Renal Function of Conscious Spontaneously Hypertensive Rats

WILLIAM H. BEIERWALTES AND WILLIAM J. ARENDSHORST

SUMMARY Renal clearance studies were performed in conscious 13-week-old spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY) before and during volume expansion by intravenous infusion of isotonic saline. Mean arterial pressure and filtration fraction were greater in SHR, whereas fractional and absolute excretion of sodium and water, glomerular filtration rate, and renal plasma flow in SHR and WKY were not statistically different. This was the case during hydropenia and volume expansion. We did not observe an exaggerated natriuresis after intravenous loading when unanesthetized SHR were compared with the response of WKY. These observations suggest that the kidneys of genetically hypertensive rats of the Okamoto-Aoki strain have adapted to an elevated renal perfusion pressure or that hypertension is required to normalize renal function so that excretion is appropriately matched with intake.

IN 1946, FARNSWORTH\textsuperscript{1} reported that patients with essential hypertension excrete salt and water more rapidly than normotensive subjects after acute volume expansion. Although this observation has been documented repeatedly,\textsuperscript{2-7} the renal mechanism(s) responsible for this "exaggerated" natriuretic response is poorly understood. Enhanced sodium excretion has been observed after intravenous administration of hyper-, iso-, or hypotonic saline and is not associated with consistent changes in glomerular filtration rate, renal plasma flow, or plasma sodium concentration.\textsuperscript{2-3} On the other hand, not all patients with essential hypertension respond to acute volume expansion with enhanced salt and water excretion.\textsuperscript{4}

To characterize renal function in a setting of chronic hypertension, various animal models have been used. Recent investigations have focused on genetically hypertensive rats to study the mechanisms involved in the pathogenesis and maintenance of essential hypertension. Genetically or spontaneously hypertensive rats (SHR) are selectively inbred, developing high blood pressure as a function of age, and offer the attractive feature that an experimental maneuver is not required to induce hypertension in a previously normotensive animal. Reports of renal function in SHR on the Okamoto-Aoki strain\textsuperscript{5} have been conflicting with regard to the kidneys' ability to excrete an acute saline load. Following intravenous administration of a salt load, sodium excretion of anesthetized SHR has been found to be less than,\textsuperscript{7} equal to,\textsuperscript{8} or greater than\textsuperscript{9} that measured in normotensive rats. Willis et al.\textsuperscript{10} reported that conscious, 13-week-old SHR excrete sodium faster than normotensive Wistar-Kyoto rats (WKY) in response to an intragastric load of saline. In contrast, we have observed recently\textsuperscript{11} that anesthetized 13-week-old SHR did not excrete sodium and water more rapidly than WKY when acute volume expansion was induced by intravenous infusion of saline.

The present study employed clearance methodology to evaluate renal function of conscious SHR and WKY at 13 weeks of age during hydropenic conditions and following intravenous infusion of a moderate saline load. Although SHR had a significantly higher mean arterial pressure than WKY, fractional and absolute excretion of sodium and water as well as glomerular filtration rate and renal plasma flow during both phases of an experiment were comparable on the average in unanesthetized SHR and WKY. Our data are consistent with the view that the kidneys of 13-week-old SHR have adapted to an elevated perfusion pressure or that hypertension is required to normalize renal function so that excretion is appropriately matched with intake.

Methods

Observations are reported on seven male, 13 ± 2 (sd)-week-old SHR of the Okamoto-Aoki strain\textsuperscript{6} and six male, normotensive WKY that were age-matched (13 ± 1 weeks). The rats were bred locally according to National Research Council guidelines.\textsuperscript{12} Spontaneously hypertensive rats were F\textsubscript{38} generation, derived from a stock of F\textsubscript{38} to F\textsubscript{38} supplied by Dr. Carl Hansen of the National Institutes of Health. The rats were deprived of their standard rat pellet diet (Purina) but were allowed free access to water overnight prior to study.

Rats were anesthetized with ether, and a femoral artery and vein were cannulated with PE-50 and PE-10 polyethylene tubing. Femoral arterial pressure was measured with a Statham P23Db pressure transducer connected to a Hewlett-Packard recorder. The bladder was exposed through a suprapubic incision and was cannulated with PE-90 tubing flared at one end. The flared end was advanced to cover the trigone and ligated to minimize dead space while allowing urine to flow freely. The...
incisions were closed by sutures. Saline (0.85% NaCl) containing $^3$H-inulin (ICN Corp.), 8.5 μCi/100 g body weight per hour, and p-aminohippurate (PAH) (Eastman Kodak), 4 mg/100 g body weight per hour, was infused intravenously at 20 μl/min. After removal of ether, rats were placed in a Lucite restraining chamber to equilibrate for at least 90 minutes before commencement of clearance measurements. During the nondiuretic period, urine was collected for 45 minutes and arterial blood was sampled at the beginning and end of the clearance period. Hematocrit was measured in heparinized capillary tubes.

