Renal Afferent Arteriole in the Spontaneously Hypertensive Rat

VINCENT H. GATTONE II, PH.D., ANDREW P. EVAN, PH.D., LYNN R. WILLIS, PH.D., AND FRIEDRICH C. LUFT, M.D.

SUMMARY We conducted morphometric studies on the afferent arteriole of spontaneous hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats to gain a better understanding of its changes with the development of hypertension. Differences may be related to the SHRs' increased renal vascular resistance. Methacrylate vascular casts were made of the renal vasculature after perfusion fixation with glutaraldehyde. These vascular casts were then examined and measurements made with the scanning electron microscope. Results from this examination of the scanning electron microscope demonstrated a smaller afferent arteriolar diameter in the SHR, compared to the WKY, for both the inner and outer cortical glomeruli. This difference was seen in the 6-week-old SHR, prior to a statistically different blood pressure from the WKY controls, as well as in the 12-week-old hypertensive SHR. However, this afferent diameter difference between rat strains was more pronounced in rats at 12 weeks of age. The tapering of the afferent arteriole (difference between proximal and distal afferent diameters) was greater in the 12-week-old SHR than in the age-matched WKY or 6-week-old SHR. We conclude that the smaller caliber afferent arterioles of the SHR may predispose and play a role in the pathogenesis of the subsequent hypertension. The increased afferent arteriolar tapering seen in the hypertensive SHR relates to the already present increased blood pressure. Wall thickness/radius ratios are not different between rat strains (SHR and WKY) at either 6 or 12 weeks of age. These results suggest increased vascular constriction or hypoplastic vessels as the cause of the smaller caliber vessels in the SHR rather than increased wall thickness. (Hypertension 5: 8-16, 1983)

KEY WORDS • renal afferent arterioles • vascular casts • SHR • morphometrics

INCREASED vascular resistance is thought to play an important role in the maintenance of hypertension in both humans and experimental animals.1 These data have led to an interest in the morphology and physiology of the vascular system during hypertension.2 3 The mesenteric vessels of the SHR show a decreased vascular compliance4 and increased norepinephrine sensitivity5 as compared to the WKY even prior to the development of hypertension. Similarly, changes have been described in the glomerular filtration barrier of the prehypertensive spontaneously hypertensive rats (SHR).3 Changes in the intrarenal vascular system could be especially significant since the kidneys have been shown to play an essential role in the development of hypertension in this rat model.6

The major resistance vessels of the kidney are the afferent arterioles. At present there is limited information concerning these vessels in the prehypertensive and hypertensive SHR. Therefore, the present study is designed to determine if there are changes occurring in the morphology of the afferent arteriole of the SHR with the onset of hypertension. The resistant blood vessels were examined through the use of methacrylate vascular casts, which allowed a morphometric analysis of the entire length of the afferent arteriole.

Our data show that the afferent arterioles of the SHR have a smaller diameter, even prior to the onset of blood pressure difference compared to the Wistar Kyoto (WKY) control. The difference in arteriolar diameter became more pronounced in the 12-week-old SHR compared to the WKY. These changes suggest that the afferent arteriole may participate in the pathogenesis of hypertension in the SHR.
AFFERENT ARTERIOLES IN SHR/Gattone et al.

Methods

Male SHR and WKY rats, 6 and 12 weeks of age, were obtained from Cox Laboratories (Indianapolis, Indiana). Mean blood pressures were measured in conscious rats by direct arterial catheterization as described in detail elsewhere. Eight rats in each group were studied and prepared as described below, except for the 12-week-old SHR group, which contained seven animals.

After the blood pressures were measured, the rats were anesthetized with pentobarbital, and a catheter (PE50 or PE90 for 6- and 12-week-old animals respectively) was inserted into the abdominal aorta below the renal arteries. The proximal aorta was ligated, and 0.9% saline (at room temperature for 10 seconds) was infused, followed by 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 (room temperature approximately 390 mOsM). To assure fixation of the vasculature in a functional state, an infusion pressure of 130–150 mm Hg was maintained for all WKY animals and the 6-week-old SHR, while 200–220 mm Hg was used for the hypertensive SHRs. Following fixation, one kidney was removed for conventional studies while a cast of the vascular system of the other kidney was prepared with Batson No. 17 compound (Polysciences Inc., Warrington, Pennsylvania). The material was infused via hand injection. The kidney preparations were handled in such a fashion that the microscopist had no knowledge of the animal group; each kidney was assigned a code number, and the code was broken only after the morphometric data were collected so that statistical analysis could be done.

