SUMMARY  The present study examined whether an alteration in renal medullary hemodynamics is associated with the development of hypertension in the spontaneously hypertensive rat (SHR). The relationships between whole kidney, cortical and papillary blood flows, and renal perfusion pressure were compared in 3- to 5-, 6- to 9-, and 12- to 16-week-old SHR and Wistar-Kyoto rats (WKY). Cortical and papillary blood flows were measured using a laser-Doppler flowmeter. Whole kidney and superficial cortical blood flows were similar in the different age groups of SHR and WKY over most of the range of perfusion pressure studied. Control papillary blood flows, determined at a renal perfusion pressure equal to the mean arterial pressure of each animal, were not significantly different in the 3- to 5- and 12- to 16-week-old SHR in comparison to values observed in age-matched WKY. In contrast, the control papillary blood flow was 30% lower in 6- to 9-week-old SHR in comparison to the value observed in WKY. Papillary blood flows were significantly less in all age groups of SHR than the corresponding flows measured in WKY when they were compared at equivalent renal perfusion pressures. These findings indicate that medullary vascular resistance is elevated even in very young SHR and suggest that alterations in vasa recta hemodynamics may participate in the development of hypertension by shifting the pressure-natriuresis relationship toward higher pressures.

(Key Words: kidney • renal medulla • autoregulation • urine concentration and dilution • laser-Doppler flowmetry)

Renal transplantation studies have indicated that renal dysfunction may underlie the development of hypertension in the spontaneously hypertensive rat (SHR).1,2 The kidneys of hypertensive patients3 and genetically hypertensive rats4-6 require an elevated renal perfusion pressure (RPP) to excrete sodium and water normally. We have demonstrated that the pressure-natriuresis relationship is shifted toward higher pressures even in very young, 3- to 5-week-old SHR.4 The factors responsible for resetting the pressure-natriuresis relationship in this model of hypertension are unknown.

Recent studies have indicated that elevations in RPP increase the delivery of sodium to the tip of the loop of Henle7 and that chemical papillectomy blunts the pressure-natriuretic response.8 These findings indicate that inhibition of tubular reabsorption in the deep nephrons contributes to the pressure-natriuretic response. Moreover, our studies indicating that elevations in RPP increase pressure and flow in the renal vasa recta circulation suggest that changes in medullary hemodynamics may signal the pressure-natriuresis response.9-11

The present study examined whether an alteration in renal medullary hemodynamics is associated with the development of hypertension in the SHR. The relationships between renal blood flow (RBF), cortical and papillary blood flows, and RPP were compared in 3- to 5-, 6- to 9-, and 12- to 16-week-old SHR and Wistar-Kyoto rats (WKY). The results indicate that renal medullary vascular resistance is elevated early in the development of hypertension and suggest that changes in medullary hemodynamics may participate in the resetting of the pressure-natriuresis relationship in the SHR.

Materials and Methods

Experiments were performed on three groups of SHR and WKY that were purchased from Harlan Laboratories (Madison, WI, USA). Group 1 consisted of

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Supported by National Heart, Lung, and Blood Institute Grants HL 29587 and HL 36279. This work was completed during Richard J. Roman’s tenure as an Established Investigator of the American Heart Association.

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fifteen 3- to 5-week-old SHR and 14 age-matched WKY. Group 2 consisted of sixteen 6- to 9-week-old SHR and 17 age-matched WKY. Group 3 consisted of fourteen 12- to 16-week-old SHR and 18 age-matched WKY. The rats were housed in stainless steel cages in an animal care facility at the Medical College of Wisconsin and were fed a rat chow containing 0.4% sodium by weight. Food and water were provided ad libitum. Surgical procedures were conducted according to established principles. The protocols employed in this study were approved by the Animal Care Committee of the Medical College of Wisconsin.

Surgical Procedures

The rats were prepared for measurement of papillary blood flow using a laser-Doppler flowmeter as we have described previously. One week before an experiment, the 6- to 9- and 12- to 14-week-old rats were anesthetized with ketamine (100 mg/kg) and acepromazine (2 mg/kg) and the left kidney was exposed through a flank incision. A small amount of renal cortical tissue overlying the papilla on the dorsal surface of the kidney was surgically removed. The kidney was reinserted into the body, the incisions were closed, and the animal was allowed 1 week for recovery. The creation of this papillary window allowed for the later exposure of the renal papilla after removal of the ureter. In the 3- to 5-week-old rats, the papilla normally protrudes from the kidney and can easily be exposed for microcirculatory studies. Therefore, the young animals did not undergo papillary window surgery before an experiment.

