Renal Cortical and Papillary Blood Flow in Spontaneously Hypertensive Rats

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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.

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...
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 stripping of 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 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.

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.

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-
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sured values at similar levels of RPP in SHR and WKY was evaluated using an unpaired t test.16

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 0 indicates a system that exhibits perfect autoregulation of RBF, whereas 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.
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-

FIGURE 2. Comparison of the relationships among renal blood flow (RBF), superficial cortical blood flow, papillary blood flow, and renal perfusion pressure (RPP) in 6- to 9-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 seven SHR and nine WKY. RBF was measured in a separate group of nine SHR and eight WKY.

FIGURE 3. Comparison of the relationships among renal blood flow (RBF), superficial cortical blood flow, papillary blood flow, and renal perfusion pressure (RPP) in 12- to 15-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 seven SHR and eight WKY. RBF was measured in a separate group of seven SHR and 10 WKY.
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 and others, who reported that GFR and RBF were markedly reduced in 6- to 9-week-old euvoilemic 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 reni
interdiction or be related to the fact that plasma lev
of renal hormones were controlled in our study
wheras 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 vaso-
constrictor hormones should only accentuate the differ-
ences 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-expand-
ed, hormonally controlled SHR and WKY. These res-
ults confirm our previous findings in Sprague-Dawley
rats. They differ from the results of one study indi-
cating that the velocity of red blood cells in the vasa
recta circulation remained constant after RPP was low-
ered 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 ex-
perimental conditions.

Recent studies in our laboratory suggest a hypothe-
sis 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 capil-
ary 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 in-
crease in medullary interstitial pressure may partici-
pate in the pressure-natriuresis response by inhibiting
vascular backleak of ions in deep nephron seg-
ments.

The present study suggests that renal medullary vac-
sular resistance is elevated in SHR. If the pressure-
natriuresis relationship is fixed by changes in medul-
ary 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 pres-
sure and inhibit sodium reabsorption. Thus, the ob-
served 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.

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References
1. Kawabe K, Wantanabe TX, Shiono K, Sokabe H. Influence on
renal blood flow of reni isografts between spontaneously hyperten-
sive and normotensive rats, utilizing the F1 hybrids. Jpn
Heart J 1979;20:886-894
2. Bianchi G, Fox U, DiFrancesco GF, Giovannetti AM, Pagetti
D. Blood pressure changes produced by kidney cross-
transplantation between spontaneously hypertensive rats and
3. Onviki P, Tarazi RC, Bravo EL. Regulation of sodium balance in
4. Roman RJ. Abnormal renal hemodynamics and pressure-
natriuresis relationship in Dahl salt-sensitive rats. Am J Physiol
1986;251:F57-F65
5. Roman RJ, Cowley AW Jr. Abnormal pressure-diuresis-
natriuresis response in spontaneously hypertensive rats. Am J
Physiol 1985;248:F199-F205
6. Roman RJ. Altered pressure-natriuresis relationship in young
spontaneously hypertensive rats. Hypertension 1987;9(suppl:
III):130-136
pressure on sodium reabsorption from the proximal tubules of
superficial and deep nephrons. Am J Physiol 1986;250:F425-
F429
8. Chen PS, Caldwell RM, Hsu CH. Role of renal papillae in the
regulation of sodium excretion during acute elevation of renal
perfusion pressure in the rat. Hypertension 1984;6:893-898
9. Takezawa K, Cowley AW Jr, Skelton M. Roman RJ. Atri-
opin III alters renal medullary hemodynamics and the pres-
sure-diuresis response in rats. Am J Physiol 1987;252:F992-
F1002
10. Roman RJ. Pressure diuresis mechanism in the control of renal
function and arterial pressure. Fed Proc 1986;45:2878-2884
11. Roman RJ. Role of increases in vasa recta pressure and flow in
the pressure diuresis response [Abstract]. Kidney Int 1985;
27:298
12. Guidelines for the care and use of laboratory animals. Revised
NIH publication no 85-23
13. Roman RJ, Smits C. Laser-Doppler determination of papillary
251:F115-F124
the study of pressure-natriuresis in the rat. Am J Physiol
1985;248:F190-F198
1981:87-88
16. Sneedor GW, Cochran WG. Statistical methods. Ames, IA:
Iowa State University Press, 1978:104-106
17. Beierwaltes WH, Arendshorst WJ, Klemmer PJ. Electrolyte
and water balance in young spontaneously hypertensive rats.
Hypertension 1982;4:908-913
18. Aoki K, Yamori Y, Ooshima A, Okamoto K. Effects of high or
low sodium intake in spontaneously hypertensive rats. Jpn
Circ J 1972;36:539-545
19. Ganguli M, Tobian L, Dahl L. Low renin papillary plasma
flow in both Dahl and Kyoto rats with spontaneous hyperten-
20. Feld LG, Van Liew JB, Galaske RG, Boylan JW. Selectivity of
renal injury and proteinuria in the spontaneously hyperten-
hyperfiltration in uninephrectomized spontaneously hyperten-
22. Chuang EL, Reineck HJ, Osgood RW, Kunau RT Jr. Stein JH.
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