Morphology of Renal Afferent Arterioles in Spontaneously Hypertensive Rats

Karin Skov, Michael J. Mulvany, and Niels Korsgaard

We present a new perfusion technique that allows arteries down to the level of capillaries to be fixed while relaxed and under a known intravascular pressure. Through a catheter inserted into the right renal artery of 12-week-old male spontaneously hypertensive rats (n=9) and control Wistar-Kyoto rats (n=11), the kidney vessels were rinsed with human plasma, relaxed by papaverine, and perfused with a casting resin containing microspheres. The microspheres (12 μm) became trapped in the glomeruli of the kidney and, together with a closing of the venous outflow, they caused the flow through the kidney to stop, so that the intravascular pressure was raised to the level of the input perfusion pressure (100 mm Hg). The resin material was allowed to harden, and the kidney was immersion-fixed and prepared for histomorphometric investigations. This technique made it possible to measure both the structurally determined lumen diameter and the corresponding media thickness under clearly defined conditions. The lumen diameter of afferent arterioles close to the glomeruli showed a 17% reduction in spontaneously hypertensive rats (15.4±0.6 μm; mean±SEM) compared with Wistar-Kyoto rat arterioles (18.5±0.3 μm, p<0.001). However, this was not due to media hypertrophy, because media cross-sectional area was smaller (p<0.001) in spontaneously hypertensive rats (210±6 μm²) compared with Wistar-Kyoto rats (274±16 μm²). We conclude that the lumen reduction in renal afferent arterioles in spontaneously hypertensive rats is not the result of an encroachment on the lumen by a hypertrophic media. (Hypertension 1992;20:821–827)

KEY WORDS • arterioles • casts, microvascular • kidney • renal circulation • perfusion • rats, inbred SHR

Even before reliable blood pressure measurements could be made, hypertension was known to be associated with structural abnormalities in all parts of the cardiovascular system, resulting in an increased relation between media thickness and lumen diameter (media:lumen ratio) in the arteries.1 However, currently it is not known whether the vascular structural changes are causes or effects of the increased blood pressure. Irrespective of the pathogenetic role of the structural changes in the extrarenal peripheral arteries, it has been repeatedly stressed2–4 that if these changes were not preceded or accompanied by functional changes in the kidney, hypertension would not ensue. A number of recent and elegant transplantation studies5–7 have, together with a similar earlier report,8 further confirmed that the kidney may play a central role in the pathogenesis of primary hypertension. The functional basis for the fact that blood pressure in these studies "follows the kidney" is, however, largely unknown. Several functional and biochemical differences have been identified between kidneys from genetically hypertensive and normotensive animals.9 Among these, the hemodynamic abnormalities have attracted special interest, because it has been shown that substantial abnormalities in renal vascular resistance in the spontaneously hypertensive rat (SHR) are present even before it is possible to measure an increased blood pressure.10,11

It has been reported that the elevated renal vascular resistance predominantly resides within the preglomerular arterioles12,13 and that these vessels in the SHR are subject to a lumen reduction of approximately 13% compared with the normotensive Wistar-Kyoto (WKY) rat.14–16 Whether this is caused by functional15,20 or structural15,16 changes in the vascular wall has been discussed. The discrepancies in these investigations may at least to some extent be explained by the notorious difficulty in measuring unequivocally the structurally determined lumen diameter of elastic structures such as the arteries. In investigations of the structural characteristics of vessels, it is important that the vessels are investigated under standardized conditions,21,22 for example, when fully relaxed and extended with a known transmural pressure. These requirements cannot be fulfilled for the investigation of the afferent arterioles of the kidney using an ordinary perfusion technique because of ignorance of prearteriolar vascular resistance; therefore, the distending pressure in the arterioles during fixation is unknown.
We therefore have developed a new perfusion technique with which it is possible, in contrast to earlier studies under standardized conditions, to fix and measure the structurally determined lumen diameter in afferent arterioles of the kidney down to the immediate precapillary level together with the corresponding values of media thickness in these vessels. Using this method, we have investigated the dimensions of the afferent arterioles in the kidneys of SHRs and WKY rats.