On the day of the experiment, the animals were anesthetized with an intraperitoneal injection of Inactin (100 mg/kg in 6- to 16-week-old rats, 50 mg/kg in 3- to 5-week-old rats) and placed on a heated table to maintain body temperature at 36.5°C. The ureter was cannulated to facilitate breathing, and cannulas were placed in the jugular vein for infusions and in the carotid and femoral arteries for measurement of arterial pressure and above and below the left renal artery. An adjustable occluder was placed on the aorta above the left renal artery, and ties were loosely placed around the mesenteric and celiac arteries and the lower aorta so that RPP could be manipulated by adjusting peripheral vascular resistance. In some animals, a flow probe (outside diameter, 1 mm in 3- to 5-week-old rats; 2 mm in other rats) was placed around the left renal artery so that RBF could be measured using an electromagnetic flowmeter (Model 501, Carolina Instruments, King, NC, USA).

The left kidney was immobilized by placing it dorsal side up in a kidney cup positioned above the abdominal aorta. The ureter, renal vein, and artery were passed through a slot cut in the bottom of the cup. The papilla was exposed by making a longitudinal incision in the ureter from the tip to the base of the papilla. The remnant ureter was then excised where it joins the kidney.

Neural and hormonal influences on renal function were controlled as previously described. Neural influences on the kidney were eliminated by acute renal denervation. The kidney was denervated by stripping the nerve fibers from the renal artery and vein and coating the hilus of the kidney with a 10% solution of phenol in ethanol. Norepinephrine (333 ng/kg/min), aldosterone (66 ng/kg/min), cortisol (33 μg/kg/min), and vasopressin (0.17 ng/kg/min), were infused intravenously to maintain plasma levels of these hormones during the experiment. The hormones 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 throughout the experiment. We have reported that plasma levels of aldosterone were elevated to 200 ng/ml in rats prepared in this manner. Plasma concentrations of vasopressin and norepinephrine were elevated to nonpressor levels, about five times the levels found in conscious rats. Plasma renin activity and plasma concentrations of angiotensin II and atriopeptin III in hormone-infused rats were similar to values measured in conscious rats.

Experimental Protocol

After the operation and a 30-minute equilibration period, the relationships among cortical and papillary blood flow, RBF, and RPP were determined. Systemic arterial pressure was first increased by approximately 25 mm Hg by tying off the celiac and mesenteric arteries. The laser-Doppler flow signals obtained from the renal cortex and the papilla were recorded as RPP was varied from 150 mm Hg down to 50 mm Hg, in steps of 10 mm Hg, by tightening the aortic clamp. The kidney was perfused at each pressure step for 5 minutes or until a steady state cortical and papillary blood flow signal was achieved.

Cortical and papillary blood flow signals were measured using a dual-channel, laser-Doppler flowmeter (Model Pfdl, Perimed KB, Stockholm, Sweden). The gain of the instrument was set on the 3 x scale, and the high frequency cutoff was 12 Hz. The papillary blood flow signal was recorded after placing the fiberoptic probe of the instrument at a 30-degree angle, 1 mm from the tip of the exposed papilla. Cortical blood flow was measured from five locations on the dorsal surface of the kidney, and the mean value at each level of RPP was recorded.

It was not practical to simultaneously measure RBF and papillary blood flow in the young rats because the flow probe interfered with access to the exposed papilla. Therefore, autoregulation of whole kidney RBF was studied in all age groups of SHR and WKY in separate groups of animals. The experimental protocol was similar to that already described, except that the papilla was not exposed.

Statistics

Data are presented as means ± 1 SE. Significance of the difference in values measured at different levels of RPP in the same animal was determined using a two-way analysis of variance followed by a Duncan multiple range test. Significance of the difference in mea-
Results

Group 1: 3- to 5-Week-Old Rats

The mean ages of the SHR and WKY were 3.7 ± 0.1 and 3.8 ± 0.1 weeks, respectively. Body weight of SHR was 68 ± 3 g, and that of WKY was 69 ± 3 g. Left kidney weights were 0.38 ± 0.02 g in SHR and 0.36 ± 0.02 g in WKY. Control mean arterial pressures measured in these Inactin-anesthetized rats before infusion of the hormone cocktail averaged 92 ± 2 mm Hg in SHR and 73 ± 2 mm Hg in WKY. Arterial pressure was not altered by infusion of the hormone cocktail solution in either group.

