Pressure-Induced Vasoconstriction of Renal Microvessels in Normotensive and Hypertensive Rats

Studies in the Isolated Perfused Hydronephrotic Kidney

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The capacity of small arteries to respond to increased intravascular pressure may be altered in hypertension. In the kidney, hypertension is associated with a compensatory shift in the autoregulatory response to pressure. To directly determine the effects of established hypertension on the renal microvascular response to changes of perfusion pressure, we evaluated pressure-induced vasoconstriction in hydronephrotic kidneys isolated from normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). Vessel diameters of interlobular arteries (ILAs) and afferent and efferent arterioles were determined by computer-assisted videomicroscopy during alterations in renal arterial pressure (RAP) from 80 to 180 mm Hg. Increased RAP induced a pressure-dependent vasoconstriction in preglomerular vessels (afferent arterioles and ILAs), but not in postglomerular vessels (efferent arterioles). The calcium antagonist nifedipine prevented pressure-induced afferent arteriolar vasoconstriction with a similar half-maximal inhibitory concentration (IC50) (WKY, 63 ± 27 vs. SHR, 60 ± 32 nM). The pressure-activation curves for ILAs in SHR and WKY were similar. In contrast, the pressure-activation curve for afferent arterioles in SHR kidneys exhibited a rightward shift, which was observed at every segment of the afferent arteriole (i.e., near ILA, at midportion, and near glomerulus). These findings demonstrate that the ILA and the afferent arteriole both possess the ability to constrict in response to increased pressure, whereas this property is lacking in the efferent arteriole. Hypertension was associated with a compensatory shift in the pressure response of the afferent arteriole, such that higher RAPs were required to elicit vasoconstriction in this vessel. (Circulation Research 1989;65:1475-1484)

The kidney exhibits a remarkable ability to maintain blood flow and glomerular filtration rate constant when renal perfusion pressure is altered.1-2 Multiple factors contribute to this autoregulatory response, including tubuloglomerular feedback3 and renal autacoids.4 Nevertheless, when these factors are blocked, renal autoregulation continues to be partially maintained, suggesting that renal autoregulation is mediated in part by intrinsic properties of the renal microvessels.5,6 Changes in vascular tone in response to alterations in perfusion pressure have been directly confirmed in several circulatory beds, including those of skeletal muscle7 and brain.8 In contrast, the responsiveness of the renal microvasculature to changes of renal arterial pressure (RAP) has not been delineated fully. Furthermore, although the kidney plays a prominent role in initiation and maintenance of hypertension, it has not been ascertained whether the pressure response of renal microvessels differs in normotensive and hypertensive animals.

The observation that both glomerular filtration rate and renal blood flow exhibit autoregulation indicates that preglomerular microvessels are primarily responsible for renal autoregulation.9 Controversy remains, however, regarding the relative contributions of the afferent arteriole and the interlobular artery (i.e., cortical radial artery10) to the preglomerular component of the renal autoregulatory response.11-13 Furthermore, although a resetting of renal perfusion pressure and flow relations has been demonstrated in hypertensive animals,14 the precise sites within the renal microvasculature responsible for this resetting of autoregulation have not been delineated.

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In the present study, we examined directly the renal microvascular responses to altered renal perfusion pressure by use of a unique in vitro model, the isolated perfused hydronephrotic kidney. With this preparation, we compared the responses of renal microvessels to changes of perfusion pressure in kidneys from normotensive and hypertensive rats under identical conditions. In addition, we evaluated the effects of nifedipine, a dihydropyridine calcium antagonist, on the pressure response of the afferent arteriole to ascertain if dihydropyridine-sensitive calcium channels are involved in this response.

**Materials and Methods**

**Preparation of Donor Animals**

Chronic hydronephrosis was established for subsequent visualization of the renal microcirculation in isolated perfused kidneys, as described previously. Six-week-old male Wistar-Kyoto rats (WKY) and stroke-prone spontaneously hypertensive rats (SHR) were anesthetized with ether. The right ureter of each animal was ligated through a flank incision. After 8–10 weeks, at which time renal tubular atrophy had progressed to a stage that allowed direct microscopic visualization of renal microvessels, the kidneys were removed for study.

On the day of harvesting the hydronephrotic kidneys, the systolic blood pressures of the conscious donor animals were measured by tail-cuff sphygmomanometry (model KN-210-1, Natsume, Tokyo, Japan). To minimize experimental errors, averages were obtained of at least five measurements.

