Enhanced Vascular Tone in the Renal Vasculature of Spontaneously Hypertensive Rats

Debebe Gebremedhin, Francisco J. Fenoy, David R. Harder, and Richard J. Roman

The renal microvascular responses of Wistar-Kyoto and spontaneously hypertensive rats to changes in perfusion pressure were compared using a juxtamedullary nephron microvascular preparation perfused in vitro with a physiological salt solution containing 5% albumin. In the spontaneously hypertensive rats, the internal diameters of arcuate and interlobular arteries and the proximal and distal afferent arterioles averaged 307±26, 52±2, 24±0.9, and 22±1.2 μm, respectively, at 80 mm Hg. They were 18–35% smaller (p<0.05) than the corresponding vessels measured in Wistar-Kyoto rats. In low calcium media, the arcuate and interlobular arteries and the proximal and distal afferent arterioles of spontaneously hypertensive rats exhibited a greater dilation than the vessels of Wistar-Kyoto rats. These observations suggest that the diameters of the preglomerular vasculature of the spontaneously hypertensive rats are reduced because of an elevated vascular tone rather than structural changes narrowing the lumen of these vessels. These results suggest that enhanced vascular tone in the preglomerular vasculature of juxtamedullary nephrons may contribute to the elevated renal medullary vascular resistance and resetting of the pressure-natriuretic relation previously observed in spontaneously hypertensive rats. (Hypertension 1990;16:648–654)

Renal transplantation studies have indicated that some form of renal dysfunction underlies the development of genetic hypertension.1,2 Nevertheless, the factors that contribute to the “resetting of the kidney in hypertension” remain unknown. Numerous differences in renal function of spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats have been described; these include an elevated renal vascular resistance,3-5 enhanced renal vascular reactivity to vasoconstrictors,4,5 enhanced tubuloglomerular feedback response,6 and structural changes that increase the wall-to-lumen ratio in the renal vasculature.7,8 Because most of these changes have been identified in the established phase of hypertension, it remains unclear whether these changes are a cause or a consequence of the disease.

We recently reported that the pressure-natriuretic response is blunted in SHR and Dahl salt-sensitive rats before the development of hypertension.9,10 In the SHR, the abnormality in the pressure-natriuretic response is associated with a reduction in papillary blood flow11 and renal interstitial hydrostatic pressure.12 These observations suggest that an elevation in renal medullary vascular resistance may participate in the resetting of the renal function toward higher pressures in SHR.11

The factors responsible for reducing papillary blood flow in SHR are unknown. Medullary blood flow is derived exclusively from the perfusion of deep nephrons. Thus, structural changes that narrow the lumen of the preglomerular vasculature of juxtamedullary nephrons or alterations in the reactivity of these vessels could be responsible for the reduction of papillary blood flow in SHR. The recent development of the in vitro perfused juxtamedullary nephron microvascular preparation by Casselas and Navar13 and Casselas et al14 now allows for direct study of the preglomerular vasculature of deep nephrons in the rat. In the present study, this preparation was used to compare the pressure–diameter relations of the preglomerular renal vasculature of SHR and WKY rats under control conditions and after blockade of vascular tone using a low calcium solution. In addition, the contribution of eicosanoids to the enhanced vascular tone in the SHR was examined by studying the effects of the cyclooxygenase inhibitor indomethacin on the pressure–diameter relations of the preglomerular juxtamedullary vasculature of SHR and WKY rats.
Methods

Experiments were performed on 28 SHR and 28 WKY rats weighing between 210 and 350 g. At the time of the study, the rats were 8–13 weeks of age. The rats were anesthetized with pentobarbital (65 mg/kg body wt i.p.), and a cannula was placed in the femoral artery for measurement of arterial pressure. Mean arterial pressure averaged 139±4 mm Hg in the SHR and 99±2 mm Hg in WKY rats. The left kidney was isolated for study of juxtamedullary vasculature according to the method of Casellas and Navar as modified by Sanchez-Ferrer et al. A double lumen catheter was inserted in the abdominal aorta and advanced into the left renal artery near the hilus of the kidney. One of the channels was connected to a syringe pump for perfusion, and the other channel was used to measure perfusion pressure. The kidney was perfused with physiological salt solution containing (mM): NaCl 116, CaCl2 2.5, KCl 3, MgCl2 0.76, KH2PO4 1.7, NaHCO3 25, glucose 11, N-(2-hydroxyethyl)piperazine-N’-2-ethanesulfonic acid (HEPES) buffer 5, and 5% bovine serum albumin. The perfusate had an osmotic pressure of 290 mosm/kg H2O. Perfusion pressure was continuously monitored using a x 62.5, and magnification to the screen was approximately x900.

