Afferent Arteriolar Responsiveness to Altered Perfusion Pressure in Renal Hypertension

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The present study was performed to determine the role of afferent arterioles in the impaired autoregulatory response shown to occur in the contralateral kidney of Goldblatt hypertensive rats. The responsiveness of juxtamедullary afferent arterioles to alterations in perfusion pressure was studied in the nonclipped kidney of two-kidney, one clip hypertensive and sham-operated rats. Systolic pressure, 5–6 weeks after clipping, averaged 184±6 mm Hg in the hypertensive rats (n=16) and 121±3 mm Hg in the sham-operated control rats (n=7). By using the in vitro blood-perfused juxtamедullary nephron technique, afferent arterioles were directly visualized, and their inside diameters were measured by videomicroscopic methods. In sham-operated kidneys perfused with blood from normotensive rats, afferent arteriolar diameter averaged 22.8 ±1.8 μm at a renal arterial perfusion pressure of 151±1 mm Hg and increased to 24.8±1.8 μm when perfusion pressure was reduced to 110±2 mm Hg. Conversely, in hypertensive kidneys perfused with blood from either hypertensive or normotensive rats, the afferent arterioles failed to vasodilate and actually exhibited a slight decrease in diameter from 24.6 ±1.3 to 23.0 ±2.3 μm in response to the same reduction in perfusion pressure. Vasodilator capability, however, could be demonstrated in response to verapamil and sodium nitroprusside, which increased afferent diameter in both the sham-operated and hypertensive groups of rats. Thus, unlike arterioles from normotensive rats, juxtamедullary afferent arterioles from two-kidney, one clip Goldblatt hypertensive rats fail to vasodilate after a reduction in perfusion pressure. This impaired autoregulatory ability might result from an intrinsic functional defect in the vasculature of the high pressure contralateral kidney. (Hypertension 1990;15:748–752)

In the normal kidney, autoregulatory capability is believed to be characteristic of nephrons at all levels of the renal cortex; however, previous studies have suggested that the contralateral or nonclipped kidney of the two-kidney, one clip (2K1C) Goldblatt hypertensive rat exhibits an impairment of autoregulatory capability. Imposition of a renal arterial stenosis on one kidney stimulates a progressive angiotensin-dependent elevation in arterial pressure that apparently leads to certain functional adaptations in the nonclipped kidney exposed to an increasing perfusion pressure. Among the changes observed is a compromised ability to maintain glomerular filtration rate or renal blood flow in response to reductions in blood pressure. Therefore, although renal blood flow and glomerular filtration rate in the contralateral kidney are maintained in the normal range while the hypertensive state persists, the impaired autoregulatory capability prevents the establishment of normal renal hemodynamic function when arterial pressure is reduced to normotensive levels. The site and mechanism of the altered vascular responsiveness have not yet been determined.

This study was designed to directly evaluate the response of afferent arterioles in the contralateral kidney of 2K1C hypertensive rats to alterations in renal perfusion pressure. Experiments were performed with the in vitro blood-perfused juxtamедullary nephron technique, which allows direct visualization of juxtamедullary afferent arterioles on the inner cortical surface. Afferent arteriolar diameter can be measured without disturbing the intimate association of renal microvascular and tubular structures. Furthermore, the tubuloglomerular feedback loop remains intact so that its role, if any, can be expressed.

**Methods**

Rats weighing 175–200 g were anesthetized with pentobarbital (40 mg/kg i.p.), and the left renal artery was constricted with a silver clip (0.25 mm i.d.). Sham-operated control rats were prepared and
were used to estimate systolic pressure.

A minimum of three records per session for each rat were used to estimate systolic pressure.