**Perfusion of Hydronephrotic Kidneys**

Donor animals were anesthetized with 5-sec-butyl-5-ethyl-thiobarbituric acid (Inactin, 100 mg/kg; Byk-Gulden, Constance, Germany), and the abdominal cavity was exposed by midline incision. The renal artery of the hydronephrotic kidney was cannulated in situ across the aorta through the abdominal cavity was exposed by midline incision. The renal artery of the hydronephrotic kidney was cannulated in situ across the aorta through the superior mesenteric artery. Warm oxygenated medium was perfused throughout the cannulation procedure. The hydronephrotic kidney was excised and placed on the stage of an inverted microscope (model K, Nikon, Tokyo, Japan) modified to accommodate a heated chamber equipped with a thin glass viewing port on the bottom surface. Kidneys were allowed to equilibrate for at least 30 minutes before initiation of experimental manipulations.

Kidneys were perfused with medium consisting of a Krebs-Ringer bicarbonate buffer containing 5 mM D-glucose, 7.5% bovine serum albumin (Bovuminar, Armour Pharmaceutical, Kankakee, Illinois), and a complement of amino acids as described previously. The perfusion apparatus is illustrated in a previous publication. The perfusion medium was saturated with a gas mixture of 95% O2/5% CO2 within a pressurized reservoir. The perfusion pressure, monitored at the level of the renal artery, was altered by adjustment of the back-pressure-type regulator (model 10BP, Fairchild Industrial Products, Winston-Salem, North Carolina), which controlled the exit of gas from the media reservoir. Perfusion flow was monitored by means of an extracorporeal electromagnetic flow probe (model 300A, Carolina Medical Electronics, King, North Carolina) placed in the perfusion circuit immediately proximal to the kidney.

**Determination of Vessel Diameters**

Microvessels were selected for observation on the basis of adequate perfusate flow. Flow was assessed by observation of the rate of collapse and recovery of the vessels in response to a temporary clamping of the perfusion line between the pressure reservoir and kidney. Vessels with diminished perfusion exhibited a sluggish and blunted response to this manipulation and were excluded from study. In practice, well over 90% of the preparations responded uniformly to pressure, and a lack of responsiveness was generally associated with an intravascular occlusion.

The regional responses of each afferent arteriole were assessed at three anatomically defined sites: 1) near the interlobular artery (ILA), 2) at the midportion of the arteriole, and 3) near the glomerulus. Segments of the efferent arteriole near its emergence from the glomerulus were measured during alterations in perfusion pressure. The responses of ILAs near the origin of selected afferent arterioles were also assessed. Based on their diameters (approximately 30 μm), these portions of the ILA were thought to represent the terminal portions of the ILA (i.e., of the superficial cortex before induction of hydronephrosis).

Vessel diameters were measured as detailed in a previous publication. In brief, video images from a video camera (model ITC-47, Ikegami, Tokyo, Japan) were recorded with a videocassette recorder and transmitted to an IBM AT computer equipped with a video acquisition and display board (model IVG-128, Datacube, Peabody, Massachusetts). Vessel diameters were estimated with an automated program custom designed to permit determination of the mean distance between parallel edges of the selected microvessels. A segment of the ILA or afferent or efferent arteriole approximately 50 μm in length was scanned at 2- to 5-second intervals. Mean vessel diameter was determined by averaging all measurements obtained during the plateau of the response.

**Experimental Protocols**

**Comparison between WKY and SHR.** Kidneys from WKY (n=14) and SHR (n=14) were used for investigation of the effects of perfusion pressure on renal microvascular tone. Initially, RAP of each preparation was maintained at 80 mm Hg. Thereafter, the pressure was raised in a stepwise fashion by 20 mm Hg increments to a maximum of 180 mm Hg. The diameters of the afferent and efferent arterioles...
and ILAs were determined for at least 1 minute at each level of RAP.

Effects of nifedipine on pressure-induced vasoconstriction. The effects of nifedipine on pressure-induced afferent arteriolar vasoconstriction were assessed in kidneys from WKY (n=6) and SHR (n=6). Afferent arteriolar diameters were determined at the perfusion pressures described above in the absence of nifedipine. Thereafter, increasing doses of nifedipine (10^{-9} to 10^{-6} M) were added directly to the perfusate. The same regions of the vessels (i.e., as previously observed in the absence of nifedipine) were then measured 10 minutes after the administration of each dose of nifedipine.

Analysis of Data
All data are expressed as the mean±SEM. Data were analyzed by one-way analysis of variance followed by Student's t test. Values of p<0.05 were considered statistically significant.

Results
Renal Microvascular Responses to Increased Perfusion Pressure
On the day the kidneys were harvested, tail-cuff systolic blood pressure (SBP) was measured in the conscious donor animals. The mean tail-cuff SBPs were 138±2 mm Hg in WKY (n=14) and 203±4 mm Hg in SHR (n=14, p<0.001).

