Altered Pressure-Natriuresis Relationship in Young Spontaneously Hypertensive Rats

RICHARD J. ROMAN

SUMMARY In the present study, the pressure-natriuretic responses of 3- to 5- and 6- to 9-week-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) were characterized to determine whether the relationship between sodium excretion and renal perfusion pressure is altered during the development of hypertension. Differences in the neural tone and renal hormone levels in SHR and WKY were minimized by denervating the kidney and fixing plasma concentrations of aldosterone, vasopressin, norepinephrine, and cortisol by intravenous infusion. Renal perfusion pressure was varied using adjustable occluders on the lower aorta. The slopes of the relationships between sodium excretion and renal perfusion pressure were not significantly different in 3- to 5-week-old SHR and WKY (0.31 ± 0.05 vs 0.42 ± 0.06 μEq/min/g kidney weight); however, the x-intercept of this relationship was significantly shifted to the right by 15 mm Hg in SHR compared to WKY. Blood pressure was moderately elevated even in the 3- to 5-week-old SHR in comparison to WKY (98 ± 5 vs 81 ± 6 mm Hg). As the degree of hypertension became more severe, the slope of the pressure-natriuresis relationship became significantly lower in 6- to 9-week-old SHR compared to the corresponding slope observed in age-matched WKY (0.16 ± 0.02 vs 0.31 ± 0.04 μEq/min/g kidney weight). These results indicate that the relationship between sodium excretion and renal perfusion pressure is altered even in very young SHR. Thus, the resetting of kidney function occurs very early and may be necessary for the development of hypertension in SHR.

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KEY WORDS • hypertension • kidney • glomerular filtration rate • pressure-diuresis • spontaneous hypertensive rat

RECENT studies from our laboratory have indicated that the pressure-natriuretic responses of adult spontaneously hypertensive rats (SHR) and hypertensive Dahl salt-sensitive rats are blunted compared to those observed in control animals.1,2 These findings were consistent with previous reports suggesting that the kidneys of SHR require an elevated arterial pressure to excrete normal quantities of sodium and water,3-5 and they support the view that renal dysfunction is necessary for the maintenance of hypertension.5-7

Renal transplantation studies have also indicated that an abnormality in renal function may also be involved in the development of hypertension in SHR.8 However, the role of the kidney in this process remains unclear because very little information is available on renal function of SHR and Wistar-Kyoto rats (WKY) less than 6 weeks old. The purpose of the present studies was to characterize renal hemodynamics and the pressure-natriuretic responses of 3- to 5- and 6- to 9-week-old SHR and WKY under conditions in which neural and hormonal influences on renal function were fixed. The results indicated that the relationship between sodium excretion and renal perfusion pressure (RPP) was shifted toward higher pressures even in 3- to 5-week-old SHR. These data suggest that the resetting of kidney function occurs very early and could be necessary for the development of hypertension in this model.

Methods

Experiments were performed on two groups of SHR and WKY that were purchased from Harlan Laboratories (Madison, WI, USA). One group consisted of ten 6- to 9-week-old SHR and eight age-matched WKY. The other group consisted of twelve 3- to 5-week-old...
SHR and eleven age-matched WKY. The rats were housed in an AAALAC-approved animal care facility at the Medical College of Wisconsin. The rats were fed a rat chow containing 0.4% sodium by weight and were allowed food and water ad libum. All surgical procedures were conducted with strict adherence to the principles described in the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (1985), and these protocols were approved by the Animal Care Committee of the Medical College of Wisconsin.

Experimental Protocols

6- to 9-Week-Old Rats

One week prior to study, the rats were anesthetized with ketamine (100 mg/kg) and acepromazine (2 mg/kg), and the right kidney and adrenal gland were surgically removed using a flank incision. The incisions were sutured closed and the animals were given 40,000 U of penicillin G and 25 mg dihydrostreptomycin by intramuscular injection to prevent infection. On the day of the experiment, the rats were anesthetized with an intraperitoneal injection of Inactin (100 mg/kg) and cannulas were placed for the intravenous infusion of drugs, collection of urine from the ureter of the left kidney, and measurement of arterial pressure from the carotid and femoral arteries. Two adjustable occluders were placed on the aorta above and below the left renal artery, and ties were loosely placed around the mesenteric and celiac arteries for later occlusion, so that RPP could be manipulated. A flow probe was placed around the left renal artery, and renal blood flow (RBF) was measured using an electromagnetic flowmeter.