After the control pressure–diameter relations were determined, the effects of removal of calcium from the bath and the perfusion solutions on vascular diameters were studied in some preparations to determine the degree of vascular tone. In these experiments, measurements were obtained after perfusion and superfusion of the preparation with a low calcium physiological salt solution for 15 minutes. The low calcium solution was identical to the physiological salt solution described earlier except that the 2.5 mM CaCl2 was not included.

In other experiments, the contribution of cyclooxygenase products to the development of vascular tone was assessed. Control pressure–diameter relations were obtained, and then indomethacin (10 μM) was added to the perfusate and the bath. After a 30-minute equilibration period, the pressure–diameter relations were redetermined.

Statistics

The results are expressed as vessel diameters in microns or as a percentage change from the control diameter of each vessel measured at 80 mm Hg when it was perfused and bathed with physiological salt solution. Mean±SEM values are presented. Significance of differences between mean values was evaluated using paired analysis of variance for repeated measures followed by Duncan’s multiple range test. A value of p<0.05 was considered statistically significant.

Drugs and Chemicals

All chemicals were analytical grade. Trypan blue, bovine serum albumin, indomethacin, and FITC-labeled gamma globulin were purchased from Sigma Chemical Co., St. Louis, Mo.
Results

The effects of changes in perfusion pressure on the diameters of arcuate and interlobular arteries of SHR and WKY rats are summarized in Figure 1. An elevation in perfusion pressure from 80 to 160 mm Hg produced different effects on the renal vessels in the two groups of rats. At 80 mm Hg, respective inner diameters of arcuate and interlobular arteries averaged 307 ±26 and 52 ±2 μm in the SHR and 365 ±23 and 64 ±3 μm in the WKY rats. The control vessel diameters were significantly different in the two groups. The diameters of arcuate and interlobular arteries of the WKY rats increased by 31% and 23%, respectively, when perfusion pressure was increased from 80 to 160 mm Hg. In the SHR, the diameters of these same vessels increased by only 15% and 13% in response to the same rise in perfusion pressure. The slopes of the pressure-diameter curves for the arcuate arteries (WKY, 2.052±0.06 μm/mm Hg, n=23; SHR, 0.675±0.01 μm/mm Hg, n=22; p<0.05) and the interlobular arteries (WKY, 0.2063±0.005 μm/mm Hg, n=28; SHR, 0.0969±0.001 μm/mm Hg, n=34; p<0.05) were significantly different in the two groups. In contrast, an elevation in perfusion pressure caused a 10% reduction in the diameters of the proximal and distal portions of the afferent arteriole in both SHR and WKY rats (Figure 2). In addition, the diameter of the afferent arteriole in SHR was significantly smaller than the corresponding vessel diameter in WKY rats at all pressures studied.

To assess the degree of vascular tone, the pressure-diameter relations of the preglomerular vasculature of SHR and WKY rats were compared when perfused and bathed with a low calcium solution. As depicted in Figure 3, all elements of the renal vasculature of the SHR exhibited a dilatory response to a nominally calcium-free solution. In contrast, in WKY rats (Figure 4) the low calcium solution dilated the proximal and distal portions of the afferent arterioles, whereas it had no significant effect on the pressure-diameter relation of arcuate and interlobular arteries. Moreover, the degree of dilation was significantly greater in the renal vasculature of SHR than in WKY rats. The diameters of the renal vessels of SHR increased by only 19±2% to 33±2% at all pressures studied, whereas those of WKY rats increased by only 3±1% to 7±1%. This difference was significant at p<0.01. Furthermore, treatment with low calcium solution completely eliminated the differences in pressure-diameter relations of the renal vasculature of SHR and WKY rats (Figure 5) that were observed when the vessels were bathed in the control period with the physiological salt solution.
containing calcium. In calcium-free solution, the diameters of all elements of the preglomerular vasculature of the SHR and WKY rats were not significantly different at any pressure measured.