An increase in RAP provoked a prompt and pronounced vasoconstriction of preglomerular vessels in both strains. In many, but not all, preparations increased RAP elicited oscillatory vasomotion (with a periodicity of 1-2 seconds), in addition to decreasing mean vessel diameter.

Interlobular artery. The responses of ILAs from WKY and SHR to increased RAP are summarized in Figure 1. Basal vessel diameters (i.e., at 80 mm Hg) did not differ between WKY (28.4±1.6 μm, n=7) and SHR (27.3±2.0 μm, n=7, p>0.5).

Stepwise increases in RAP from 80 to 180 mm Hg caused progressive decreases in the diameters of vessels from WKY and SHR. At 180 mm Hg, vessel diameters were identical (WKY, 22.4±1.6 vs. SHR, 22.2±1.6 μm, p>0.5).

When these data were expressed as the percent changes from the basal diameter, the ILA of SHR and WKY exhibited similar responses (Figure 1). As RAP increased, stepwise decrements in vessel diameters were observed. At 180 mm Hg, responses were not significantly different (p>0.4) although the mean response of SHR (-18.6±3.0%) tended to be less than that of WKY (-21.4±1.6%).

Afferent arteriole (near ILA and at midportion).
The mean responses of segments of afferent arterioles near the ILA, at the midportion, and near the glomerulus in kidneys from WKY and SHR are summarized in Figure 2. The basal diameters of segments near the ILA were nearly identical in WKY (20.2±0.4 μm, n=20) and SHR (19.2±0.9 μm, n=20).
In contrast with the response of the ILA, the pressure responses of the efferent arteriole from WKY and SHR differed. In WKY, when RAP was increased from 80 to 100 mm Hg, efferent arteriolar diameter near the ILA and at the midportion decreased significantly from 20.2±0.4 to 18.9±0.4 \( \mu m \) (\( p<0.001 \)) and from 20.4±0.6 to 19.2±0.7 \( \mu m \) (\( p<0.001 \)), respectively. The same change in RAP failed to produce a significant change in the diameters of vessels from SHR. Thus, at 80 and 100 mm Hg the diameters of the efferent arterioles of SHR were 19.2±0.9 and 18.9±0.9 \( \mu m \) near the ILA (\( p>0.05 \)) and 18.3±0.6 and 18.1±0.6 \( \mu m \) at the midportion (\( p>0.1 \)), respectively. An increase in RAP to 120 mm Hg produced a significant vasoconstriction in both segments of the efferent arteriole in SHR kidneys (near ILA, 18.3±0.9 \( \mu m \), \( p<0.005 \); midportion, 17.4±0.4 \( \mu m \), \( p<0.001 \)). As RAP was increased further to 160 mm Hg, efferent arterioles from WKY and SHR exhibited progressive vasoconstrictor responses. In both strains, RAP of 160 mm Hg elicited a maximal vasoconstrictor response. Thus, increasing RAP from 160 to 180 mm Hg caused no further decrease in efferent arteriolar diameters in WKY (near ILA, 15.9±0.4 vs. 15.7±0.4 \( \mu m \), \( p>0.05 \); midportion, 16.2±0.5 vs. 16.2±0.5 \( \mu m \), \( p>0.05 \)) or SHR (near ILA, 15.6±0.9 vs. 15.4±0.8 \( \mu m \), \( p>0.05 \); midportion, 14.9±0.5 vs. 14.8±0.4 \( \mu m \), \( p>0.05 \)).

When these data were expressed as the change in vessel diameter, the differences in the responsiveness of efferent arterioles from WKY and SHR were readily apparent (Figure 2). In the lower range of RAP (i.e., 80–120 mm Hg), the responses of efferent arterioles of SHR were significantly blunted as compared with those of WKY. Thus, at 100 mm Hg the decrement in efferent arteriolar diameter was greater in WKY (near ILA, −6.6±0.9%; midportion, −6.3±1.3% than SHR (near ILA, −1.6±0.9%, \( p<0.005 \); midportion, −0.9±0.5%, \( p<0.005 \)). Furthermore, the RAP at which half-maximal vasodepressor responses were observed was higher in SHR (near ILA, 133±3 mm Hg; midportion, 133±3 mm Hg) than WKY (near ILA, 115±3 mm Hg, \( p<0.001 \); midportion, 114±4 mm Hg, \( p<0.005 \)). Despite such diminished responses of the efferent arteriole of SHR in the lower pressure range, the vasoconstrictor responses obtained at 180 mm Hg did not differ between WKY (near ILA, −22.3±1.6%; midportion, −20.2±1.6%) and SHR (near ILA, −19.9±1.6%, \( p>0.2 \); midportion, −18.5±2.7%, \( p>0.5 \)).