During the second phase of each experiment, isotonic saline containing $^3$H-inulin and PAH was infused intravenously to administer 3 ml/100 g body weight in 30 minutes, after which volume expansion was maintained by infusing at a rate averaging 3 ml/100 g body weight per hour. Following a 30-minute equilibrium period, urine was collected for two consecutive 30-minute periods. At the conclusion of an experiment, both kidneys were excised, cleared of perirenal fat, decapsulated, and weighed immediately.

Glomerular filtration rate (GFR) was measured by the clearance of inulin, and renal plasma flow (RPF) was determined from the clearance and extraction of PAH; the latter was assumed to be 0.85, a value we observe in anesthetized SHR and WKY. Filtration fraction was cal-

<table>
<thead>
<tr>
<th>Table 1 Renal Function in Conscious Normotensive WKY and Hypertensive SHR Rats during Nondiuretic and Volume Expansion Conditions</th>
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<tr>
<td><strong>Arterial pressure</strong> (mm Hg)</td>
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<td>--------------------------------</td>
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<tr>
<td>116 ± 14</td>
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<tr>
<td><strong>Urine flow</strong> (μl/min · kidney)</td>
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<td>10.6 ± 6.6</td>
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<td>9.6 ± 4.6</td>
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<td><strong>Sodium excretion</strong> (μEq/min · kidney)</td>
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<td>0.46 ± 0.38</td>
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<td>0.41 ± 0.25</td>
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<tr>
<td><strong>Glomerular filtration rate</strong> (ml/min · kidney)</td>
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<td>1.07 ± 0.27</td>
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<tr>
<td><strong>Renal plasma flow</strong> (ml/min · kidney)</td>
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<tr>
<td>5.37 ± 1.05</td>
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<td><strong>Filtration fraction</strong> (ratio)</td>
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<td><strong>Hematocrit</strong> (ml/100 ml)</td>
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<td><strong>Plasma protein concentration</strong> (g/100 ml)</td>
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<tr>
<td><strong>Age</strong> (weeks)</td>
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<td><strong>Number of rats</strong></td>
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</table>

Values are means ± 1 sd. KW = kidney weight.

* Indicates the significance of comparisons between WKY and SHR values.
† Indicates the significance between comparisons between volume expansion and nondiuretic values. Where no P value is indicated, $P > 0.05$. 
calculated as the ratio of GFR/RPF. Radioactivity in samples of urine and plasma was measured for 20 minutes in a liquid scintillation spectrometer. Counts per minute for all samples were at least 6 times background. Concentration of PAH was determined by an adaptation of the method of Bratton and Marshall.\textsuperscript{13} Urinary sodium concentration was analyzed with a Zeiss PMQ II flame-emission spectrophotometer. Plasma protein concentration was determined by an adaptation of the Lowry technique,\textsuperscript{14} using rat plasma total protein standards.

Student's $t$-tests for paired and unpaired variates were performed for analysis of significance. Results are reported as being statistically significant when $P$ values were less than 0.05.

Results

Results are presented in Table 1. Mean arterial pressure was significantly higher in unanesthetized 13-week-old SHR than in WKY of similar age. Although the groups were age-matched, mean body and kidney weights were slightly but significantly greater for SHR than WKY: body weight, $228 \pm 16$ (SD) g vs. $203 \pm 22$ g, $P < 0.05$; weight of both kidneys, $1.65 \pm 0.11$ g vs. $1.47 \pm 0.14$ g, $P < 0.05$. For this reason, clearance determinations are presented in Table 1 both as per kidney and per gram kidney weight.

During hydropenia, mean urine flow rate and urinary sodium excretion were similar in SHR and WKY. No statistical differences were observed between SHR and WKY when these values were expressed as per kidney or per gram kidney weight. Data for fractional excretion of water and sodium also were comparable in these unanesthetized normo- and hypertensive rats during the nondiuretic period. GFR tended to be higher in SHR, but this small difference was not statistically significant. RPF averaged about 5 ml/min per g kidney weight and was essentially the same in SHR and WKY. Although GFR and RPF were similar in normo- and hypertensive rats, filtration fraction was statistically greater in SHR. Hematocrit and plasma protein concentration of arterial blood were similar in SHR and WKY during hydropenia.