To determine the contribution of cyclooxygenase products to the elevated vascular tone in the SHR, the effects of indomethacin on the renal vasculature of SHR and WKY rats were studied and are presented in Table 1. At all levels of pressure studied, indomethacin had no effect on the pressure-diameter relations of the renal vasculature of SHR. In the WKY rats, however, indomethacin produced a substantial increase (3–14%) in diameter of the preglomerular renal vasculature at all levels of perfusion pressure studied.

**Discussion**

Renal transplantation studies have indicated that renal dysfunction underlies the development of genetic models of hypertension. The kidneys of genetically hypertensive rats require an elevated perfusion pressure to excrete equivalent amounts of sodium as normotensive controls. However, the factors responsible for resetting the kidney in hypertension are unknown. The recent finding that papillary blood flow is reduced in SHR before the development of hypertension suggests that an elevation in renal medullary vascular resistance may participate in the development of this disease. The present study examined whether structural changes or alterations in the vascular tone of the preglomerular vasculature of juxtamedullary nephrons could explain the decrements in renal medullary hemodynamics and the pressure-natriuretic response in the kidney of SHR.

The results of the present study indicate that there are significant differences in the pressure-diameter...
relations in the preglomerular vasculature of juxta-medullary nephrons in SHR and WKY rats. The diameters of the arcuate and interlobular arteries and the proximal and distal portions of the afferent arterioles in SHR were all significantly smaller than the diameters of the corresponding vessels in WKY rats at all perfusion pressures studied. In addition, the increases in the diameters of the arcuate and interlobular arteries of the SHR in response to an elevation in perfusion pressure were significantly smaller than those seen in WKY rats. This indicates that either the renal vasculature of SHR generates more active tone in response to elevations in perfusion pressure or there are structural changes in the preglomerular vasculature of SHR that makes them less compliant.

To determine whether the diameters of the preglomerular vasculature of deep nephrons of SHR were smaller than those observed in WKY rats because of structural changes, the effects of a low calcium solution on the renal vasculature of SHR and WKY rats were compared. In SHR, removal of calcium from the perfusion solution dilated all elements of the renal vasculature, indicating the presence of active vascular tone. In WKY rats, removal of calcium from the bath and perfusate had no effect on the arcuate and interlobular arteries and produced significantly less dilation of the afferent arterioles than that seen in SHR. Thus, the low calcium solution completely eliminated the differences in pressure-diameter relations of the preglomerular vasculature of SHR and WKY rats (Figure 5) that were

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**Figure 5.** Line graphs showing comparison of effects of low calcium solution on pressure-diameter relations of arcuate and interlobular arteries and proximal and distal portions of afferent arterioles of spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. *Indicates significant difference from diameter measured at 80 mm Hg. There is no significant difference in vessel diameters in SHR and WKY rats at any of the pressures studied.

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<th>Table 1. Effects of Indomethacin (10 μM) on Pressure-Diameter Relations in Preglomerular Vasculature of Spontaneously Hypertensive Rats and Wistar-Kyoto Rats</th>
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Values are mean±SEM of vascular diameters expressed in microns. n, number of vessels in each group. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