Efferent arteriole (near glomerulus). The diameters of vessel segments near the glomerulus during basal conditions (i.e., at 80 mm Hg) were similar in WKY (18.2±0.8 \( \mu m \), \( n=8 \)) and SHR kidneys (18.0±0.6 \( \mu m \), \( n=8 \)). In contrast with responses in other segments, the vasoconstrictor responses near the glomerulus were substantially diminished in both strains (Figure 2). When RAP was raised to 180 mm Hg, the efferent arteriolar diameters decreased modestly to 16.9±0.9 \( \mu m \) in WKY kidneys (\( p<0.025 \)) and to 16.6±0.8 \( \mu m \) in SHR kidneys (\( p<0.01 \)), corresponding respectively to 7.4±2.5% and 8.1±2.0% decrements from the basal diameters. Thus, the responses of the segments near the glomerulus were significantly diminished as compared with those of segments near the ILA (WKY, \( p<0.005 \); SHR, \( p<0.01 \)) or at the midportion (WKY, \( p<0.001 \); SHR, \( p<0.05 \)). Although this region of the efferent arteriole exhibited a blunted response to pressure in comparison with the more proximal portions of the vessel, similar alterations in the arteriolar responses of SHR kidneys were observed. Thus, the threshold pressure that elicited significant apparent arteriolar vasoconstriction was higher for SHR (140 mm Hg) than for WKY kidneys (120 mm Hg). Furthermore, the RAP that induced half-maximal vasoconstriction was also greater in SHR (135±7 mm Hg) than in WKY kidneys (115±6 mm Hg, \( p=0.05 \)).

Efferent arteriole (Figure 3). The basal efferent arteriolar diameters of the two strains of rats were nearly identical (WKY, 17.8±1.1 \( \mu m \), \( n=5 \) vs. SHR, 17.6±1.1 \( \mu m \), \( n=7 \), \( p>0.5 \)). Efferent arterioles did not constrict in response to elevated RAP in either strain. Indeed, efferent arterioles were progressively distended as RAP increased. In WKY, efferent arteriolar diameters were significantly greater at RAP levels of 160 mm Hg (19.1±1.1 \( \mu m \), \( p<0.025 \)) and 180 mm Hg (19.0±1.3 \( \mu m \), \( p<0.025 \)).

![Figure 3](http://circres.ahajournals.org/doi/figure/10.1161/01.RES.65.6.1478)
FIGURE 4. Effects of elevated renal arterial pressure on total RVR of kidneys from WKY (n=14) and SHR (n=14). Note shift in response of RVR to pressure in SHR kidneys. Significant increases in total RVR were observed at pressures above 120 mm Hg in WKY (p<0.001) and 180 mm Hg in SHR (p<0.005). Results are mean±SEM. RVR, renal vascular resistance; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats. *p<0.05 vs. WKY.

In SHR, statistically significant increases in efferent arteriolar diameter were observed only at 180 mm Hg (18.1±1.1 μm, p<0.01). When these data were expressed as the percent changes from the basal diameters, it was evident that 180 mm Hg caused a greater distension of the efferent arteriole in WKY (11.6±1.6%) than in SHR (3.1±0.8%, p<0.001).

Total renal vascular resistance. The changes of total renal vascular resistance (RVR) are summarized in Figure 4. At 80 mm Hg, basal RVR of kidneys from SHR [6.2±0.9 mm Hg/(ml/min), n=14] was modestly higher than that of WKY [5.8±0.6 mm Hg/(ml/min), n=14], although no statistical difference was attained (p>0.5). When RAP was raised, RVR increased significantly in both strains. The response of RVR in kidneys from SHR, however, was shifted to a higher pressure level; in WKY, significant increases in RVR were observed at 120 [6.0±0.6 mm Hg/(ml/min), p<0.001], 140 [6.2±0.6 mm Hg/(ml/min), p<0.001], 160 [6.4±0.6 mm Hg/(ml/min), p<0.001], and 180 mm Hg [6.7±0.6 mm Hg/(ml/min), p<0.001], whereas in SHR, a significant increase was obtained only at 180 mm Hg [6.6±0.9 mm Hg/(ml/min), p<0.005]. In addition, a greater increase in RVR was obtained in WKY (16.5±2.6%) than in SHR kidneys (9.4±2.2%, p<0.05) at 180 mm Hg.

Effects of Nifedipine on Pressure-Induced Vasoconstriction

The effects of nifedipine on pressure-induced afferent arteriolar vasoconstriction were assessed in kidneys from WKY (SBP 137±2 mm Hg, n=6) and SHR (SBP 194±6 mm Hg, n=6). Since the responsiveness of the distal segment near the glomerulus was diminished, diameters of the more proximal segment between its origin at the ILA and its midportion were determined. Figure 5 depicts representative tracings illustrating the ability of nifedipine to inhibit pressure-induced afferent arteriolar vasoconstriction of SHR. Before administration of nifedipine, the afferent arteriole constricted in response to the graded increase in RAP. In the presence of nifedipine (10^-6 M), the pressure-induced afferent arteriolar vasoconstriction was completely abolished.