Methods

Animals

Fourteen SHRs and 14 WKY rats, 11 weeks old, were obtained from Moellegaards Breeding Center, L. Skensved, Denmark. They were maintained on a normal sodium chow, and water was provided ad libitum. One week before operation, systolic blood pressure was measured with the tail-cuff procedure in conscious animals. The operation was performed when rats were 12 weeks old. The rats were anesthetized with methohexitol (Brietal, Lilly; 1%, 7.5 mg/kg body wt i.p.). Normotensive and hypertensive animals were treated in the same way throughout the study. All procedures were in accordance with our institutional guidelines. An additional number of about 30 rats was used to explore and critically evaluate the Microfil filling technique and vasodilation procedure before the study.

Surgery

After the abdominal wall was opened and the major arteries were cleaned of fat, the right renal artery was catheterized with a polyethylene tube via the superior mesenteric artery. The kidney was transfused with plasma solution (human plasma obtained from a local hospital; 2.50 mg bovine albumin [Sigma] per milliliter plasma; and 0.004 ml heparin per milliliter plasma, 5,000 IU [DAK, Denmark], at room temperature) to wash out the blood. The perfusion was done for 10 minutes under constant pressure (100 mm Hg) applied from a suitably elevated bottle. Ligatures around the aorta, proximal and distal to the origin of the right renal artery, and the right suprarenal artery were tied in rapid succession. Ligatures were tied around the inferior vena cava proximal and distal to the right renal vein, and the vena cava was cut below the right renal vein to allow escape of blood and perfusate. Immediate and homogeneous blanching of the kidney on opening the inferior vena cava indicated good perfusion; kidneys that were badly perfused were excluded from further investigations. After plasma perfusion was started, the rat was killed, while still anesthetized, by opening the thorax and removing the heart. In the heart, the atria were cut off and both ventricles were opened from base to apex. The ostia of the large vessels were cleaned; the heart was gently blotted with towels to remove excessive water and was weighed immediately; and ventricle/body weight ratio was determined.

Microfil Infusion

To relax the vasculature, the perfusate was, after plasma perfusion, switched to a papaverine solution (2 mg papaverine per milliliter saline, Mecobenzon, Denmark) at constant pressure applied in the same way as the plasma. It was consistently observed that the kidney turned even more pale during this perfusion, probably because of relaxation of the vessels. After 5 minutes of perfusion with papaverine, the perfusate was switched using a three-way cock to a silicone rubber solution (Microfil, MV-130, Canton-Biomedical Products, Boulder, Colo.) containing latex beads (Sigma latex beads, 11.9±1.9 μm [SD], Sigma specification: approximately 200,000 microspheres per milliliter Microfil). This mixture was also infused at a constant pressure of 100 mm Hg. Care was taken not to introduce air into the system. The freshly prepared solution was infused through the catheter in a reduced viscous form to match the viscosity of plasma by the addition of more diluent. The low viscosity prolonged the time of hardening, but pilot studies showed that it did not have any influence on the hardening process or the final hardness. Microfil was chosen as perfusion medium because 1) it is able to fill smaller vessels, 2) it can be diluted so that it has the viscosity of plasma, 3) it is not filtrated in the kidneys, and 4) it has a reasonable working time before hardening (approximately 15 minutes). Before the infusion of Microfil solution, the Microfil diluent to which microspheres were added was treated in an ultrasonic bath for 1–1.5 hours to mix the latex beads completely, because our pilot studies have shown that the microspheres had a tendency to cluster together in the Microfil. The microspheres were checked microscopically for complete segregation and for distortion, which was never the case, before further mixing and use. After 5 minutes perfusion with Microfil solution, the hole in the inferior vena cava was closed to stop residual shunt flow through the outer medullary and subcortical zones, and all larger arteries around the kidney were clamped. Thus, mainly because of the entrapment of the latex beads in the afferent arterioles (Figure 1), all vessels containing Microfil were inflated under the same pressure. The Microfil was allowed to harden under these conditions for 2 hours while the kidney was kept moistened with saline. The kidney was removed and immersion-fixed in 3% formaldehyde plus 1% glutaraldehyde in 3/4 Tyrode’s buffer for a minimum of 3 days.