Differences in neural and endocrine influences on renal function of SHR and WKY were minimized as previously described.1, 2, 9, 10 The left kidney was denervated and the remaining left adrenal gland was removed to prevent the release of catecholamines and steroids during the experiment. Plasma concentrations of aldosterone, cortisol, vasopressin, and norepinephrine were maintained at fixed levels by intravenous infusion of norepinephrine (333 ng/kg/min), aldosterone (66 ng/kg/min), cortisol (33 μg/kg/min), and vasopressin (0.17 ng/kg/min). These hormones were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The drugs were dissolved in a 0.9% sodium chloride solution containing 1% albumin that was infused at a rate of 33 μl/min/100 g body weight during the experiment. [3H]inulin (2 μCi/ml) was included in the infusion solution to permit the measurement of glomerular filtration rate (GFR). We have previously reported that plasma levels of aldosterone were elevated 10-fold in Sprague-Dawley rats prepared in this manner.9 Plasma concentrations of norepinephrine and vasopressin were elevated to nonpressor levels, about five times the values found in conscious rats.9 Plasma renin activity,9 plasma angiotensin II concentration,10 and plasma levels of atrial natriuretic peptide10 measured in hormone-infused rats were similar to values reported in conscious rats.

After a 1-hour equilibration period, the relationship between sodium excretion and RPP was characterized as follows. The RPP was first lowered to approximately 100 mm Hg in SHR and WKY by occluding the aorta above the kidney. Urine and plasma samples were collected during two 20-minute clearance periods. The aortic clamp was released to allow RPP to return to the control level of arterial pressure in the WKY and approximately 130 mm Hg in the SHR. Urine and plasma samples were again collected during two additional 20-minute periods. The mesenteric and celiac arteries were then tied off and the aortic clamp below the kidney tightened to elevate RPP to approximately 150 mm Hg in WKY. In SHR, the aortic clamp was released to allow RPP to rise to the control level of arterial pressure, approximately 155 mm Hg. Urine and plasma samples were again collected during two additional 20-minute clearance periods. In SHR, the ties on the mesenteric and celiac arteries were then tied off to elevate RPP to approximately 175 mm Hg, and urine and plasma samples were collected during two final 20-minute periods.

3- to 5-Week-Old Rats

These very small rats did not tolerate surgery and therefore were not uninephrectomized prior to an experiment. On the day of the acute study, they were anesthetized using a 50 mg/kg intraperitoneal dose of Inactin. Cannulas were placed in the jugular vein for infusions, in the femoral and carotid arteries for measurement of RPP, and in both ureters for collection of urine. Loose ties were placed around the aorta and the celiac and mesenteric arteries to allow for manipulation of RPP. Both adrenal glands were removed surgically, and the left and right kidneys were denervated. The rats were infused with hormone cocktail at the doses described above. The rate of infusion was adjusted for the lower body weight of these younger rats. It was not possible to measure RBF using an electromagnetic flowmeter in these rats because the renal artery was too small. Therefore, effective RBF was calculated from the renal clearance of [3H]inulin. Measured values were not corrected for the extraction of [3H]PAH.

Since basal urine flow was very low in these animals, RPP was initially set at the control arterial pressure during the first 40-minute control clearance period. The RPP was then increased 20 mm Hg above control level by tying off the mesenteric and celiac arteries, and urine and plasma samples were collected during a second 40-minute experimental clearance period. Perfusion pressure was increased further by tying off the abdominal aorta below the kidneys, and samples of urine and plasma were again collected during a final 40-minute clearance period.