Figure 6 depicts the effects of nifedipine on pressure-induced afferent arteriolar vasoconstriction in WKY (n=7) and SHR kidneys (n=6). In the absence of nifedipine, afferent arteriolar diameters decreased in a stepwise manner from 19.6±0.6 to 16.0±0.5 μm (p<0.001) in WKY and from 20.4±0.9 to 15.5±0.5 μm (p<0.001) in SHR as RAP was increased. The administration of increasing doses of nifedipine from 10^-9 to 10^-6 M did not alter basal afferent arteriolar diameters in WKY (10^-9 M, 20.1±0.5; 10^-8 M, 20.0±0.6; 10^-7 M, 19.8±0.6; and 10^-6 M, 20.1±0.5 μm, p>0.2) and SHR (10^-9 M, 20.6±1.6; 10^-8 M, 20.4±1.1; 10^-7 M, 20.4±1.0; and 10^-6 M, 20.5±0.9 μm, p>0.5). Nevertheless, nifedipine substantially inhibited pressure-induced afferent arteriolar vasoconstriction in a dose-dependent manner in both WKY and SHR (Figure 6). At the concentration of 10^-6 M, nifedipine completely abolished the pressure-induced afferent arteriolar vasoconstriction in both strains (WKY, p>0.5; SHR, p>0.1).

Figure 7 summarizes the inhibition by nifedipine of pressure-induced vasoconstriction in WKY and SHR kidneys. The data from Figure 6 are expressed as percent inhibition of the vasoconstriction elicited at 180 mm Hg and plotted as a function of nifedipine concentration.
concentration. Nifedipine reversed the vasoconstrictor responses of the afferent arteriole of WKY and SHR in a similar fashion. Thus, the concentrations at which half-maximal inhibition was observed (IC₅₀) were nearly identical (WKY, 63±27 nM vs. SHR, 60±32 nM, p>0.5).

**Discussion**

Sustained hypertension substantially alters the renal response to alterations in renal perfusion pressure, shifting the threshold pressure of both autoregulation of renal blood flow and pressure-induced natriuresis to higher pressures in hypertensive animals. This resetting of the renal response to pressure represents a renal adaptation to hypertension. Although differences in the responsiveness of renal microvessels to various hormones have been demonstrated in normotensive and hypertensive animals, the effects of hypertension on renal microvascular responsiveness during acute changes of perfusion pressure have not been directly ascertained. The present study characterizes for the first time, in a controlled in vitro setting, the responsiveness of the renal microvessels to changes of RAP in kidneys from normotensive and hypertensive rats. Our results demonstrate in a quantitative manner the preferential vasoconstriction of preglomerular microvessels (i.e., the ILA and afferent arteriole) in response to changes of perfusion pressure. Furthermore, direct assessment of the microvascular response to elevated RAP suggests that the renal hemodynamic adaptation in hypertension involves an alteration in the responsiveness of the afferent arteriole.

Pressure-induced vasoconstriction of renal microvessels constitutes an important determinant of renal autoregulation. It is extremely difficult to assess directly the renal microvascular response to pressure in situ. Furthermore, when arterial pressure is altered in vivo, concomitant changes in neural and hormonal determinants of the renal microvascular tone tend to counter the induced changes, thereby confounding the interpretation of results. Thus, the isolated perfused hydronephrotic kidney is uniquely suited to the study of the renal microvascular responsiveness to alterations in perfusion pressure. The renal microvessels can be visualized...
in an intact in situ setting, yet under in vitro conditions in which RAP is under direct experimental control. Extrarenal neural and humoral influences on renal vascular tone are eliminated. Furthermore, because hydrenephrosis induces tubular atrophy, tubuloglomerular feedback (TGF) is absent in this model.

Our observations with this model demonstrate in a direct and conclusive manner that the renal microvessels possess a prominent capacity to respond to elevated RAP. Aukland and Øien predicted that intact renal autoregulation requires a myogenic reduction in vessel diameter of 25%. In the present study, the maximal pressure-induced vasoconstrictor responses of preglomerular microvessels (excluding the segments near the glomerulus) ranged from 18.5% to 22.3%, suggesting that pressure-induced vasoconstrictor capacities are well preserved in this model. In contrast, total RVR increased to a lesser extent than would be required to achieve complete renal autoregulation (Figure 4). It should be noted that these kidneys are devoid of TGF and were perfused with cell-free media. As suggested previously, the reduced viscosity of the cell-free perfusate greatly reduces both the basal resistance and changes in resistance associated with decreased vessel diameter. Thus, the diminished RVR response in spite of a marked microvascular vasoconstriction may reflect the low viscosity of the perfusion medium.