In a series of independent experiments, we investigated the effect of the procedure used on the lumen dimensions of mesenteric small arteries. This vascular bed was selected because the vessels are transparent and luminal diameter can be readily measured; this would be impossible for the renal vascular bed. The mesenteric bed of anesthetized rats was exteriorized, and the inner diameter of eight selected mesenteric arteries from six animals was measured using intravital microscopy at different times during the perfusion with plasma and papaverine solution. Thereafter, the diameters were measured during Microfil perfusion, when the flow had stopped, after hardening, after 24 hours in fixative, and finally after histological processing. The data of these experiments are shown in Table 1. It can be seen that the lumen diameter measured in the fixed section was very similar to that seen after papaverine perfusion. The only significant change in lumen diameter observed occurred when papaverine was infused.

Light Microscopy

The right kidney from each animal was divided longitudinally from its convexity toward the hilus. The
Relative lumen diameter is given as a percentage of the lumen diameter when perfused with papaverine solution. Values are mean±SEM, calculated on the basis of measurements in eight vessels from six Wistar rats.

### Table 1. Relative Lumen Diameter in Mesenteric Small Arteries at Various Stages of Morphometric Method

<table>
<thead>
<tr>
<th>Stage</th>
<th>Relative lumen diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma perfusion</td>
<td>65±6</td>
</tr>
<tr>
<td>Perfusion with papaverine</td>
<td>100</td>
</tr>
<tr>
<td>Microfil perfusion</td>
<td>92±6</td>
</tr>
<tr>
<td>Microfil</td>
<td>101±6</td>
</tr>
<tr>
<td>Fixed vessel</td>
<td>99±5</td>
</tr>
<tr>
<td>In histological section</td>
<td>97±7</td>
</tr>
</tbody>
</table>

Figure 1. Schematic drawing shows a glomerulus in which latex beads have become trapped in glomerular capillaries. Plugging of capillaries with beads resulted in a precapillary pressure rise to perfusion pressure level. Furthermore, the latex beads in the afferent arteriole were used for identification of this arteriole. D, distal part of the arteriole; P, proximal part.

dorsal half of the kidney was cut into six pieces at right angles to the corticomedullary junction to optimize the number of afferent arterioles cross-sectioned. The tissue pieces were preembedded in agar to keep orientation, dehydrated through a graded series of ethanol solutions, and embedded in glycol methacrylate (GMA, Historesin, LKB, Bromma, Sweden). Resultant blocks were cut in serial 2-μm-thick sections on a microtome (Supercut 2065, Leica, Reichert-Jung, Wetzlar, FRG), placed on glass slides, stained with Giemsa, and coded so that the examiner (K.S.) was unaware of the source of the specimen.

### Identification of Afferent Arterioles

With the use of two microscopes (BH2, Olympus, Tokyo) equipped with mirrors, the pictures of the sections were projected on a table top (Figure 2A). Under oil immersion lenses (×100), each vessel in two adjacent sections was first traced on paper for future identification and then measured with a ruler at a total magnification of ×1,650 (Figure 2B). Only vessels that were situated in the renal cortex, contained Microfil, and had a diameter less than 30 μm were included. The arterioles were categorized into two groups relative to their locations to the glomeruli: 1) distal arterioles, which were observed to be in close relation to the glomerulus, and 2) proximal arterioles, which were all other afferent arterioles measured. On average, 34 (range, 24–50) profiles of afferent arterioles were measured in each kidney. Branching and irregular vessels were excluded. Also excluded were vessels in which the smooth muscle cell nuclei were oriented perpendicular to the vessel axis, because they represented tangential sections through a vascular bend. Furthermore, the arterioles found had to fulfill at least one of the following criteria to be classified as an afferent arteriole: 1) a microsphere present in the lumen or in a glomerulus, providing that it was possible in serial sections to show that the microsphere had passed through the vessel to that glomerulus; 2) an internal elastic lamina present, because this lamina is constantly absent in efferent arterioles even where many smooth muscle cells are seen in the vascular wall24 as indicated in Table 2, about half of the proximal afferent arterioles were identified by their internal elastic lamina; however, because the internal elastic lamina disappears from all the afferent arterioles close to the entrance into the glomerulus,24 the number of distal afferent arterioles identified by this criteria is very low; 3) a close relation to an interlobular artery, if necessary, demonstrated in serial sections; 4) epithelioid cells, which are primarily seen in the distal part of afferent arterioles,24 present in the media wall; 5) Microfil present in the arteriole but absent in the glomerular capillaries, indicating that the efferent part in that region had not been filled. Table 2 shows the distribution between these categories in the identification of the vessels. Note that criterion 4 was not unequivocal, since epithelioid cells are seen in a few efferent arterioles.24 However, this criterion was only used in 18.4% (Table 2), and any error introduced here would be minor.

