Role of Gender on Renal Interstitial Hydrostatic Pressure and Sodium Excretion in Rats

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The objective of this study was to compare renal interstitial hydrostatic pressure (RIHP) and sodium excretion in female and male Sprague-Dawley (SD) rats. The RIHP and pressure natriuresis responses were determined in female (n = 13) and male (n = 8) SD rats. Renal perfusion pressure (RPP) was controlled at two levels (100 and 120 mm Hg). Clearances were taken at each level and RIHP was measured with a chronically implanted polyethylene matrix in all rats. At the lower RPP level, RIHP was similar in both groups of rats (5.2 ± 0.2 mm Hg for female, and 5.5 ± 0.4 mm Hg for male), whereas fractional excretion of sodium (FE\textsubscript{Na}) was significantly lower (P < .05) in male (1.10 ± 0.27%) as compared to female (2.23 ± 0.32%) rats at similar lower RPP. Allowing RPP to increase from 100 to 120 mm Hg resulted in similar increases in FE\textsubscript{Na} (ΔFE\textsubscript{Na}), urine flow rate (ΔV), and RIHP (ΔRIHP) in both groups of rats. The ΔFE\textsubscript{Na}, ΔV, and ΔRIHP were 1.67 ± 0.43%, 38.45 ± 4.74 μL/min/g kidney weight, and 2.7 ± 0.2 mm Hg for female, and 1.79 ± 0.42%, 30.40 ± 4.37 μL/min/g kidney weight, and 2.8 ± 0.3 mm Hg for male rats. In conclusion, RIHP is similar in female and male SD rats at similar RPP levels. Both female and male SD rats increase RIHP and sodium excretion similarly in response to increases in RPP. The lower basal FE\textsubscript{Na} in male as compared to female rats may play an important role in the more significant elevation of blood pressure in males with age. Am J Hypertens 2001; 14:893–896 © 2001 American Journal of Hypertension, Ltd.

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Recent studies have shown that in a normotensive population blood pressure (BP) is higher in men than in women at similar ages.\textsuperscript{1–3} With aging, BP increases in both men and women; however, mean BP is significantly higher in men between the ages of 20 and 70 years as compared to women at similar age.\textsuperscript{1–3} Also, the incidence of uncontrolled hypertension is greater in men as compared to women.\textsuperscript{3} However, the mechanisms that contribute to this gender difference are not known.

Pressure natriuresis is an important mechanism in the regulation of BP and plasma volume.\textsuperscript{4} Increases in renal perfusion pressure (RPP) result in increases in renal interstitial hydrostatic pressure (RIHP), urinary sodium excretion, and urine flow rate in male normotensive Sprague-Dawley (SD) and Wistar-Kyoto (WKY) rats.\textsuperscript{4,5} When the increases in RIHP that are produced by increases in RPP are attenuated by acute renal decapsulation in male normotensive rats, the increases in sodium and water excretion that are observed with increases in RPP are almost completely abolished.\textsuperscript{5,6} Therefore, in normotensive rats, increases in RIHP are required for the full expression of pressure natriuretic and diuretic responses, suggesting a causal relationship between increases in RIHP and sodium and water excretion. Pressure natriuresis and diuretic responses are significantly attenuated in hypertensive rats leading to less sodium excretion and thus sodium retention.\textsuperscript{5} These attenuated natriuretic and diuretic responses are associated with a blunted increase in RIHP.\textsuperscript{5}

Although extensively studied in male rats, the pressure natriuresis and diuresis responses are less characterized in female rats. Moreover, a systematic comparison of the relationships between RPP, RIHP, and sodium and water excretions have not been reported in male and female SD rats. Therefore, the objective of these experiments was to study the relationship between RPP, RIHP, and sodium and water excretions in female and male normotensive SD rats to evaluate the possible role of gender on these relationships.
Methods
All rats in these studies were SD rats purchased from Harlan Sprague-Dawley (Indianapolis, IN). All rats were fed a normal Purina Rat Chow containing 0.1 mEq sodium/g and had free access to food and water.