Analytical Techniques

Concentrations of [3H]inulin and [3H]PAH in urine and plasma samples were measured using a liquid scintillation spectrophotometer. Urine flow rate was deter-
mined gravimetrically from the timed urine collections. Sodium and potassium concentrations of the samples were measured by flame photometry. The GFR, RBF, and absolute and fractional excretion of sodium and water were calculated using standard clearance formulas we have described previously.9

Statistics
Significance of differences in values measured at different levels of RPP in the same animal was determined using a two-way analysis of variance followed by a Bonferroni test for repeated measures.118 Significance of differences in measured values at similar levels of RPP in SHR and WKY was evaluated using an unpaired t test.12 Linear regression analysis was used to calculate the slopes and x-intercepts of the lines relating urine flow, sodium excretion, and RPP in individual rats. Differences in the mean slopes and x-intercepts of these lines between groups of SHR and WKY were compared using an unpaired t test.12 Probability less than 0.05, determined by a two-tailed test, was considered significant.

Results
3- to 5-Week-Old Rats
The mean ages of the SHR and WKY in this study averaged 3.8 ± 0.2 and 3.9 ± 0.2 weeks, respectively. Mean body weight of SHR was 55 ± 5 g, and that of WKY was 60 ± 5 g. Arterial pressure measured after cannulation of the femoral artery was 94 ± 5 mm Hg in SHR and 79 ± 5 mm Hg in WKY. After infusion of the hormone cocktail, the mean arterial pressure rose to 103 ± 3 mm Hg in SHR and to 85 ± 6 mm Hg in WKY.

The pressure-diuresis and pressure-natriuresis relationships observed are presented in Figure 1. Control urine flows and sodium excretions measured at their spontaneous level of arterial pressure and factored per gram of kidney weight (kwt) were not different in SHR and WKY and averaged approximately 8 μl/min/g kwt and 1 μEq/min/g kwt, respectively. At an elevated RPP of 130 mm Hg, urine flow and sodium excretion were significantly greater in WKY than in SHR. The slope of the relationship between urine flow and RPP tended to be slightly, but not significantly, greater for WKY than SHR (2.51 ± 0.91 vs 1.82 ± 0.34 μl/min/g kwt). Similarly, the slope of the relationship between sodium excretion and RPP was not significantly different between SHR and WKY (0.31 ± 0.05 vs 0.42 ± 0.06 μEq/min/g kwt). The x-intercept of this relationship, however, was significantly shifted to the right by approximately 15 mm Hg in SHR as compared to WKY (99 ± 5 vs 84 ± 6 mm Hg).

The effects on renal hemodynamics of changing RPP are presented in Figure 2. Control levels of GFR were not significantly different and averaged 1.21 ± 0.09 ml/min/g kwt in SHR and 0.95 ± 0.20 ml/min/g kwt in WKY. Glomerular filtration rate was well autoregulated in both groups, and no differences in GFR could be detected in SHR and WKY at any RPP studied. Similarly, no differences in effective RBF were detected in SHR and WKY at any RPP studied (see Figure 2). In both groups RBF averaged approximately 3.5 ml/min/g kwt.

6- to 9-Week-Old Rats
The ages of SHR and WKY in this study were similar and averaged 7.2 ± 0.4 and 6.8 ± 0.3 weeks, respectively. Body weights of the SHR and WKY averaged 147 ± 11 and 141 ± 10 g, respectively. Arterial pressure measured after cannulation of the femoral artery averaged 132 ± 3 mm Hg in SHR and 108 ± 8 mm Hg in WKY. After infusion of hormone cocktail, arterial pressure rose to 162 ± 4 mm Hg in SHR and to 132 ± 4 mm Hg in WKY.

The relationships between urine flow, sodium excretion, and RPP in 6- to 9-week-old SHR and WKY are presented in Figure 3. Control urine flows and sodium excretions measured at their spontaneous level...
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of arterial pressure in SHR and WKY were not significantly different, and averaged 55 µl/min/g kwt and 10 µEq/min/g kwt, respectively. When the kidneys of SHR and WKY were perfused at approximately 130 or 150 mm Hg, the WKY excreted twice as much sodium and water as the SHR. Over the pressure range of 100 to 160 mm Hg, the slope of the line relating urine flow and RPP averaged 0.83 ± 0.11 µl/min/g kwt for SHR. The slope of this line was significantly lower than that (1.87 ± 0.26 µl/min/g kwt) observed for WKY. Likewise, the slope depicting the relationship of sodium excretion to RPP was significantly lower for SHR than for WKY (0.16 ± 0.04 vs 0.31 ± 0.02 µEq/min/g kwt).