*Significant difference (p<0.05) from the control values measured at the same level of perfusion pressure within a group.
vascular reactivity to vasoconstrictors are elevated in vascular tone in SHR. In contrast, indomethacin of other studies indicating that the wall-to-lumen Hypertrophy of the muscle layers in renal vessels, indeed, could contribute to the elevated renal vascular tone in SHR and still be consistent with present results in low calcium solutions as long as the thickening of the vascular wall does not narrow the lumen of maximally dilated vessels. This possibility is supported by the recent report that the hypertrophy of the wall of renal vessels in SHR does not encroach on the lumen. Recent studies have indicated that a tubuloglomerular feedback response mediates the pressure-dependent contraction of distal afferent arterioles in the isolated perfused juxtamedullary nephron preparation and contributes importantly to the autoregulation of glomerular capillary pressure. Other studies by Dilley and Arendshorst have indicated that an abnormality in the tubuloglomerular feedback response may be responsible for a portion of the elevated renal vascular resistance in SHR. Our findings of an elevated vascular tone in the distal afferent arterioles of juxtamedullary nephrons in SHR are consistent with this hypothesis; however, the relative contribution of changes in the myogenic response, tubuloglomerular feedback, or levels of autacoids to this abnormality remains to be determined. To examine the possibility that the augmented vascular tone in the renal vasculature of SHR was due to an altered production of eicosanoids, the effects of the cyclooxygenase inhibitor indomethacin on the pressure-diameter relations were studied. Indomethacin had no effect on the response of the renal vasculature of SHR to elevations in perfusion pressure. This finding argues against the view that an elevated production of vasoconstrictor cyclooxygenase products (i.e., thromboxane, endoperoxides, and prostaglandin F₂α) accounts for the enhanced renal vascular tone in SHR. In contrast, indomethacin significantly increased vascular diameters in WKY rats and accentuated the differences in the pressure-diameter relations in the preglomerular vasculature of SHR and WKY rats. These findings indicate that contractile cyclooxygenase products probably contribute to the development of active myogenic vascular tone in the juxtamedullary vasculature of WKY rats; the lack of a dilatory response to indomethacin in SHR further suggests that either these contractile eicosanoids are not produced or the responsiveness of the renal vessels to these substances may be reduced in hypertensive rats. Previous studies have indicated that the pressure-natriuretic response is altered in SHR and that this is associated with a reduction in papillary blood flow. Furthermore, studies on the mechanism of pressure-natriuresis indicated that it is associated with changes in renal medullary hemodynamics, renal interstitial pressure, and inhibition of tubular reabsorption in deep nephrons. The present finding suggests that an elevated vascular tone in the preglomerular vasculature of juxtamedullary nephrons of SHR may account for the elevated renal medullary vascular resistance in this model of hypertension. The increase in preglomerular vascular resistance in deep nephrons would be expected to lower pressures in the vasa recta circulation and reduce renal medullary interstitial pressure. These changes would dictate that the kidney of the SHR requires an elevated perfusion pressure to normalize vasa recta capillary pressure, renal medullary interstitial hydrostatic pressure, and sodium excretion. A recent publication by Hayashi et al indicated that in the hydronephrotic kidney, the preglomerular vasculature of SHR and WKY rats exhibits similar myogenic vasoconstrictor responses to elevations in perfusion pressure; however, the SHR required a higher pressure to elicit these responses. These results suggest that the renal vasculature of the SHR adapts to the hypertension and argue against the view that an elevated renal vascular reactivity participates in the development of hypertension. The results of the present study are not consistent with those obtained in the hydronephrotic kidney model and probably reflect differences in vascular reactivity in the different experimental preparations. In the hydronephrotic kidney, renal tubular tissue is destroyed; thus, the tubuloglomerular feedback response is absent, and the influence of parenchymal factors on renal vascular tone may also be altered. On the other hand, it should also be recognized that the in vitro physiological salt solution-perfused juxtamedullary microvascular preparation also has limitations, as the kidney is not perfused with blood. Recent preliminary work in our laboratory suggests that our preparation may not autoregulate single nephron glomerular filtration rate and renal blood flow as efficiently as an intact kidney. Nevertheless, tubular-vascular interactions are preserved, and we have shown that both tubuloglomerular feedback and myogenic mechanisms contribute to the vascular response to increases in perfusion pressure in this preparation. In summary, the results of the present study indicate that vascular tone is elevated in the preglomerular vasculature of juxtamedullary nephrons of SHR perfused in vitro with a physiological salt solution. These changes in vascular tone, if they exist in vivo, could contribute to the previously observed alterations in vasa recta hemodynamics and the resetting of the pressure-natriuretic relation in SHR.
References


KEY WORDS • renovascular hypertension • microcirculation • glomerular function • hemodynamics • kidney
Enhanced vascular tone in the renal vasculature of spontaneously hypertensive rats.
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Hypertension. 1990;16:648-654
doi: 10.1161/01.HYP.16.6.648

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

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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