Polyethylene Matrix Implantation
The implantation procedure of the polyethylene (PE) matrix has been previously described. The RIHP was measured directly and continuously with a PE matrix that was implanted in the left kidney of rats when they were 11 to 16 weeks old.

In these experiments, the relationship between RIHP, and sodium excretion were studied in two groups of SD rats. Female SD rats (n = 13): On the day when the acute pressure natriuresis studies were performed these rats were 17 to 23 weeks old. Male SD rats (n = 8): On the day when the acute pressure natriuresis studies were performed these rats were 12 to 19 weeks old.

Surgical Procedure for Acute Pressure Natriuresis Experiments
On the day of the acute experiment, rats were anesthetized with inactin (100 mg/kg) and catheters were placed in the trachea (PE-240; Clay Adams, Parsippany, NJ), and left jugular vein (PE-50) for intravenous infusion of 1 mL/100 g body weight/h of saline and 1 mL/100 g body weight/h of a solution of 3% inulin and 6.25% bovine albumin in saline. A PE-50 catheter was implanted in the left carotid artery for mean arterial pressure (MAP) measurement and blood withdrawal. A PE-50 catheter was implanted in the left femoral artery for the measurement of RPP. A PE-90 catheter with a flared tip was placed in the bladder for urine collection. A small adjustable clamp was placed around the abdominal aorta above both renal arteries and was used to control RPP. The rats were allowed to recover after completion of the surgical procedures. Then RPP was controlled at the lower level (approximately 100 mm Hg) by tightening the adjustable clamp around the abdominal aorta and thus reducing RPP. Ten minutes after setting RPP at the lower level, the first clearance period of 30 min was started. During the 30-min clearance period, MAP and RIHP were measured and recorded continuously. At the end of this period approximately 1 mL of blood was withdrawn from the left carotid artery for plasma electrolytes, phosphate, and inulin measurements. At this time the adjustable clamp was loosened to allow RPP to increase and then controlled at the higher level (approximately 120 mm Hg). The second clearance period of 30 min was started 10 min after setting RPP at the higher level. Again during the second clearance period, MAP and RIHP were measured and recorded continuously. At the end of this period, about 1 mL of blood was withdrawn from the left carotid artery for plasma electrolytes, phosphate, and inulin measurements. All rats were killed by air embolism at the end of the experiment while still under deep anesthesia, and both kidneys were excised and weighed. This method of death is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

Glomerular filtration rate (GFR) was calculated from the clearance of inulin, and inulin concentrations were measured by the anthrone method. Sodium concentrations in plasma and urine were measured using flame photometry (NOVA Biomedical model NOVA 1+, Waltham, MA). Phosphate concentrations in plasma and urine were measured according to the method of Chen et al.

Standard paired Student’s t tests were used for comparisons between the first and second clearance periods in the same group of rats. Standard unpaired Student’s t tests were used for group comparisons at equivalent periods. All data are means ± SE, and P < .05 was accepted as a statistically significant difference.