During the second phase of the experiments, we evaluated the renal response to an acute load of isotonic saline infused intravenously at a rate of 3 ml/100 g body weight per hour. These data also are summarized in Table 1. As a result of the volume expansion, hematocrit and plasma protein concentration were reduced significantly but comparably in SHR and WKY. Mean arterial pressure remained higher in SHR because pressure in both groups of rats was unchanged from values recorded during hydropenia. GFR tended to increase about 10% following expansion; this difference was statistically significant in both SHR and WKY when expressed per kidney, but not as per gram kidney weight for WKY. Volume expansion produced a 30% increase in RPF, which was significant in SHR and WKY. Filtration fraction did not change during volume expansion, remaining greater in SHR. The changes in water and sodium excretion in the individual experiments are shown in Figures 1 and 2, respectively.

Following 1 hour of volume expansion, urine flow rate had increased approximately 5-fold, and urinary sodium excretion had risen about 14 times (Table 1). These steady state values do not reflect the initial responses to expansion; however, the data in Figures 3 and 4 demonstrate that water and sodium excretion were also compa-
The means ± 1 SE. The means are not statistically different (P > 0.05) within a period.

Discussion

This study reports for the first time clearance measurements of renal function of conscious SHR and normotensive WKY during hydropenia and acute volume expansion. There were no consistent differences in urinary excretion of water and sodium, glomerular filtration rate, and renal plasma flow between unanesthetized 13-week-old SHR and WKY. This was the case during hydropenia and also after a moderate intravenous load of isotonic saline. Following volume expansion, the increases in sodium excretion for SHR and WKY were statistically significant when compared to the previous nondiuretic period, but the responses of the two groups were not significantly different from each other. The only measurable difference in renal function between conscious SHR and WKY was in filtration fraction, which was higher in SHR. These observations are consonant with our data obtained under comparable conditions for 13-week-old SHR and WKY that were anesthetized and prepared for micropuncture.\textsuperscript{11}

Willis et al.\textsuperscript{10} also have measured sodium excretion in conscious 13-week-old SHR and WKY. During a preexpansion period, sodium excretion was comparable in their two groups of rats. However, after intragastric administration of isotonic saline, the natriuretic response of SHR was twice that of WKY, while creatinine excretion was similar in both groups. Urine flow rate, GFR, and RPF, as well as arterial pressure, were not measured during their acute experiments.

Differences in experimental protocol may explain the disparity between our results and those of Willis et al. The natriuretic response may be dependent on the route of saline administration, i.e., intragastric vs. intravenous, and/or the rate and magnitude of expansion. We infused a saline load intravenously because it produced a rapid and reproducible degree of volume expansion that was comparable in normo- and hypertensive rats, and we have conducted micropuncture studies in anesthetized SHR and WKY using this protocol.\textsuperscript{11} Another reason was that studies of acute saline loading in patients with essential hypertension have employed this route of administration. The rate and magnitude of expansion also differed between the two studies. We infused isotonic saline continuously at a rate of 3 ml/100 g body weight per hour, whereas Willis et al. injected isotonic saline, 2 ml/100 g body weight, intragastrically, so intestinal absorption determined the rate and degree of expansion. Based on sodium excretion data, our rats had a larger expansion of extracellular fluid volume. Our natriuretic response was 4-5 times greater than that which they observed. Another difference was baseline sodium excretion prior to volume expansion. Sodium excretion in their study was roughly one-third that found during hydropenia in the present study. Our value was similar to the mean obtained in balance studies (ref. 10, unpublished observations).

The ability of the kidneys to excrete an acute saline load also has been investigated in other models of hypertension. Conscious rats made hypertensive by the Grollman technique exhibit an exaggerated natriuresis in response to an oral load of hypertonic saline,\textsuperscript{15} however, intraperitoneal administration does not elicit this response.\textsuperscript{16} Conscious rats in which hypertension was induced by renal encapsulation\textsuperscript{17} or chronic administration of mineralocorticoids and salt\textsuperscript{15,18} also respond to intragastric saline loading with an exaggerated natriuresis. A similar response to oral loading of isotonic saline has been reported for unanesthetized rats with two-kidney Goldblatt hyper-
tension. After approximately 10 weeks of hypertension, the exaggerated natriuresis was not observed.

Peters et al. also have studied conscious rats with two-kidney Goldblatt hypertension before and after intravenous infusion of an acute load. In response to expansion, the unclipped kidney excreted 25-30% more sodium than a kidney of normotensive rats, but excretion by the clipped kidney was decreased to a similar extent. Thus bilateral excretion of water and sodium as well as GFR were comparable between normotensive and hypertensive rats during both states of hydration. Peters et al. suggested that the discrepancy between intravenous and oral loading was related to the volume of distribution of the administered saline load as a consequence of the route of administration. They pointed out that hypertensive humans with renal arterial stenosis do not exhibit an exaggerated natriuresis following oral loading of saline. Thus hypertensive rats usually exhibit an exaggerated natriuresis after receiving an intragastric load of saline. In contrast, this response is not generally observed following intravenous administration of saline.