If an insufficient number of afferent arterioles was found in the kidney sections, and if the Microfil perfusion, as judged microscopically, had been poor, the animal was eliminated from further consideration. Poorly perfused areas of the kidney were seen as wedge-shaped areas without Microfil content. Of the original 28 animals, eight were discarded, leaving nine SHRs and 11 WKY rats at the end of the experiment.

The degree of Microfil filling was judged, for each kidney, after the perfusion on the basis of the kidney surface coloring. We did not find any relation between the degree of Microfil filling and either the later-estimated lumen size or whether the animal was hypertensive or normotensive.

### Morphometry

Two perpendicular lines were drawn on paper and were superimposed and centered on the image of the vessel to be measured (Figure 2B). The longest inner diameter, defined as the maximum distance across the vessel from one adluminal side of the internal elastic lamina to the other, was found. The inner diameter of the vessel perpendicular to the longest axis was measured. Assuming that the elliptical form of the vessel...
was due to an oblique sectioning, this measured diameter was the true internal diameter of the vessel relaxed and fixed under a transmural pressure of 100 mm Hg. Furthermore, the outer diameter of the vessels, which were taken as the border between media and adventitia, was measured at the same axis. Vessel wall thickness and media cross-sectional area were calculated as \((\text{o.d.} - \text{i.d.})/2\) and \(\pi(\text{o.d.}/2)^2 - (\text{i.d.}/2)^2\), respectively. Measurement resolution was approximately 1 mm, corresponding to 0.6 \(\mu\)m.

Statistics
All results are presented as mean\(\pm\)SEM. Significance of differences between SHR and WKY parameters in Table 3 was assessed by Student's two-tailed \(t\) test. The parameters in Table 4 were analyzed statistically with a two-way analysis of variance. Probability levels less than 5% were considered significant.

Results
Table 3 shows rat characteristics. One week before perfusion of the left kidney, the systolic blood pressure of SHRs (11 weeks old) was significantly greater (46%) than that of WKY rats. Body weight at the age of 12 weeks was lower in SHRs, and heart weight was greater, leading to a significantly increased heart-to-body weight ratio. No differences were found in kidney weights between the animal strains comparing left with left and right with right kidneys. However, the right kidney within rat strains was heavier than the left kidney \((p<0.01, \text{paired two-way analysis of variance})\). The left kidney was weighed after having been cleaned for fat tissue, immersion-fixed, and gently blotted for excessive moisture with towels; the right kidney was weighed after having been perfused with Microfil, immersion-fixed, cleaned, and blotted.

Table 4 presents the results of the morphometric measurements of the afferent arterioles. Two-way analysis of variance indicated that the lumen diameter of the vessels was smaller in SHRs than WKY rats in both proximal and distal arterioles. Furthermore, the analysis showed a not unexpected reduction of the lumen diameter toward the glomerulus in both rat strains. The degree of reduction or tapering along the length of the arterioles was calculated and expressed as percentage of tapering (Table 4), which was found to be slightly larger in SHRs compared with WKY rats. Although the decreasing lumen diameter along the length of the afferent arterioles was

Table 2. Identification of Arterioles

<table>
<thead>
<tr>
<th>Identification criteria</th>
<th>Distal arterioles</th>
<th>Proximal arterioles</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55.3</td>
<td>19.6</td>
<td>34.6</td>
</tr>
<tr>
<td>2</td>
<td>3.8</td>
<td>47.4</td>
<td>29.1</td>
</tr>
<tr>
<td>3</td>
<td>15.0</td>
<td>32.2</td>
<td>25.0</td>
</tr>
<tr>
<td>4</td>
<td>18.4</td>
<td>0.3</td>
<td>7.8</td>
</tr>
<tr>
<td>5</td>
<td>7.5</td>
<td>0.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Identification criteria refer to criteria listed in "Methods." Distal arterioles are vessels in close relation to a glomerulus; proximal arterioles are all other afferent arterioles. Values are percent of arterioles identified by the identification criteria.