Results
The results of these experiments are shown in Fig. 1 and Table 1. At the lower RPP level (100 mm Hg), RIHP was 5.2 ± 0.2 mm Hg for female and was 5.5 ± 0.4 mm Hg for male SD rats (P = NS). However, at this basal RPP level, both urinary sodium excretion (UNaV) and fractional excretion of sodium (FENa) were significantly higher in female as compared to male rats. The UNaV and FENa were 3.95 ± 0.54 µEq/min/g kidney weight and 2.23 ± 0.52% for female and 1.95 ± 0.25 µEq/min/g kidney weight (P < .05) and 1.10 ± 0.27% (P < .05) for male rats. However, the increases in fractional excretion of sodium (ΔFENa), urine flow rate (ΔV), and ΔRIHP were similar (P = NS) in both groups when RPP was allowed to increase from the lower to the higher level. The ΔFENa, ΔV, and ΔRIHP were 1.67 ± 0.37%, 38.45 ± 4.74 µL/min/g kidney weight, and 2.7 ± 0.2 mm Hg for female rats, and 1.79 ± 0.42%, 30.40 ± 4.37 µL/min/g kidney weight, and 2.8 ± 0.3 mm Hg for male rats. The data collected from the male rats (n = 8) were further examined to determine whether there were any differences in sodium excretion, RIHP, or MAP based on age within this group. The male group of SD rats was divided into two groups. One group contained rats of aged 12 to 13 weeks. (n = 4; average body weight, 320 g), another group contained rats aged 17 to 18 weeks of age (n = 4; average body weight, 384 g). Comparisons between these two groups of male rats indicated that RIHP and MAP were similar in both groups at equivalent RPP. However, FENa was significantly lower in the 12- to 13-weeks group (FENa = 0.45 ± 0.10%; n = 4) as compared to the 17- to 18-weeks, group (FENa = 1.74 ± 0.22%; n = 4; P < .05) at the lower RPP of 100 mm Hg. At the higher RPP of 120 mm Hg, FENa was similar in both groups, FENa = 2.39 ± 0.64% for the 12- to 13-weeks group, and FENa = 3.38 ± 0.41% for the 17- to 18-weeks group. Also at this higher
RPP, MAP, and RIHP were similar for both groups of male rats. As shown in Table 1, fractional excretion of phosphate (FEPi) increased significantly (from 31.94 ± 5.34% to 42.71 ± 4.71%; P < .05) in female rats; whereas it did not change in male rats (20.13 ± 5.61% to 28.47 ± 3.47%) as RPP was allowed to increase from the low to high level. The GFR was well autoregulated in both groups with the changes in RPP (Table 1). The weight of both kidneys was 2.00 ± 0.06 g for female rats, which was significantly lower (P < .05) than 2.89 ± 0.17 g for male rats.

**Discussion**

The results of the present study show that sodium excretion in male SD rats is significantly lower than that in female SD rats at similar lower RPP (Fig. 1 and Table 1). These data are consistent with a study by Reckelhoff et al9 in which male Okamoto spontaneously hypertensive rats (SHR) were shown to excrete less sodium at equivalent BP than female SHR. Data collected in a normotensive population have shown that BP is higher in men than in women at similar ages.1–3 With aging BP increases in both men and women; however, mean BP is significantly higher in men between the ages of 20 and 70 years as compared to women at similar age.1–3 The mechanisms that are responsible for this gender difference in BP are not known. Because sodium retention is one of the major factors known to cause hypertension, the enhanced sodium reabsorption in male compared to female SD rats demonstrated in the present study may play an important role in the more significant elevation of BP in males with age. Food intake was not measured in this study and all rats had free access to food and water before the acute experiment. Also, on the day of the acute experiment all rats were given the same rate of intravenous infusions of saline (per body weight). Therefore, based on the experimental design of this study, one cannot rule out the possibility that the lower basal sodium excretion in the male rats may indicate that sodium intake in the male rats was lower than in the

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**FIG. 1.** Renal interstitial hydrostatic pressure (RIHP, top) and fractional excretion of sodium (FENa, bottom) at two renal perfusion pressure (RPP) levels (99 to 101 mm Hg and 118 to 121 mm Hg) in female (F) and male (M) Sprague-Dawley (SD) rats. *Significant difference (P < .05) between low and high RPP levels in the same group of rats compared with Student’s paired t test. †Significant difference (P < .05) between female and male rats at equivalent periods (similar RPP levels) as compared with Student’s unpaired t test.

