Pressure-Induced Constriction of the Afferent Arteriole of Spontaneously Hypertensive Rats

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In uncomplicated essential hypertension, renal blood flow, glomerular filtration rate, and glomerular capillary pressure are within the normal range despite elevated renal perfusion pressure, suggesting abnormally high resistance of the preglomerular vessels. Among various preglomerular vascular segments, the afferent arteriole (Af-Art) is thought to be the site responsible for most resistance. However, little is known about the vascular reactivity of the Af-Art or its alteration in hypertension. In this study, we tested the hypothesis that pressure-induced constriction is exaggerated in Af-Arts from spontaneously hypertensive rats (SHRs).

Single Af-Arts were microdissected from kidneys of SHRs and normotensive control Wistar-Kyoto (WKY) rats and were microperfused in vitro. When pressure in the Af-Art was increased stepwise from 20 to 80 mm Hg, luminal diameter increased similarly in both WKY and SHR Af-Arts (from 10.0±0.8 to 18.6±1.9 μm and from 10.1±1.2 to 16.9±1.5 μm, respectively). However, when pressure was further increased to 140 mm Hg, the diameter remained unchanged in WKY Af-Arts (19.2±1.9 μm), whereas it decreased significantly to 11.1±0.9 μm in those from SHRs. We conclude that pressure-induced constriction is exaggerated in SHR Af-Arts, which may contribute to the development and maintenance of hypertension. (Hypertension 1992;19[suppl II]:II-164-II-167)

In patients with uncomplicated essential hypertension and in spontaneously hypertensive rats (SHRs), despite elevated renal perfusion pressure (aortic pressure), renal blood flow is usually reported as normal or somewhat decreased with normal or slightly reduced glomerular filtration rate, demonstrating increased renal vascular resistance.1 Although other vascular beds exhibit elevated resistance, the increase in renal vascular resistance seems to be crucial for blood pressure to remain elevated. As stated by Guyton2 and Tobian,3 unless renal vascular resistance is elevated, hypertension will not develop regardless of the degree of elevated resistance in other vascular beds; rather, a natriuretic response at the kidney causes blood pressure to normalize.

Micropuncture studies have shown that despite higher renal perfusion pressure, glomerular capillary pressure in the SHR is the same as in its normotensive control, the Wistar-Kyoto (WKY) rat.4 Therefore, the increased resistance seems to reside primarily in the preglomerular vessels, somewhere between the renal artery and the afferent arteriole (Af-Art). Among the various preglomerular vascular segments, the Af-Art is thought to account for most preglomerular resistance. However, there is little information about the reactivity of this small resistance vessel or its alteration in hypertension.

To study the vascular reactivity of the Af-Art directly, we have recently established a preparation in which a microdissected Af-Art is microperfused in vitro.5 This preparation has the advantage of allowing us to observe Af-Art response directly while controlling pressure in the Af-Art. Using this preparation, we carried out the present study to test the hypothesis that pressure-induced constriction is exaggerated in the SHR.

Methods

Experiments were performed with 12- to 14-week-old male SHRs and WKY rats (Charles River Laboratories, Inc., Wilmington, Mass.) weighing 289±9 g (n=8) and 281±11 g (n=7), respectively. Rats were anesthetized with intraperitoneal ketamine (60 mg/kg), and arterial pressure was measured via a cannula placed in the femoral artery; mean arterial pressure averaged 133±5 mm Hg in SHRs and 94±5 mm Hg in WKY rats. After pressure was measured, the aorta was catheterized below the renal arteries and clamped with a hemostat above the kidneys. The kidneys were perfused with cold medium 199
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FIGURE 1. Photomicrograph shows example of pressure-induced myogenic contraction in isolated microperfused afferent arteriole of a spontaneously hypertensive rat. Arrowheads indicate sites of constriction.

(GIBCO Laboratory, Grand Island, N.Y.) containing 5% bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, Mo.) and then were removed and sliced along the corticomedullary axis. Slices were placed in ice-cold medium 199 containing 5% BSA and microdissected with thin steel needles and sharpened forceps (No. 5, Dumont; Fine Science Tools Inc., Belmont, Calif.) under a stereomicroscope (SZH, Olympus, Overland Park, Kan.) at magnifications up to ×100.