Table 3. Characteristics of Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SHR ((n=9))</th>
<th>WKY ((n=11))</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure ((\text{mm Hg}))</td>
<td>177±5</td>
<td>121±3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body weight ((\text{g}))</td>
<td>284±1</td>
<td>297±3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart/body weight ((\text{mg/g}))</td>
<td>3.20±0.07</td>
<td>2.91±0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Right kidney weight ((\text{g}))</td>
<td>1.33±0.21</td>
<td>1.44±0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Left kidney weight ((\text{g}))</td>
<td>1.04±0.08</td>
<td>1.18±0.12</td>
<td>NS</td>
</tr>
</tbody>
</table>

SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; \(p\), significance levels by two-tailed \(t\) test. Values are mean\(\pm\)SEM.
arteriole was found to be accompanied by an equivalent reduction in media thickness, no differences in media thickness could be demonstrated between SHRs and WKY rats. In accordance with these findings, the measured outer diameter of the vessels was found to be significantly smaller, both between SHRs and WKY rats and between proximal and distal arterioles within the rat strains.

Based on the measurements of lumen diameter, media thickness, and outer diameter, the media: lumen ratio and media cross-sectional area (equivalent to media volume per segment length) were calculated. The results of these calculations are also shown in Table 4. There was a tendency \( (p=0.072) \) for the media: lumen ratio of SHR vessels to be greater than that in WKY vessels. However, this media: lumen ratio was, within the strains, fairly constant along the length of the vessels. As expected, the media cross-sectional area decreased with decreasing size of arterioles within both SHRs and WKY rats. The media cross-sectional area was, however, significantly reduced in SHRs compared with WKY rats in both proximal (10%) and distal (23%) afferent arterioles.

### Discussion

A number of studies have estimated the diameter of the renal afferent arterioles in SHRs and WKY rats. Hsu and Slavicek\(^{14}\) used a microsphere technique in which a mixture of microspheres (15.1±3, 16.8±3, 22.3±3 \( \mu \)m) was injected into the circulatory system of the animals, and then the average lumen diameter was estimated on the basis of distribution curves obtained by measuring the diameters of the microspheres trapped within the afferent arterioles. In 11-week-old SHRs, they estimated the lumen diameter of the afferent arteriole on average to be 17.7 \( \mu \)m, which was significantly lower than the 19.5 \( \mu \)m found in WKY rats. These figures were somewhat larger than those found by Gattone and coworkers\(^{16}\) and recently also by Kimura et al\(^{17,18}\) using a simple perfusion fixation followed by a vascular casting technique after which the tissue was digested and the casts were measured on a scanning electron microscope. In 12-week-old\(^{16}\) and 20-week-old\(^{17,18}\) SHRs, the arteriolar lumen diameter was 11–14 \( \mu \)m in the immediate preglobular arterioles, in contrast to the significantly larger (14–17 \( \mu \)m) arterioles of the WKY rat. Some of this discrepancy in the estimates of lumen diameters may be explained by the fact that, by the microsphere technique,\(^{14}\) the measured lumen diameter represents only a portion of the arteriole, which will not necessarily represent the mean diameter of the vessel. As shown by Gattone and coworkers\(^{16}\) and confirmed by the present investigation, a substantial and differing tapering of the afferent arteriole exists within and between SHRs and WKY rats. However, we believe that the conditions under which the arteries are fixed and investigated are the major reason for the discrepancy between the measurements referred to above.