| Table 1. Renal responses to changes in RPP in female and male groups of Sprague-Dawley (SD) rats |
|-----------------|-----------------|
|                  | Female SD (n = 13) | Male SD (n = 8) |
| RPP (mm Hg)     | 99 ± 1           | 101 ± 1          | 121 ± 2*       |
| RIHP (mm Hg)    | 5.2 ± 0.2        | 7.9 ± 0.4*       | 5.5 ± 0.4      | 8.3 ± 0.5*    |
| MAP (mm Hg)     | 119 ± 2          | 118 ± 1          | 124 ± 2        | 121 ± 2      |
| GFR (mL/min/g KW)| 1.36 ± 0.15      | 1.61 ± 0.13      | 1.29 ± 0.17    | 1.40 ± 0.22  |
| UNaV (µEq/min/g KW)| 3.95 ± 0.54  | 9.06 ± 1.15*     | 1.95 ± 0.51†   | 6.07 ± 1.16* |
| FENa (%)        | 2.23 ± 0.32      | 3.90 ± 0.51*     | 1.10 ± 0.27†   | 2.89 ± 0.40* |
| FEPi (%)        | 31.94 ± 5.34     | 42.71 ± 4.71*    | 20.13 ± 5.61   | 28.47 ± 3.47 |
| V (µL/min/g KW) | 26.05 ± 3.12     | 64.50 ± 6.24*    | 15.10 ± 5.79   | 45.51 ± 8.72* |

RPP = renal perfusion pressure; RIHP = renal interstitial hydrostatic pressure; MAP = mean arterial pressure; GFR = glomerular filtration rate; KW = kidney weight; UNaV = urinary sodium excretion; FENa = fractional excretion of sodium; FEPi = fractional excretion of phosphate; V = urine flow rate.

Values are mean ± SE.

* Significant difference (P < .05) between low and high RPP periods in the same group of rats compared with Student’s paired t test.

† Significant difference between female and male SD rat group at equivalent periods (similar RPP level) compared with Student’s unpaired t test.
female rats. However, it should be noted that the average body weight of the male group was 352 g, which was significantly higher than the average body weight of the female group of 276 g on the day of the acute experiment. Therefore, it is likely that the male rats consumed more food and had higher sodium intake than the female rats.

The mechanisms that are responsible for this gender difference in sodium excretion are not known; however, it appears that different levels of testosterone may be involved. Reckelhoff et al.9 demonstrated that androgens play a role in mediating the exacerbation of hypertension in the male SHR. They suggested that testosterone or its metabolites might directly enhance the tubular reabsorption of sodium or indirectly by activation of the renin angiotensin system. Testosterone levels have been demonstrated to increase by the age of 8 weeks and reach a peak at 12 weeks of age in male rats.9 Androgen receptors have been demonstrated to be present in the nuclei of proximal tubule cells.9

Consistent with the localization of androgens receptors, the present study also suggests that the gender difference in sodium excretion may involve differences in proximal tubular reabsorption of sodium. Because phosphate is almost exclusively reabsorbed in the proximal tubule, it has been used as an index for proximal tubular reabsorption.10 In the present study, there was a tendency for a higher FE_{Pi} in the female rats as compared to male rats at equivalent RPP level (Table 1). Furthermore, there was a significant phosphaturia in female but not in male rats as RPP was allowed to increase. Taken together, these data suggest that there might be an overall elevation of proximal tubular reabsorption of sodium in the male as compared to the female SD rats, which would contribute to the lower sodium excretion in male SD rats.

It is important to note that in this study there were no significant differences in BP between female and male rats at this age range (12 to 23 weeks). Moreover, in the present study female and male SD rats displayed similar RIHP and pressure natriuresis responses. Yet, enhanced sodium reabsorption was evident in male as compared to female rats at the basal lower RPP level. Therefore, it is unlikely that at this age (12 to 19 weeks) an altered RIHP or a shift in the pressure natriuresis response contribute to the enhanced sodium reabsorption that was observed in male SD rats in this study.

In conclusion, sodium excretion in male normotensive rats is significantly lower than that in female rats at similar lower RPP, although RIHP is similar in female and male rats at similar RPP levels. Both female and male rats increase RIHP and sodium excretion similarly in response to increases in RPP. The lower basal sodium excretion in male as compared to female rats may play an important role in the more significant elevation of BP that is observed in males with age.

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References