A comparison of the renal blood flow autoregulatory data in SHR and WKY is presented in Figure 1. Control renal blood flows, outer cortical blood flows, and papillary blood flows measured in SHR at a RPP of 90 mm Hg and in WKY at their control RPP of 70 mm Hg were not significantly different. Whole kidney blood flow and superficial cortical blood flow were autoregulated to a limited extent in SHR over a range of pressures from 80 to 100 mm Hg and from 80 to 120 mm Hg in WKY. In the normal range of pressures for these animals (60–100 mm Hg), RBF was poorly autoregulated in both SHR and WKY. The RBF autoregulatory index, calculated as the percent change in blood flow divided by the percent change in RPP in this range of pressures, was similar in SHR and WKY and averaged 0.75 ± 0.12. According to this analysis, an autoregulatory index of 1 is indicative of a system with a fixed vascular resistance that does not autoregulate. RBF factored per gram kidney weight was highly variable in these young SHR and WKY. This was primarily due to differences in the weight of the kidneys rather than to differences in the measured blood flow. As a consequence, we were unable to detect significant differences in RBF or cortical blood flow in SHR and WKY, even though RBF tended to be lower in SHR at all RPPs studied.

Papillary blood flow was not autoregulated in 3- to 5-week-old SHR or WKY. Over the range of pressures from 60 to 100 mm Hg, the papillary blood flow autoregulatory index averaged 1.20 ± 0.17 in SHR and 1.72 ± 0.23 in WKY. Papillary blood flows were significantly lower in SHR than in WKY when the kidneys were perfused at equivalent RPP over the range of pressures from 80 to 130 mm Hg.

Group 2: 6- to 9-Week-Old Rats

The mean age of the SHR was 7.7 ± 0.2 weeks, and that of the WKY was 7.9 ± 0.3 weeks. Body weight of the SHR rats averaged 167 ± 9 g, and that of the WKY was 144 ± 12 g. Left kidney weight was 0.84 ± 0.03 g in SHR and 0.74 ± 0.06 g in WKY. Control mean arterial pressure was significantly higher in SHR than in WKY and averaged 121 ± 1 and 94 ± 2 mm Hg, respectively.

A comparison of the relationships among RBF, cortical and papillary blood flow, and RPP in SHR and WKY are presented in Figure 2. Control RBF measured at a RPP equal to the mean arterial pressure of the rats was similar in SHR and WKY. In both groups, RBF was well autoregulated in the range of pressures from 80 to 150 mm Hg. No differences in RBF were detected in SHR and WKY at any level of RPP studied. The autoregulatory index was similar in both groups and averaged 0.27 ± 0.06. This value was significantly lower than the RBF autoregulatory index observed in the 3- to 5-week-old rats.

Control superficial cortical blood flow measured in SHR and WKY was also similar. In WKY, cortical blood flow was autoregulated as efficiently as whole kidney blood flow down to an RPP of 80 mm Hg. In SHR, cortical blood flow was only autoregulated down to an RPP of 110 mm Hg, which is higher than the lower limit for autoregulation of whole kidney RBF.

FIGURE 1. Comparison of the relationships among renal blood flow (RBF), superficial cortical blood flow, papillary blood flow, and renal perfusion pressure (RPP) in 3- to 5-week-old SHR and WKY. Asterisk indicates a significant difference from the control value () measured at the animals' spontaneous level of RPP. Dagger indicates a significant difference in the measured values at a similar level of RPP. Cortical and papillary blood flows were studied in eight SHR and eight WKY. RBF was measured in a separate group of seven SHR and six WKY.
that was observed in these animals. In general, the relationships between cortical blood flow and RPP in SHR and WKY were similar except at an RPP of 80 mm Hg.

Marked differences were observed in the laser-Doppler flow signals recorded from the renal papilla of SHR and WKY. Control papillary blood flow was 30% lower in SHR than in WKY, even though the control RPP was 30 mm Hg greater in these animals. When RPP was lowered to 94 mm Hg, a pressure equivalent to the control RPP of WKY, papillary blood flow in SHR was less than half that observed in WKY. Similar to the results in the 3- to 5-week-old rats, papillary blood flow was not autoregulated in 6- to 9-week-old SHR and WKY. The autoregulatory index calculated from the papillary blood flow data was 1.35 ± 0.15 in SHR and 0.86 ± 0.07 in WKY.