Methods of microdissection and perfusion of the Af-Art have been described elsewhere.5,6 Briefly, a single Af-Art with its glomerulus intact was microdissected from the outer third of the cortex and transferred to a temperature-regulated chamber mounted on the stage of an inverted microscope (IMT-2, Olympus). The Af-Art was cannulated using an array of glass pipettes as described previously.5 Perfusion pressure was measured with the Landis technique, using a fine pipette (tip diameter, 2 μm) introduced into the Af-Art through the opening of the perfusion pipette. The Af-Art was perfused with medium 199–5% BSA (oxygenated with 95% O2–5% CO2 to pH 7.4), and perfusion pressure was adjusted to 20 mm Hg.

The bath was identical to the arteriolar perfusate and was exchanged continuously. Microdissection and cannulation of the Af-Art were completed within 90 minutes at 8°C, after which the temperature of the bath was gradually raised to 37°C for the remainder of the experiment. Once the temperature had stabilized, a 30-minute equilibration period was allowed before any measurements were taken. After the equilibration period, the perfusion pressure was increased stepwise every 20 mm Hg until it reached 140 mm Hg, with each pressure lasting 10 minutes. Images of Af-Art were displayed at magnifications up to ×1,980 and recorded with a video system consisting of a camera adaptor with a ×3.3 photo eyepiece, black-and-white charge-coupled camera (Dage-MTI, Inc., Michigan City, Ind.), monitor (Javelin Electronics, Torrance, Calif.), and video recorder (EDV 9500, Sony). The diameter at the most constricted point was measured during the last 1 minute of a 10-minute period for each pressure with an image-analysis system (Fryer, Carpentersville, Ill.).

Values are expressed as mean±SEM. A repeated-measures analysis of variance was used to test whether there was an interaction between rat type (SHR, WKY) and pressure levels. Because an interaction was detected (p<0.003), a two-sample t test was done to examine whether diameters at each pressure differ between the two groups. A value of p<0.007 was considered significant because of the Bonferroni adjustment for multiple comparisons.

Results

Figure 1 illustrates the pressure-induced constriction of an SHR Af-Art. As perfusion pressure increased from 20 to 60 mm Hg, luminal diameter increased passively and stayed at that level until 100 mm Hg. However, when the pressure was increased to 120 mm Hg, constriction appeared at the proximal segment. As pressure increased further to 140 and then 160 mm Hg, constriction became stronger and stronger. The site of the strongest constriction was always seen in the proximal segment farther than 30 μm from the vascular pole. (In some Af-Arts, constriction was seen in more distal segments; however, it was much weaker.)

Figure 2 shows the relation between arteriolar luminal diameter and perfusion pressure. When pressure was increased from 20 to 80 mm Hg, luminal diameter increased passively and stayed at that level until 100 mm Hg. However, when the pressure was increased to 120 mm Hg, constriction appeared at the proximal segment. As pressure increased further to 140 and then 160 mm Hg, constriction became stronger and stronger. The site of the strongest constriction was always seen in the proximal segment farther than 30 μm from the vascular pole. (In some Af-Arts, constriction was seen in more distal segments; however, it was much weaker.)

Figure 2 shows the relation between arteriolar luminal diameter and perfusion pressure. When pressure was increased from 20 to 80 mm Hg, luminal diameter increased similarly in both WKY and SHR Af-Arts (10.0±0.8 to 18.6±1.3 μm and 10.1±1.2 to 16.9±1.5 μm, respectively). When pressure was fur-
FIGURE 2. Line graph shows relation between perfusion pressure and luminal diameter in microperfused afferent arteriole of spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats. *p<0.003 SHR vs. WKY.

The tone of the Af-Art is regulated by many factors, including myogenic response; tubuloglomerular feedback; endocrine, paracrine, and autocrine hormones; and the sympathetic nervous system. Alterations of any one factor or inappropriate interactions among them could lead to abnormally high arteriolar resistance, as seen in hypertension. In vivo studies have shown that both tubuloglomerular feedback and sympathetic nerve activity are enhanced in SHRs, whereas renal denervation normalizes the tubuloglomerular feedback. It has also been reported that renal vasculature of SHRs exhibits exaggerated responses to vasoconstrictors such as angiotensin II, norepinephrine, vasopressin, and thromboxane. Because the Af-Art is a small resistance vessel in the kidney, it is thought to be the major site responsible for such abnormalities. However, only a few reports have directly examined the reactivity of the Af-Art in SHRs. Using microperfusion techniques, we have demonstrated that pressure-induced constriction of the Af-Art is exaggerated in SHRs, which may contribute to elevated renal vascular resistance either by itself or by interaction with other factors that control Af-Art tone.