On the day of the experiment, the kidney donor (360 ±8 g) was anesthetized with pentobarbital, and the right renal artery was cannulated through the superior mesenteric artery. The kidney was immediately perfused with a Tyrode’s solution containing 52 g/l bovine serum albumin (Sigma Chemical Co., St. Louis, Missouri) at 25°C and pH 7.4.8 The perfused kidney was removed and sectioned longitudinally leaving the papilla intact in the perfused dorsal two-thirds portion. Cuts were made along the lateral fornice, and the papilla was reflected back to expose the underlying pelvic cavity. The pelvic mucosa and adipose and connective tissue were dissected away to expose the underlying branches of the renal vasculature and the tubular structures of juxtamedullary nephrons. Tight ligatures were placed around the distal ends of the main arterial branches supplying the superficial vasculature.

After surgical preparation, the Tyrode’s perfusate was replaced with reconstituted blood collected from a suitable donor rat. The source of the donor blood varied according to the experimental protocol; however, in each case, the blood was collected and prepared in an identical fashion. When blood was collected from the kidney donor, the renal artery of the clipped kidney was ligated before the contralateral kidney was cannulated. Arterial blood was collected by a carotid cannula into a syringe containing heparin (2,000 units). The blood was centrifuged, and the white blood cell and platelet fraction were discarded. Plasma colloid osmotic pressure was measured and adjusted to 18 mm Hg by the addition of albumin. The plasma was passed through 5.0 and 0.45 μm filters. Erythrocytes were added to the filtered plasma to achieve a hematocrit of 33%. This reconstituted blood was filtered through a 10 μm nylon mesh and oxygenated in a closed reservoir pressurized with a 95% O2 and 5% CO2 gas mixture. Renal perfusion pressure measured by a cannula fixed in the superfusion buffer was replaced with an identical one containing verapamil (50 μM) and sodium nitroprusside (380 mM). This solution was permitted to bathe the inner cortical surface for 3 minutes at 110 mm Hg before the perfusion pressure was returned to 150 mm Hg.

**Normal Kidney and Normal Blood**

Further experiments were performed in an identical manner except that the kidneys of sham-operated rats were used and perfused with blood obtained from the normotensive sham-operated kidney donor (sham group).

**Hypertensive Kidney and Blood From a Normotensive Donor**

A final group of experiments was performed to determine if the response of juxtamedullary afferent arterioles from hypertensive rats to changes in perfusion pressure was dependent on circulating factors present in blood from hypertensive rats. The non-clipped contralateral kidneys of hypertensive rats were perfused with blood from normotensive rats (HN group). Perfusion pressure was reduced from 150 to 110 and then 80 mm Hg in successive 3-minute intervals. After 3 minutes at 80 mm Hg, the superfusion buffer was replaced with a solution containing verapamil and sodium nitroprusside. After 3 minutes of verapamil exposure, perfusion pressure was elevated to 110 and 150 mm Hg in successive 3-minute intervals.

**Data Analysis**

Vascular inside diameter was measured using either a video caliper (Vista Electronics, Ramona, California) or an image-shearing monitor (model 901, Instrumentation for Physiology and Medicine, Inc., San Diego, California) calibrated with a stage micrometer (smallest division, 2 μm). Diameter measurements were precise to within 1 μm. The diameter
of each vessel was measured from a single site at 12-second intervals throughout the experiment. The plateau vessel diameter for each experimental period was calculated by averaging the five determinations recorded during the last minute of that period. Statistical comparisons within each series were made using a one-way analysis of variance for repeated measures combined with the Newman-Keuls multiple range test. Comparisons across treatment groups were made using an unpaired t test. p values of less than 0.05 were considered significant. All data are reported as mean±SEM.