Group 3: 12- to 16-Week-Old Rats

The ages of the SHR and WKY were similar and averaged 15.0 ± 0.4 and 15.1 ± 0.4 weeks, respectively. Mean body weight of the SHR was 278 ± 7 g and that of WKY was 302 ± 10 g. Left kidney weight was 1.27 ± 0.03 g in SHR and 1.38 ± 0.05 g in WKY. Control mean arterial pressure measured prior to infusion of the hormone cocktail was 157 ± 3 mm Hg in SHR and 113 ± 2 mm Hg in WKY.

The blood flow results for the 12- to 16-week-old SHR and WKY are presented in Figure 3. Control RBF was slightly lower but not significantly different in SHR and WKY, averaging 5.7 ± 0.6 and 6.3 ± 0.7 ml/min/g kidney weight, respectively. In both SHR and WKY, RBF was autoregulated over the range of pressures from 100 to 150 mm Hg. The efficiency of autoregulation was greater in WKY than in SHR. The autoregulatory index was 0.22 ± 0.09 in WKY and 0.42 ± 0.14 in SHR. In WKY, the lower limit of RBF autoregulation was 80 mm Hg. In SHR, the lower limit for autoregulation of RBF was 100 mm Hg.

Similar results were observed in regard to the auto-
regulation of superficial cortical blood flow in these rats. The control cortical blood flows measured in SHR and WKY were not significantly different. Cortical blood flow tended to be slightly lower in SHR than in WKY, but in both groups it was autoregulated down to an RPP below 100 mm Hg.

The control blood flow signals recorded from the papilla of SHR and WKY were not significantly different. In both SHR and WKY, papillary blood flow was poorly autoregulated and varied directly with RPP. The papillary blood flow autoregulatory index in both groups was similar and averaged 1.09 ± 0.06. For any given level of RPP, in the range of pressures from 80 to 180 mm Hg, papillary blood flow was significantly lower in SHR than in WKY by about 30%.

Discussion

We have recently demonstrated that the relationship between sodium excretion and RPP is altered very early in, or prior to, the development of hypertension in SHR and Dahl salt-sensitive rats. The present study examined whether changes in papillary blood flow were associated with resetting the pressure-natriuresis relationship during the development of hypertension in SHR. The results indicate that the relationship between RPP and papillary blood flow is altered early in the development of hypertension in the SHR. Papillary blood flows measured at equivalent RPP were significantly lower by about 30% in all age groups of SHR compared with values measured in WKY (see Figures 1–3). These findings indicate that medullary vascular resistance is elevated in SHR. An expected consequence of this elevated vascular resistance is that arterial pressure would have to rise to normalize blood flow in the vasa recta of the SHR. In the present study, mean arterial pressure was already elevated by 19 mm Hg in the 3- to 5-week-old SHR in comparison to the pressure observed in age-matched WKY. Control papillary blood flows were similar in 3- to 5- and 12- to 16-week-old SHR and WKY when the kidneys were perfused at a RPP equal to their different mean arterial pressures. Thus, in the very young and adult SHR, in which arterial pressure is changing slowly, the degree of hypertension appears to be appropriate to restore normal perfusion of the papilla and maintain sodium balance.

In contrast, control papillary blood flow was significantly lower in the 6- to 9-week-old SHR compared with age-matched WKY (see Figure 2). The reason for this difference is that medullary vascular resistance declined and papillary blood flow increased markedly in the WKY as they matured. The relationship between papillary blood flow and RPP, however, remained relatively constant in the SHR as they matured. In this regard, Beierwaltes et al. demonstrated that the development of hypertension in SHR was associated with greater sodium and water retention than was observed in WKY. Others have shown that increasing sodium intake in young SHR accelerated the development and the severity of hypertension. Taken together, these observations suggest that the low papillary blood flow in the 6- to 9-week-old SHR may be related to their inability to maintain normal sodium balance and to the rapid development of hypertension.

The role of changes in papillary blood flow in the development of hypertension has not been widely studied. Ganguli et al. reported that papillary blood flow, measured using the albumin accumulation technique, was lower in 17-week-old SHR compared with WKY. In the present study, control papillary blood flows were not different in 12- to 16-week-old SHR and WKY. We used younger rats than did Ganguli et al. Sustained hypertension may have caused greater vascular damage in the older rats used in the previous study. This possibility is consistent with data indicating that hypertension selectively injures juxtamedullary glomeruli. Overall, our results strongly support the original conclusion of Ganguli et al. that changes in the medullary circulation alter tubular sodium reabsorption and participate in the development of hypertension in this model.