The effects of changes in RPP on GFR and RBF in these animals are presented in Figure 4. Control level of GFR was 30% lower in SHR than in WKY. GFR was autoregulated in both groups over a broad range of RPPs. At any level of RPP, GFR was significantly lower in SHR than in WKY. In contrast, RBF was similar in SHR and WKY and averaged approximately 4.5 ml/min/g kwt at any of RPPs studied (see Figure 4).

The relationships between absolute sodium excretion (not factored per gram of kidney weight) and RPP measured in the young SHR and WKY were compared with previous results obtained in adult rats and are presented in Figure 5. The data obtained in the different age groups of WKY fell along a single curve. The data from 3- to 5- and 6- to 9-week-old SHR also fell along a single curve. However, the results from the adult SHR were shifted to the right relative to the results in the young SHR. Over the range of pressures from 110 to 150 mm Hg in WKY, from 100 to 160 mm Hg in young SHR, and from 130 to 190 mm Hg in the adult SHR, the data in Figure 5 could be well fit by

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**Figure 2.** Effect of changes in renal perfusion pressure on glomerular filtration rate (GFR) and renal blood flow in 3- to 5-week-old SHR and WKY. Values are means ± 1 SE from 10 to 12 rats per group. Asterisk indicates a significant difference from the control value (○) measured at the animal's spontaneous level of arterial pressure. Dagger indicates a significant difference in measured values in SHR and WKY at a similar level of renal perfusion pressure. Kidney weight (kwt) averaged 0.74 ± 0.03 g in SHR and 0.72 ± 0.04 g in WKY.

**Figure 3.** Relationships between urine flow, sodium excretion and renal perfusion pressure in 6- to 9-week-old SHR and WKY. Values are means ± 1 SE from 10 rats per group. Asterisk indicates a significant difference from the control value (○) measured at the animal's spontaneous level of arterial pressure. Dagger indicates a significant difference in measured values in SHR and WKY at a similar level of renal perfusion pressure. Kidney weight (kwt) averaged 1.29 ± 0.10 g in SHR and 1.33 ± 0.13 g in WKY.
straight lines ($r > 0.95$). The slope of line describing the pressure-natriuresis relationship in young SHR was 43% less than the corresponding slope observed in WKY ($0.23 \pm 0.06$ vs $0.41 \pm 0.02 \mu$Eq/min/mm Hg). The slope of the relationship in the adult SHR averaged only $0.12 \pm 0.02 \mu$Eq/min/mm Hg, which was 48% less than the slope observed in the young SHR and was 71% less than that observed in WKY.

Discussion

The hypothesis proposed by Guyton and associates that the kidney plays a dominant role in the long-term control of arterial pressure was based on the pressure-diuresis phenomenon. According to this theory, hypertension could only develop if the relationship between sodium excretion and RPP was shifted toward higher pressures. Considerable evidence has accumulated suggesting that the neural and hormonal control of the kidney differs in SHR and WKY. However, the pressure-natriuresis relationships of young SHR and WKY have not been characterized to determine whether these abnormalities are sufficient to actually alter renal function and play a role in the development of hypertension.

In the present study, the slopes of the relationship between sodium excretion and RPP were not significantly different in 3- to 5-week-old SHR and WKY (see Figure 1). However, the overall relationship was significantly shifted to the right by 15 mm Hg in the SHR. Later in the development of hypertension, the slope of the relationship between sodium excretion and RPP became significantly reduced in 6- to 9-week-old SHR compared to the corresponding slope observed in WKY (see Figure 3). In both 3- to 5- and 6- to 9-week-old SHR, urine flow and sodium excretion were lower than that measured in age-matched WKY when their kidneys were perfused at the same level of RPP. The finding that the pressure-natriuresis relationship in 3- to 5-week-old SHR was shifted in the absence of significant differences in renal hemodynamics (see Figure 2) supports our view that this abnormality is present very early and may be responsible for the development of hypertension in this model.