Arteries are contractile elastic structures whose dimensions consequently will depend on the smooth muscle cell activity within the vascular wall, the viscoelastic properties of the vessel, and the distending transmural pressure. This fact becomes especially important when morphological comparisons between vessels are made, because such comparisons, of course, would be less informative if the vessels were not investigated under identical, controlled, and constant conditions.\(^{1,21,22}\) The classic way to investigate the dimensions of vessels in situ is after a simple perfusion fixation using different kinds of fixatives perfused at constant flow\(^{9,27,28}\) or pressure\(^{16–18}\) in histological sections or after making casts of the vasculature to measure vessel lumen diameters. However, because the transmural pressure to which a given vessel is subjected during fixation will depend on the vascular resistance upstream, and because that resistance is obviously unknown, the measurements of vessel structure under the conditions given by the simple perfusion fixation technique will not be unequivocal. Furthermore, any direct contractile effect of the fixatives on the smooth muscle cells used during fixation must also be taken into consideration to make a distinction between structural versus functional influence on vascular structure.

### Table 4. Characteristics of Proximal and Distal Afferent Arterioles of Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vessel</th>
<th>SHR (n=9)</th>
<th>WKY (n=11)</th>
<th>p (strain)</th>
<th>p (art)</th>
<th>p (x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen diameter (( \mu )m)</td>
<td>Distal</td>
<td>15.4±0.6</td>
<td>18.5±0.3</td>
<td>&lt;0.001</td>
<td>0.012</td>
<td>0.615</td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>17.2±0.4</td>
<td>19.7±0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tapering (%)</td>
<td>Distal</td>
<td>10.6±3.6</td>
<td>7.5±2.6</td>
<td>0.450</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>1.6±0.1</td>
<td>4.1±0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media thickness (( \mu )m)</td>
<td>Distal</td>
<td>3.6±0.1</td>
<td>3.9±0.2</td>
<td>0.245</td>
<td>0.026</td>
<td>0.373</td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>1.1±0.1</td>
<td>4.1±0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer diameter (( \mu )m)</td>
<td>Distal</td>
<td>22.5±0.5</td>
<td>26.3±0.7</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.274</td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>25.4±0.4</td>
<td>28.0±0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media/lumen (%)</td>
<td>Distal</td>
<td>27.0±2.3</td>
<td>23.3±1.2</td>
<td>0.072</td>
<td>0.729</td>
<td>0.769</td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>25.9±1.5</td>
<td>23.1±1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media area (( \mu )m²)</td>
<td>Distal</td>
<td>210±6</td>
<td>274±16</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.194</td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>279±11</td>
<td>311±11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats. Tapering indicates the calculated degree of lumen reduction from proximal to distal arterioles and is expressed as percent tapering. \( p(\text{strain}) \), \( p(\text{art}) \), and \( p(x) \) show level of significance between SHR and WKY rats, level of significance between proximal and distal vessels, and interaction with a two-way analysis of variance, respectively. For degree of tapering, the comparison was made with Student’s \( t \) test; the \( p \) value for this test is shown under \( p(\text{strain}) \). Values are mean±SEM.
With the method presented here, in which all precapillary vessels are fixed relaxed with a high dose of papaverine and with a known transmural pressure, it is possible to evade these problems. The mechanical plugging of the capillaries by the latex beads (Figure 1), together with a closure of the venous outlet, increased the pressure in the relaxed precapillary vessels to the level of the perfusion pressure. As has been shown by Nilsson, the lumen diameter of small arteries will, after an initial rapid increase, be almost constant at pressures greater than approximately 80 mm Hg. At a distending pressure greater than this level, the vessels in two different species such as the SHR and WKY rat will be maximally dilated independent of possible (and so far unproven) differences in viscoelastic properties. Therefore, the discussion of whether the vascular beds under investigation must be perfused at the in vivo mean blood pressure or at some fixed level becomes less important. Also, the influence of possible interexperimental and intraexperimental differences in distension pressure on the measured parameters will be reduced. Furthermore, constant pressure will make comparisons between studies easier.