Results
Measurements of systolic pressure in conscious rats demonstrated progressive development of hypertension in the clipped rats. Systolic pressure of sham-operated control rats averaged 121±3 mm Hg (n=7), whereas systolic pressure of the 2K1C rats had increased significantly to 184±6 mm Hg (n=16; p<0.01). As previously demonstrated for normal kidneys, afferent arteriolar diameter in the sham-operated rats increased 8±4% (p<0.05) from 20.9±1.9 to 22.4±2.1 μm when perfusion pressure was reduced from 151±1 to 110±2 mm Hg (Figure 1, open circles). In contrast, afferent arterioles from the nonclipped kidneys of hypertensive rats perfused with their own blood (HH group) exhibited a decrease in diameter of 7±2% (p<0.05) from 27.7±1.2 to 25.7±1.4 μm when perfusion pressure was reduced to the same degree (154±2 to 108±1 mm Hg; p>0.05). As perfusion pressure was increased to 152±2 mm Hg, afferent diameter remained relatively constant (n=16; p>0.05). During vasodilator exposure at a perfusion pressure of 150 mm Hg, afferent diameter was increased relative to control (no vasodilators) by 4.9±1 μm in the sham-operated group of rats and 2.6±1 μm in the HH group of rats. The absolute vasodilator-induced change in vessel caliber was not different between groups because of the large range of vessel diameters. When the change in vessel caliber data was evaluated as a percentage of their respective control values, however, significant differences were observed. Afferent arterioles from sham-operated kidneys increased in diameter by 23±4% at 150 mm Hg in response to the vasodilators. This is contrasted with only a 10±3% increase in diameter observed in the hypertensive group of rats (p<0.01).

Additional experiments were conducted to determine the afferent arteriolar responses of 2K1C rat kidneys to changes in perfusion pressure while being perfused with blood from normotensive rats (HN, Figure 2). In these experiments, afferent arteriolar diameter declined 5±1% when renal perfusion pressure was reduced from 152±1 to 108±2 mm Hg (p<0.05). This change in vessel diameter was nearly identical to the response observed in vessels from the HH group but directionally opposite to the responses of vessels in sham-operated rat kidneys (p<0.01). A further reduction in perfusion pressure to 79±1 mm Hg did not produce any further change in vessel diameter.
The results of the present study demonstrate that juxtamedullary afferent arterioles of the contralateral kidney in 2K1C hypertensive rats fail to vasodilate in response to acute reductions in perfusion pressure. Indeed, these afferent arterioles responded with slight reductions in arteriolar diameter, indicative of passive responses to reductions in transmural pressure. This is in direct contrast to the rapid vasodilator responses observed in sham-operated control rats or in normal kidneys. The reason for this difference is not readily apparent. Earlier experiments were conducted with in vivo micropuncture techniques, and therefore, the kidneys were perfused with blood having elevated angiotensin concentrations and plasma renin activity. In the present experiments, the nonclipped kidney was perfused with blood collected from either hypertensive (HH) or normotensive rats (HN) to determine the possible role of circulating factors unique to hypertensive rats. Because the source of perfusate blood had no discernible influence on afferent arteriolar responses to perfusion-pressure alterations, it appears that the lack of autoregulatory responsiveness was because of an intrinsic derangement and not specifically caused by circulating factors. This intrinsic derangement is also not caused by a nonspecific inability to vasodilate because the response to verapamil and sodium nitroprusside clearly demonstrates the existence of active contractile tone in the afferent arteriolar smooth muscle of hypertensive rats.