Farman and Bonvalet volume expanded anesthetized SHR and normotensive Wistar rats at 9 and 18-30 weeks of age with intravenous isotonic or hypertonic saline to 1.5% body weight and found that sodium excretion, urine flow, and GFR increased in both groups of rats. Since the increase in sodium excretion by SHR was less than by normotensive rats, they concluded that abnormal sodium retention may be causally related to the hypertension. Unfortunately, the appropriate genetic control rat, the normotensive WKY, was not studied.

Mullins and Banks reported that anesthetized female SHR and WKY obtained from the same supplier had similar absolute and fractional excretion of sodium before and after intravenous infusion of isotonic saline to 4.5% body weight at 12 and 16 weeks of age. When a second group of SHR was volume expanded, an enhanced sodium excretion was noted at 16 weeks of age, but not at 12 weeks. Their data were interpreted as suggesting that a defect in sodium excretion is not a factor in the development or maintenance of hypertension in SHR.

Our values for GFR, RPF, and urine flow during hydropenia were comparable to those previously published for conscious normotensive rats and rats with two-kidney Goldblatt hypertension. During volume expansion, Peters et al. reported GFR and urine flow values similar to ours, although their RPF was 40-60% less than we observed. Urine flow in our conscious rats was greater than usually observed in anesthetized rats, probably due to the effect of surgery and anesthesia on release of antidiuretic hormone. We report an elevated filtration fraction in SHR during hydropenia and volume expansion. Although RPF tended to be lower and GFR tended to be higher in SHR, these differences were not statistically significant. The mean values for filtration fraction, 0.24 in SHR and 0.20 in WKY, were less than we usually observe (unpublished observations) in anesthetized SHR and WKY during comparable hydropenic conditions, 0.32 and 0.28, respectively. The greater RPF in unanesthetized rats was responsible for the lower filtration fraction, since GFR was similar. Increased filtration fraction has been positively correlated with increases in diastolic blood pressure and reductions in RBF. An increased filtration fraction is not always seen in essential hypertension or may be present with GFR and RBF in the normal range. In anesthetized SHR, filtration fraction has been found to be elevated (unpublished data, ref. 9) or not statistically different from WKY.

An exaggerated natriuretic response in humans with essential hypertension has stimulated studies of extracellular fluid volume, which appears to be variable, e.g., either normovolemic or slightly volume contracted with normal plasma renin activity, or hypervolemic with low plasma renin activity. Hypervolemic hypertension subjects have a higher ratio of plasma to interstitial fluid volume than normovolemic hypertensive or normotensive control subjects. Salt restriction abolishes this difference. Krakoff et al. found that hypervolemic, low renin hypertensive patients respond to acute volume expansion with an exaggerated natriuresis. A mild exaggerated natriuresis follows acute volume expansion in normotensive subjects who have been chronically volume expanded by consumption of a diet with a very high salt content. In this regard, SHR weighing between 70 and 300 g have a plasma volume (39 ml/kg body weight) similar to that of weight-matched Wistar rats, and conscious unstressed SHR at 13 weeks of age appear to have normal plasma renin activity, although this is an area of controversy. Thus the SHR may be analogous to humans with essential hypertension who are normovolemic with normal plasma renin activity and do not exhibit an exaggerated natriuresis after acute volume expansion.

It is clear that factors other than arterial pressure per se have prime effects on renal excretion of salt and water after acute saline loading in a setting of hypertension. Our results demonstrate that excretion is not directly proportional to arterial pressure when SHR and WKY are compared. This does not imply that changes in renal perfusion pressure do not influence renal excretion, which, on the contrary, has been well documented. One group of SHR studied by Mullins and Banks exhibited an enhanced natriuretic response while another equally hypertensive group of SHR did not. In addition, Brookhaven salt-sensitive normotensive and hypertensive rats had an exaggerated natriuresis after oral administration of isotonic saline, but not hypertonic saline.

Guyton et al. have postulated that essential hypertension results from a "resetting" of the arterial pressure required to balance renal excretion of fluid and electrolytes with intake. In this context, renal function in hypertensive subjects may be considered to be abnormal, although excretion equals intake. In our unanesthetized SHR, GFR, RPF, sodium excretion, and urine flow were comparable with values for age-matched normotensive WKY, while arterial pressure and filtration fraction were significantly elevated in SHR. Inducing a diuresis and natriuresis by a moderately rapid infusion of isotonic saline did not unmask any covert renal abnormality in genetically hypertensive rats, as excretion was appropriate for the administered load. Our results are consistent with
the view that the kidneys of 13-week-old SHR have adapted to an elevated perfusion pressure or that hypertension is required to normalize renal function so that excretion is appropriately matched with intake.

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This update has been corrected in the online version of the article, which is available at http://circres.ahajournals.org/content/42/5/721.abstract.