been shown to be a result of a stimulatory effect of female sex hormones. The effects of the female sex hormones on the production of nitric oxide in Dahl S rats are unknown. However, it is possible that female sex hormones may act to improve renal function in Dahl S rats by enhancing the production of nitric oxide and thus may protect against the development of hypertension. This possibility is supported by the observation that ovariectomy in Dahl S female rats resulted in a blunted pressure-natriuresis relationship and an exaggerated hypertension compared with intact females.

In summary, increases in dietary sodium caused the development of sodium-dependent hypertension in Dahl S rats that was blunted in females. A decrease in dietary sodium resulted in a rapid reversal of hypertension in both males and females. Female rats maintained on a low-NaCl diet developed spon-
taneous hypertension, which was sodium independent. Ovariectomy resulted in an exaggerated development of both sodium-dependent and -independent hypertension in female Dahl S rats. Reducing dietary sodium from 8% to 0.15% NaCl in ovariectomized females did not result in a reversal of Dahl S hypertension. Our findings indicate that there are sex differences in the development of sodium-dependent hypertension and spontaneous hypertension in Dahl S rats, and the female sex hormones act to suppress both sodium-dependent and -independent increases in blood pressure. The results of this study have led to the conclusion that female Dahl S rats are less sensitive to sodium-dependent hypertension but more sensitive to sodium-independent hypertension than males. We speculate that removal of sex hormones selectively activates the mechanism(s) responsible for sodium-independent hypertension in female Dahl S rat.

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