In the present study, papillary blood flows were measured after the ureter was removed to expose the papilla. Exposure of the papilla has been reported to elevate papillary blood flow and increase the intrarenal production of prostaglandins. It is not known whether exposure of the papilla has different effects on renal medullary hemodynamics in SHR and WKY. Nor is it clear whether this maneuver accentuated or diminished the differences in papillary blood flow that were observed in SHR and WKY in the present study.

Control renal blood flows were similar in all age groups of SHR and WKY, even though control RPPs were elevated in SHR (see Figures 1–3). This finding indicates that basal renal vascular resistance is elevated in all age groups of SHR. This new finding in young SHR extends the results of previous studies indicating that renal vascular resistance and reactivity are elevated in adult SHR.

To our knowledge, autoregulation of RBF has not been studied in young SHR. We found that 6- to 9- and 12- to 16-week-old SHR autoregulate RBF down to normotensive pressures (100 mm Hg) as efficiently as WKY. The 3- to 5-week-old SHR and WKY did not autoregulate RBF as well as did older animals. However, no significant differences in RBF or cortical blood flow were detected in young SHR and WKY at any RPP studied. These results are in general agreement with previous results in adult SHR and suggest that a defect in whole kidney RBF is not responsible for the abnormal pressure-natriuresis relationship in these animals.

RBF was not significantly different in 6- to 9-week-old SHR and WKY in the present study. This finding differs from those of Dilley and colleagues, who reported that GFR and RBF were markedly reduced in 6- to 9-week-old euvoicemic SHR compared with WKY. They suggested that the renal vasoconstriction in the SHR was due to an enhanced tubuloglomerular feedback response. Since SHR have been reported to have an elevated renal sympathetic tone and exhibit an enhanced vascular reac-
tivity to catecholamines, the difference in the results may depend on the presence or absence of an intact renal innervation or be related to the fact that plasma levels of renal hormones were controlled in our study, whereas these factors were not fixed in the previous studies. In addition, the source of the rats differed in the studies and different methods were used to measure RBF. Regardless of the reason for the discrepancy, any excess renal vasoconstriction in SHR due to an elevated renal sympathetic tone or circulating levels of vasoconstrictor hormones should only accentuate the differences in papillary blood flow in SHR and WKY reported in the present study.

To our knowledge, autoregulation of papillary blood flow in SHR and WKY has not been studied previously, and only a few studies have addressed this issue in normal animals. The present results indicate that papillary blood flow was not autoregulated as well as was cortical blood flow or RBF in volume-expanded, hormonally controlled SHR and WKY. These results confirm our previous findings in Sprague-Dawley rats. They differ from the results of one study indicating that the velocity of red blood cells in the vasa recta circulation remained constant after RPP was lowered from 120 to 80 mm Hg in hydropenic Sprague-Dawley rats. The reason for the discrepant results is unknown. Papillary blood flow could be altered in the absence of changes in red blood cell velocity if the number of perfused vasa recta capillaries is altered by changes in RPP. It is also possible that the efficiency of papillary blood flow autoregulation may vary with the sodium and water balance of an animal and other experimental conditions.

Recent studies in our laboratory suggest a hypothesis for the mechanism of pressure diuresis that can relate changes in papillary blood flow and sodium and water excretion. The present findings suggest that increases in RPP are transmitted in part to the vasa recta. In other studies, we found that vasa recta capillary pressure increased from 6 to 16 mm Hg after RPP was varied from 100 to 150 mm Hg. Elevations in vasa recta capillary pressure of this magnitude may inhibit water reuptake from the medullary interstitium and increase medullary interstitial pressure. An increase in medullary interstitial pressure may participate in the pressure-natriuresis response by inhibiting tubular backleak of ions in deep nephron segments.

The present study suggests that renal medullary vascular resistance is elevated in SHR. If the pressure-natriuresis response is mediated by changes in medullary hemodynamics, at a given RPP, vasa recta capillary and medullary interstitial pressure should be lower and sodium reabsorption in the deep nephrons should be elevated in SHR compared with WKY. Moreover, the pressure-natriuresis response of SHR should be blunted, since they would require a greater increase in RPP to elevate medullary interstitial pressure and inhibit sodium reabsorption. Thus, the observed changes in medullary hemodynamics in young SHR could explain the shift in the pressure-natriuresis relationship and may participate in the development of hypertension in these animals.

Acknowledgment
The authors thank Michelle Rossa for assisting in the preparation of this manuscript.

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Renal cortical and papillary blood flow in spontaneously hypertensive rats.
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Hypertension. 1988;11:657-663
doi: 10.1161/01.HYP.11.6.657

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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