Although preglomerular vessels are thought to be primarily responsible for autoregulatory response, controversy remains concerning the relative role of the afferent arteriole and the ILA. Tønder and Aukland demonstrated that the pressure within the ILA in the outer cortex was substantially lower than that of the aorta. Furthermore, Källskog et al. demonstrated that within the autoregulatory range, the pressure in the superficial ILA was maintained relatively unchanged. These observations indicate that the ILA contributes importantly to renal autoregulation. In contrast, Carmines and colleagues reported that in juxtamedullary nephrons, afferent arteriolar pressure was variably regulated, whereas interlobular arterial pressure was not. Thus, these observations suggest a predominant contribution of the afferent arteriole to renal autoregulation, at least in the juxtamedullary portion of the renal microcirculation. Of interest, Carmines et al. demonstrated the presence of pressure-induced vasoconstriction in both afferent arterioles and ILA. Thus, the lack of pressure regulation within this segment of the ILA may simply reflect the larger diameter of this vessel near the arcuate artery (approximately 50 μm). Since vascular resistance is inversely related to the fourth power of the radius, it follows that a pressure-induced vasoconstriction of the smaller caliber segments of this vessel (i.e., in the outer cortex) may contribute more to autoregulation than the larger segments (i.e., in the juxtamedullary region).

The present study provides additional evidence for an important role of the ILA in renal hemodynamic response to alterations in RAP. As RAP was raised from 80 to 160 mm Hg, the diameters of the afferent arteriole and the ILA decreased with a concomitant rise in total RVR. The pressure-induced vasoconstrictor response of the afferent arteriole attained maximal levels at 160 mm Hg, and further increases in RAP produced no further decreases in the diameter of this vessel (Figure 2). In contrast, as RAP increased from 160 to 180 mm Hg, the total RVR increased (Figure 4). Thus, there was a dissociation between afferent arteriolar response and total RVR. In the pressure range from 160 to 180 mm Hg, however, the ILA exhibited a further vasoconstriction (Figure 1). In concert, these observations suggest that the ILA contributes prominently to pressure-induced alterations in total RVR particularly in the higher range of RAP. It should be noted that the ILAs observed in the present study are terminal portions with relatively small diameters (approximately 30 μm). In contrast, the responsiveness of more proximal portions of the ILA (diameters approximately 76.0±3.0 μm) are diminished in this model (unpublished observations). It is likely that the same transmural pressure gradient results in a greater distending force in larger vessels (i.e., due to the Laplace relationship between wall tension and radius). Taken together, these findings are consistent with the postulate that the segments of the ILA in the superficial cortex contribute importantly to the regulation of renal hemodynamics.

The present study clearly demonstrates pronounced segmental heterogeneity in the response of the afferent arteriole to pressure. Both strains of rats manifested a blunted response to elevated perfusion pressure at the segment near the glomerulus. Thus, this segment of the afferent arteriole vasoconstricted only modestly in response to elevation of RAP from 80 to 180 mm Hg (7.4±2.5% and 8.1±2.0% decreases in vessel diameters in WKY and SHR, respectively). In contrast, the same change in RAP resulted in pronounced decreases in the diameters of the segments of the afferent arteriole near the ILA (−22.3±1.6% and −19.9±1.6% in WKY and SHR, respectively) and at the midportion (−20.2±1.6% and −18.5±2.7% in WKY and SHR, respectively). These findings are in accord with the observation by Steinhausen et al. that reduction in RAP in vivo causes less vasodilation in the afferent arteriolar segment near the glomerulus than in the more proximal portions of this vessel. In contrast with these observations in vivo and in vitro hydrenephrotic kidneys, Carmines reported that in the perfused juxtamedullary nephron preparation the portion of the afferent arteriole nearest the glomerulus manifests the major resistance adjustment to interventions in RAP. These divergent findings may be attributable to differences in the role of TGF in these two models. In the juxtamedullary nephron, TGF is intact, whereas the
tubular atrophy associated with chronic hydropnephrosis disrupts this mechanism.23 The segment of the afferent arteriole near the glomerulus is thought to be the primary site of TGF regulation.29 In concert, these observations suggest that the afferent arteriolar response to elevated pressure involves both intrinsic and extrinsic mechanisms. The portion of this vessel near the glomerulus may be under predominant control by TGF, whereas more proximal segments exhibit an intrinsic (i.e., myogenic) vasoconstrictor response to elevated pressure.