In contrast to our findings, Hayashi et al., who used the isolated perfused hydronephrotic kidney, and Gebremedhin et al., who used the juxtamedullary nephron perfused in vitro, reported that increasing renal perfusion pressure induced a comparable decrease in Af-Art diameter in both SHRs and WKY rats, although basal luminal diameter (with renal perfusion pressure at 80 mm Hg) was significantly smaller in SHRs. In these studies, however, the pressure in the Af-Art was not known. Because the renal vasculature upstream from the Af-Art (such as the interlobular artery) showed pressure-induced constriction, it may have secondarily affected the dynamics of the Af-Art, thus accounting for the different results from ours.

The myogenic response and macula densa–mediated tubuloglomerular feedback are the two intrinsic mechanisms of renal autoregulation. Microperfusing both a rabbit Af-Art and its attached macula densa segment, we have recently shown that increasing NaCl concentration at the macula densa constrains the Af-Art predominantly in the distal segment. As seen in the present study, on the other hand, pressure-induced constriction was strongest in the proximal segment of the Af-Art farther than 30 μm upstream from the vascular pole. Such segmental constriction is consistent with the in vivo finding of Steinhausen et al. that decreasing renal perfusion pressure increased the luminal diameter of the proximal but not the distal Af-Art in the rat split hydronephrotic kidney (devoid of tubuloglomerular feedback). In addition, using the juxtamedullary nephron perfused in vitro, Moore and Casellas have recently shown that blocking tubuloglomerular feedback attenuated most of the pressure-induced constriction in the distal Af-Art but only part of it in the proximal (upstream) segment. Taken together, these results suggest that the predominant site of the pressure-induced response is the proximal Af-Art, whereas the macula densa–mediated response occurs mainly in the distal segment.

Isolated Af-Arts from WKY rats did not constrict significantly when intraluminal pressure was raised, which is consistent with the findings of Edwards in normal rabbits and Yuan et al. in normal Sprague-Dawley rats. The lack of pressure-induced constriction apparently contrasts with the autoregulation of renal blood flow and glomerular filtration rate observed in vivo. The reason why these isolated arterioles exhibit little, if any, pressure-induced constriction remains speculative at present. One possibility is the absence of the macula densa in these preparations. Micropuncture studies have reported that when tubuloglomerular feedback was blocked by interruption of tubular flow to the macula densa, stop-flow pressure (an index of glomerular capillary pressure) was virtually dependent on renal perfusion pressure, whereas it was well autoregulated when tubuloglomerular feedback was maximally activated by infusion of an isotonic solution into Henle's loop at a high fixed rate. This may suggest that the macula densa signal is required for the Af-Art to fully respond to perfusion pressure. The lack of pressure-induced constriction in the isolated Af-Art could also be due to the absence of red blood cells in the
arteriolar perfusate,\textsuperscript{17} to interstitial pressure, or to circulating vasoactive substances.

Both functional and structural changes of resistance vessels seem to be involved in the elevated vascular resistance seen in SHRs.\textsuperscript{1} It has been postulated that vessels with structural changes (increased wall-to-lumen ratio) would generate a greater contractile force, which in turn may contribute to high vascular resistance.\textsuperscript{21} Among many other alterations observed in various vascular beds of SHRs, the functional role of the endothelium has recently been studied extensively.\textsuperscript{22,23} It has been reported that relaxation of vascular strips induced by endothelium-dependent vasodilators such as acetylcholine is impaired in SHRs, probably because of decreased and increased activity of endothelium-derived relaxing and contracting factors, respectively. Whether the exaggerated pressure-induced constriction we observed in the SHR Af-Art is due to changes in structure or function (such as those in the endothelium) remains to be fully elucidated.

In summary, the present study demonstrates that pressure-induced constriction is exaggerated in the SHR Af-Art. This increased contractility may be involved in elevated resistance of preglomerular vessels, thereby contributing to the development and maintenance of hypertension. The physiological relevance of our findings, as well as the mechanisms responsible, awaits further investigation.

References

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