It is well recognized that angiotensin II plays a major role in the establishment and maintenance of renal vascular hypertension. Blockade of the renin-angiotensin system with renin inhibitors, converting enzyme inhibitors, or angiotensin receptor antagonists is known to reduce arterial pressure. This suggests that angiotensin II contributes to the increased renal vascular resistance present in the nonclipped kidney. Indeed, angiotensin II might also have contributed to the vasoconstrictor-like reduction in vessel caliber seen in hypertensive kidneys in response to reductions in perfusion pressure. If angiotensin or plasma renin were directly linked to the impaired autoregulatory response to reductions in perfusion pressure, however, some restoration should have been observed in the kidneys perfused with blood from normotensive rats. In fact, kidneys from both 2K1C groups responded similarly to reductions in perfusion pressure, regardless of whether they were perfused with blood from hypertensive or normotensive rats. Therefore, it appears that a sustained elevation in arterial pressure induces a functional alteration in the renal microvasculature that is not readily reversible, and is responsible for a higher renal vascular resistance and an impaired autoregulatory capability. One in vivo consequence of such effects is that a higher steady-state systemic arterial
caliber (data not shown). The vasodilator response to verapamil was similar to that reported for the vasodilator solution in rats in the HH group. At a perfusion pressure of 108±1 mm Hg, verapamil increased vessel diameter by 7±2% (p<0.05). Further elevation of perfusion pressure to 150±2 mm Hg caused an additional dilation of 7±2% (p<0.01) to a final diameter of 22.1±1.7 μm. When compared with the control diameter at 150 mm Hg (without verapamil), exposure to verapamil caused a vasodilator response of 7±2% from 20.7±1.7 to 22.1±2.0 μm in the HH group of rats. This value is similar to the value in the HH group but different from that in the sham-operated group of rats (p<0.02).

**Discussion**

Previous studies have demonstrated that development of hypertension in the 2K1C Goldblatt rat model is associated with an impaired ability of the contralateral kidney to autoregulate renal blood flow and glomerular filtration rate. Ploto and coworkers observed that superficial nephron glomerular filtration rate was better preserved than whole kidney glomerular filtration rate in response to decreases in perfusion pressure. This suggests that deeper nephrons must be more severely affected by prolonged elevations in arterial pressure. Support for this notion comes from the work of Stumpe et al who reported that juxtamedullary nephrons of the contralateral kidney do not autoregulate superficial nephron glomerular filtration rate; however, these determinations were made under conditions where tubular flow past the macula densa was blocked and, thus, a failure to autoregulate superficial nephron glomerular filtration rate might be because of a tubuloglomerular feedback-induced preglomerular vasodilation. To evaluate this issue further, we used the in vitro blood-perfused juxtamedullary nephron technique, which is ideally suited for directly viewing the responses of individual preglomerular microvascular structures, and assessed afferent arteriolar responsiveness to reductions in perfusion pressure.

The results of the present study demonstrate that juxtamedullary afferent arterioles of the contralateral kidney in 2K1C hypertensive rats fail to vasodilate in response to acute reductions in perfusion pressure. Indeed, these afferent arterioles responded with slight reductions in arteriolar diameter, indicative of passive responses to reductions in transmural pressure. This is in direct contrast to the rapid vasodilator responses observed in sham-operated control rats or in normal kidneys. The reason for this difference is not readily apparent. Earlier experiments were conducted with in vivo micropuncture techniques, and therefore, the kidneys were perfused with blood having elevated angiotensin concentrations and plasma renin activity. In the present experiments, the nonclipped kidney was perfused with blood collected from either hypertensive (HH) or normotensive rats (HN) to determine the possible role of circulating factors unique to hypertensive rats. Because the source of perfusate blood had no discernible influence on afferent arteriolar responses to perfusion-pressure alterations, it appears that the lack of autoregulatory responsiveness was because of an intrinsic derangement and not specifically caused by circulating factors. This intrinsic derangement is also not caused by a nonspecific inability to vasodilate because the response to verapamil and sodium nitroprusside clearly demonstrates the existence of active contractile tone in the afferent arteriolar smooth muscle of hypertensive rats.

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pressure is required to maintain normal levels of renal blood flow and glomerular filtration rate. Additionally, the impaired autoregulatory capability might alter the sodium excretory response to changes in perfusion pressure or volume expansion. This was demonstrated in a study by Mackenzie et al.15 in which administration of an acute saline load increased fractional sodium excretion in the nonclipped kidney at spontaneous arterial pressure to an extent greater than in normal kidneys or nonclipped kidneys with reduced renal perfusion pressure.

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**References**


**KEY WORDS** • arterioles • Goldblatt hypertension • blood pressure • autoregulation
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