Our findings of regional heterogeneity of the afferent arteriolar response may be relevant to the controversy centering on the relative contribution of TGF and myogenic mechanisms to the autoregulation of renal blood flow. Sanchez-Ferrer et al20 have recently examined pressure-induced vasoconstriction in the in vitro perfused juxtamedullary nephron. They reported that the pressure-induced decrement in afferent arteriolar diameter and regulation of glomerular pressure were completely abolished by pharmacological or surgical disruption of TGF. Based on these findings, the authors concluded that TGF-induced vasoconstriction represents the sole mechanism whereby afferent arteriolar tone is modulated by elevated pressure. Our findings do not support this conclusion. Our demonstration that afferent arterioles of the hydropnephrotic kidney constrict in response to pressure elevation despite the absence of TGF mechanism clearly indicates an important contribution of non-TGF-mediated vasoconstriction in the pressure response to the renal microvasculature. One possible explanation for these apparent discrepancies relates to the region of the afferent arteriole under observation. We find a diminished intrinsic response of the afferent arteriole near the glomerulus, whereas this site is the major region affected by TGF.29 Furthermore, we studied vessels arising from small caliber (approximately 30 μm) ILAs. These afferent arterioles had a mean basal diameter of approximately 20 μm. In contrast, the afferent arterioles observed in the juxtamedullary nephron preparation arise primarily from the arcuate artery and have basal diameters of 32 μm. Thus, these differences may relate to regional factors and to the influence of vessel diameter on myogenic responsiveness. We believe that our present findings, together with those cited above,26,30 are in accord with the formulation that TGF-mediated and myogenic vasoconstriction act in concert to maintain renal autoregulation.21

Sustained hypertension influences several aspects of the renal response to elevated renal perfusion pressure. Autoregulation of renal blood flow and the pressure-natriuresis relation are both reset toward higher pressure level in hypertensive animals.14,18,19 In the present study, we have demonstrated that the RAP at which a significant increase in total RVR is attained is higher in kidneys isolated from SHR (180 mm Hg) than in those from WKY (120 mm Hg, Figure 4). This observation indicates a resetting of the relation between RAP and total RVR in our experimental model.

There is little information on the functional modification of the responsiveness of microvessels to changes of perfusion pressure in hypertensive animals. Previous investigations demonstrated that arterioles from gracilis muscle in SHR exhibited a higher threshold for pressure-induced vasoconstriction than in WKY, indicating the shift of pressure-diameter relation at arteriolar levels in the microcirculatory bed of skeletal muscle.2 In the present study, we have demonstrated a shift to the right in the pressure response curves of afferent arterioles in kidneys from SHR (Figure 2). The threshold RAP that elicited a significant afferent arteriole vasoconstriction was 20 mm Hg higher in SHR than in WKY. Furthermore, the pressure at which half-maximum vasoconstrictor response was elicited was also shifted to a higher pressure level in SHR at every segment of the afferent arteriole (Figure 2). These changes in the responsiveness of the afferent arteriole paralleled similar alterations in the response of the total RVR to RAP (Figure 4). In contrast, ILAs exhibited no shift in pressure-induced activation in kidneys from hypertensive animals. Thus, the present study, in the adaptive changes in the responsiveness of renal microvessels to changes of RAP in hypertensive animals, were restricted to the afferent arteriole. To our knowledge, this finding constitutes the first direct demonstration that renal arteriolar behavior adapts to a higher pressure level in hypertensive animals.

Although the present study clearly demonstrates a shift in the relation between RAP and pressure-induced afferent arteriolar tone, an influence of preafferent arteriolar resistance cannot be ruled out. Sustained hypertension causes structural changes that could decrease vessel diameters and increase passive resistance of larger vessels.31 Thus, a greater pressure drop in preafferent arteries may be responsible for the observed shift in the relation between RAP and afferent arteriolar vasoconstriction in kidneys from SHR. Nevertheless, the ILA that we examined exhibited no shift in pressure-induced response (Figure 1). Furthermore, the basal (i.e., at 80 mm Hg) vessel diameters of the ILA were identical (WKY, 28.4±1.6 vs. SHR, 27.3±2.0 μm, p>0.5). These observations militate against a difference in luminal pressure in WKY and SHR microvessels, and suggest that changes in the response to pressure observed in kidneys from SHR represent a true functional adaptation of the renal microcirculation.