The structural or functional changes in the kidneys of SHR that shift the pressure-natriuresis relationship have not been identified. Since RBF and GFR were similar in the 3- to 5-week-old SHR and WKY, differences in the renal vascular sensitivity to vasoconstrictors and/or intrinsic defects in the renal microvasculature cannot explain the observed shift in the pressure-natriuresis relationship. Increases in renal vascular tone caused by alterations in renal nerve activity also do not explain our results, since the kidneys in our study were denervated. Nor is it likely that the shift in the pressure-natriuresis relationship was due to renal vascular damage secondary to sustained hypertension, since arterial pressure averaged only 98 mm Hg in the 3- to 5-week-old SHR. On the other hand, intrinsic differences in the responsiveness of the renal tubules of young SHR to sodium- and water-retaining hormones or differences in the regulation of
sodium and water reabsorption by intrarenal autacoids (i.e., angiotensin II, kinins, and eicosanoids) might contribute to the abnormal pressure-natriuresis relationship in these animals.

Significant differences were observed in renal hemodynamics in 6- to 9-week-old SHR and WKY. The GFR was lower in SHR than in WKY (see Figure 4). These results confirm the recent finding of Dilley et al. indicating that GFR and single nephron GFR were reduced in SHR at this age, perhaps because of an elevated tubuloglomerular feedback responsiveness. Our findings also agree well with previous anatomical studies indicating that the early development of hypertension in 6-week-old SHR was associated with significant changes in glomerular capillaries. Thus, an age-related fall in GFR may contribute to the reduction in the slope of the pressure-natriuresis relationship in SHR as they mature from 3 to 6 weeks of age (Figure 1 vs Figure 3).

In contrast to the previous results of Dilley et al., we did not find that RBF was lower in 6- to 9-week-old SHR compared to WKY. The difference may be that in the present experiments, the rats were studied with neural and hormonal influences on the kidney fixed, whereas these factors were not controlled in the previous study. In addition, RBF was measured using an electromagnetic flowmeter in the present study, whereas it was estimated from the renal clearance of PAH in the previous study. Moreover, the source of the SHR used in the two studies differed. Whatever factor was responsible for the difference in renal vascular resistance in the two studies, it should be emphasized that any excessive vasoconstriction in the kidneys of SHR relative to that in WKY would only further accentuate the differences in pressure-natriuresis relationships of SHR and WKY that were identified in the present study.

In 3- to 5-week-old SHR, the slope of the relationship between sodium excretion and RPP was shifted toward higher pressures (see Figure 1), and the slope of the relationship was found to be significantly less in 6- to 9-week-old SHR than in WKY (see Figure 3). The consequence of these changes in the pressure-natriuresis relationship is that for a given neural and endocrine background, RPP would have to be elevated in SHR in order to achieve the same rate of sodium excretion as that seen in WKY. If renal nerve activity and/or the levels of circulating sodium and water remaining hormones are higher in SHR than in WKY, as has been reported, these differences would further magnify the differences in the pressure-natriuresis relationship observed in SHR and WKY with neural and hormonal influences on the kidney fixed. When the rats were very young (3 to 5 weeks old) and their food and sodium intake was small (around 0.2 mEq/day, 0.1 μEq/min sodium excretion), the expected difference in arterial pressure in SHR and WKY would be small (10 mm Hg; see Figure 5). However, as the rats matured and consumed more sodium, arterial pressure would have to rise to a greater extent in the SHR than in the WKY to maintain sodium balance. Therefore, the difference in blood pressure in SHR and WKY should become progressively greater with age. This idea is consistent with previous studies indicating that SHR between the ages of 3 and 7 weeks retained significantly more sodium than WKY and with reports indicating that increasing the sodium intake of a young SHR increased the rate of rise and severity of hypertension. The slope of the pressure-natriuresis relationship was found to be further diminished and shifted to the right in the adult SHR (see Figure 5). This shift in the pressure-natriuresis relationship may reflect renal vascular damage secondary to the established hypertension in these animals.

In summary, the relationship between sodium excretion and RPP was shifted to the right in 3- to 5-week-old SHR compared to WKY, and the slope of this relationship was blunted in 6- to 9- and 12- to 16-week-old SHR relative to WKY. These data indicate that the resetting of kidney function occurs very early and may be necessary for the development of hypertension in SHR. The factors responsible for altering the pressure-natriuresis relationship in SHR remain to be determined.

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R J Roman

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