A number of factors, however, could also have influenced the transmural pressure in our setup. A clotting of particles or plastic material in the catheter during perfusion would lead to a drop of pressure in the distal arteries. The probability that this happened was minimal, because the perfusion time from one kidney to another was constant. Furthermore, all kidneys with the slightest morphological indication of a bad perfusion were discarded and were not used for further investigations. The transmural distension pressure given by the difference between intramural and extramural pressure could, especially in an encapsulated organ such as the kidney, be influenced by a filtration of the perfusion medium. Therefore, it was not necessary in our experiment to correct for eccentricity or to exclude measuring on sections with large sectioning angles, as has been done earlier when vessel dimensions were determined from measurements of vessel wall or lumen cross-sectional area.

Thus, regarding the lumen diameter of the afferent arterioles, we have found, in agreement with what has been reported earlier, that it was smaller in the SHR compared with the WKY rat. The absolute luminal diameter measured is larger in our experiment than found previously, probably because the smooth muscle cells were fully relaxed and the distending pressure was larger. Furthermore, we have also found, as reported by Gattone and coworkers, a tendency for an increased tapering of arterioles in SHRs compared with WKY rats. However, as argued above and in contrast to earlier reports, we can conclude that the reduced lumen diameter is caused by structural changes in the afferent arterioles, because any functionally determined contribution to a lumen reduction has been eliminated. Based on the Poiseuille equation, the reduced diameter could account for a 54% increase in minimum renal vascular resistance. This conclusion therefore fully supports the hemodynamic findings in which the renal vascular resistance at maximal vasodilation was increased by 15–36% in the SHR depending on the conditions under which it was measured.

In extrarenal arteries in SHRs, a structurally determined lumen reduction is also observed in the established phase of hypertension compared with the normotensive WKY rat. In these vessels, the structural changes are associated with an increased media thickness-to-lumen diameter ratio (media : lumen ratio) partly explained by the reduced lumen diameter but also consistently associated with media hypertrophy. In the casting experiments of the kidneys, in which the surrounding tissue is cleared, it is not possible to tell whether observed luminal changes are associated with structural changes in the tunica media, information that is essential in the investigation of vessel structure in hypertension. Some indications can be obtained by making histological sections in corresponding perfusion-fixed material, as has been done by Gattone and coworkers, although the identification of specific vessels in such sections might be difficult. Measured in that way, Gattone found only small changes in the media : lumen ratio, suggesting that there might be no media hypertrophy. These indications have recently been confirmed to some extent in a morphometric investigation of perfusion-fixed and isolated arterioles from 21-week-old SHRs.

In our experiment, in contrast to these earlier studies, it was possible in the same histological sections to measure corresponding values of media thickness and lumen diameter. The media thickness was measured in the same plane as the lumen diameter. Fully in agreement with the earlier reports, we found only a slight tendency for the media : lumen ratio to be increased in the SHR. This, however, as also inferred by Gattone et
al. was not found to be associated with an encroaching, hypertrophic response in the media, as has been reported for the extrarenal and larger renal arteries. In fact, on the contrary, the media cross-sectional area was according to our results significantly smaller in SHR renal afferent arterioles as compared with the WKY rat, perhaps indicating inherent, structural abnormalities of these vessels not caused by a secondary hypertrophic response to the increased pressure. This possibility is supported by reports that have shown that both renal vascular resistance and vascular lumen diameter are changed even before measurable changes are found in the blood pressure. This therefore could explain why blood pressure “follows the kidney,” as has been shown in transplantation studies, but further studies combining the morphometric and transplantation techniques are needed to confirm this.

Acknowledgments

We thank Professor Steen Olsen for critical evaluation and Dr. Eva Ehrnrooth for practical assistance during the pilot project. Anette Espeersen is thanked for expert technical assistance, and the Departments of Pathology, Neuropathology, and Forensic Medicine, Aarhus City Hospital, Aarhus, for facilities.

References

2. Guyton AC: Renal function curve: A key to understanding the pathogenesis of hypertension. Hypertension 1987;10:1–6
Morphology of renal afferent arterioles in spontaneously hypertensive rats.
K Skov, M J Mulvany and N Korsgaard

Hypertension. 1992;20:821-827
doi: 10.1161/01.HYP.20.6.821

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
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:
http://hyper.ahajournals.org/content/20/6/821

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/