The differing responsiveness of preglomerular and postglomerular microvessels merits comment. Gilmore et al32, using hamster cheek pouch implants of renal tissues, observed that the renal microvascular response to increased transmural pressure was restricted to the renal preglomerular microvessels. More recently, Steinhausen et al27 reported
that reduction of RAP caused vasodilation of preglomerular, but not postglomerular, microvessels in in vivo hydronephrotic kidneys, suggesting indirectly that pressure-dependent tone was restricted to preglomerular vessels. Finally, Edwards33 demonstrated that isolated, pressurized afferent arterioles exhibited myogenic responsiveness, whereas isolated effluent arterioles did not. Thus, the present finding that pressure-induced vasoconstriction of in situ vessels of the in vitro perfused kidney is restricted to preglomerular vessels is in agreement with conclusions based on studies with isolated microvessels and in vivo observations. In concert, these diverse observations indicate that an intrinsic difference in the responsiveness of preglomerular and postglomerular vessels is a characteristic of the renal microcirculation.

The mechanisms mediating pressure-induced vasoconstriction and the lack of responsiveness of postglomerular vessels remain incompletely defined. Harder et al.34,35 recently reported that membrane depolarization of vascular smooth muscle cell was involved in pressure-induced vasoconstriction in diverse experimental preparations including isolated dog ILA and cat middle cerebral artery. Furthermore, he demonstrated that verapamil inhibited the pressure-induced vasoconstriction,34 suggesting an involvement of potential-dependent calcium channels. Previous observations from our laboratory36-39 and others40 suggest that these channels play a more predominant role in the activation of preglomerular, compared with postglomerular, microvessels. Such a formulation may explain, in part, the preferential involvement of preglomerular vessels in pressure-induced vasoconstriction. Preliminary findings in our laboratory also indicate that KCl-induced vasoconstriction is more prominent in afferent than efferent arterioles,41 an observation that is consistent with the premise that membrane depolarization may exert a greater vasoconstriction in preglomerular microvessels.

If such membrane depolarization mediates the pressure-induced activation of the afferent arteriole, in analogy with the effects in larger vessels mentioned above, calcium antagonists should inhibit this response. Indeed, we have previously reported that diltiazem inhibits pressure-induced vasoconstriction of the afferent arterioles of Sprague-Dawley rats.42 Furthermore, the present study demonstrates that the inhibition of pressure-induced afferent arteriole in both normotensive (WKY) and hypertensive (SHR) rats in a dose-dependent manner (Figure 6). Since preglomerular microvessels contribute to renal autoregulation,43,44 the inhibition of pressure-induced vasoconstriction of the afferent arteriole may partly be responsible for the loss of renal autoregulation by calcium antagonists.45,46 Of interest, nifedipine inhibited the pressure-induced afferent arteriolar vasoconstriction in a similar fashion in WKY and SHR kidneys (Figure 7). Thus, the IC50 for pressure-induced afferent arteriolar activation was nearly identical (WKY, 63±27 vs. SHR, 60±32 nM). In contrast, previous studies have demonstrated that when vasoconstriction was induced by norepinephrine, mesenteric arteries from SHR were more sensitive to the inhibitory action of nifedipine than those from WKY.45 These disparate findings indicate that the ability of calcium antagonists to inhibit vasoconstriction is dependent on the vasoconstrictor stimulus and type of vessels used.45,46

Although the pharmacological effects of nifedipine on pressure-induced afferent arteriolar vasoconstriction are identical in normotensive and hypertensive animals, calcium antagonists may alter renal hemodynamics in hypertensive animals more than normotensive animals. Arendshorst and Beierwaltes47 found that the elevated afferent arteriolar resistance observed in SHR was abolished by aortic clamping, suggesting a prominent pressure-induced vascular tone of this vessel in vivo. In the present study, we have demonstrated that activation of the afferent arteriole by pressure is shifted by 20 mm Hg in SHR, while the difference in SBP (measured by tail-cuff method) between SHR and WKY is 65 mm Hg. In concert, these observations suggest that in vivo, the contribution of pressure-induced vascular tone to renal vascular resistance may be greater in SHR than WKY. Thus, the ability of calcium antagonists to reverse pressure-induced afferent arteriolar vasoconstriction may be a contributing factor to the frequently observed finding that these agents increase glomerular filtration rate of hypertensive, but not normotensive, animals.36,48

In conclusion, we have demonstrated that renal preglomerular, but not postglomerular, microvessels exhibit pressure-induced vasoconstrictor properties. Furthermore, we have demonstrated that the afferent arterioles of normotensive and genetically hypertensive animals respond differently to changes of RAP. The latter observation may suggest the resetting of pressure-vasoconstriction relation in hypertensive animals. Finally, we have demonstrated conclusively that calcium antagonists attenuate pressure-induced afferent arteriolar vasoconstriction, suggesting the participation of dihydropyridine-sensitive calcium channels in mediation of pressure-induced vasoconstriction of this microvessel.

References

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28. Carmines PK: Responses of the renal microvasculature to changes in renal arterial pressure (abstract). *FASEB J* 1989; M19:A381


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