The renal vascular casts were allowed to cure in saline for at least 20 hours at room temperature, and thereafter were digested in 30% potassium hydroxide at approximately 80°C until all the tissue was removed or loosened. The casts were allowed to cool, rinsed several times in distilled water, then air dried. If necessary, specimens were briefly sonicated during the rinsing stage to remove any loose tissue. Subsequently, the casts were dissected apart and random segments oriented and mounted on stubs with silver paste (Pelco Inc., Tustin, California). These samples were sputter-coated with Au/Pd with a Hummer V (Technics Inc., Alexandria, Virginia) and examined with an AMR 1000A scanning electron microscope operated at 20 kV. Photographs were recorded on Polaroid type 55 P/N film.

Diameters of the afferent arterioles were measured directly on the visual cathode ray tube (CRT) at a constant magnification (×450) from the coded specimens. A keyboard data and measuring system on the SEM served as a reference for, and assisted in, the collection of the morphometric data. Measurements of both proximal and distal afferent arteriolar regions were made. The lengths of the afferent arterioles varied at all levels of the cortex, but the afferent could be broadly grouped as long (> 100 μm), or short (< 100 μm). For the long afferents, the proximal afferent diameter (PAD) was measured 90 to 150 μm from the glomerulus, while for the short afferents, the PAD was measured 15 μm from the interlobular artery. To validate the comparability of these measurement locations so that all PAD values could be pooled, we performed serial diameter determinations through the 90–150 μm portion of long afferents. There was a negligible difference in the diameter of the afferent arteriole over that distance. Values of the PAD from short afferents were also comparable to those from the long afferents. Therefore, we concluded that there was negligible tapering over that portion of the afferent arteriole used for the measurement of PAD. Thus, consistency of afferent arteriolar diameter allowed us to use all afferent arterioles, regardless of their length, in the statistical analysis of the PAD. The distal afferent arteriole diameter (DAD) was measured at the narrowest point of the afferent arteriolar within 20 μm of the glomerular. The location of each glomerulus was noted and recorded as either juxtamedullary, inner, mid, or outer cortex. The data from juxtamedullary and inner cortical glomeruli were combined (as the inner group), while measurements from mid- and outer cortical groups were termed the outer group. For each animal, a minimum of 25 afferent arterioles were measured. The means of the measurements, i.e., PAD and DAD, per glomerular group from each animal, were then submitted for statistical analysis.

Appropriate steps were taken to assure that the measurements acquired from the scanning electron microscope were accurate. The accuracy of the magnification was checked using a 600-mesh high transmission grid (Marivac Ltd., Halifax, Nova Scotia) and a standardizing specimen (Secondary Images, Winchester, Ohio). In addition, the 600-mesh grid was used to check for image distortion. The only aberration found in the visual CRT was a minimal nonrectilinearity in the horizontal axis, which was noted at the corners and very edge of the screen. The working distance (12 mm), spot size, and magnification used during the measurements limited the depth of field to approximately 130 μm, so only one afferent arteriole would be clearly visible at any time.

To determine if the random orientation of the afferent arteriole could result in an over- or undermeasurement of its dimensions due to a perspective error, we measured the diameter of a 90 μm wire that was formed into a ‘‘tangle.’’ Initially, measurements were made along arcs that were close to the horizontal plane of the scanning electron microscope and thereby simulated the procedure used for the afferent arterioles. We found the diameter of the wire to be identical at all points measured. Next we measured arcs of wire that extended through the depth of focus of the scanning electron microscope in order to detect any illusionary measurements that would be associated with its depth of focus. A maximum error of 7% was associated with measurements taken along such arcs (top to bottom) when the focus of the entire field was not changed. However, if points along the same arc were refocused (making appropriate adjustment in magnification) and measured, there was a perspective error of less than...
4%. Examination of flat segments of the same wire showed distortion (8%) only at the corners of the visual CRT due to nonrectilinearity in the scanning electron microscope. The data from these measurements were obtained from a wire of known diameter and of a random orientation, indicating that there was minimal distortion of our measurements of the vascular casts when the vessel was near the center of the CRT or refocused as needed.

Segments of the opposite kidney that was not casted, was dehydrated and embedded in JB-4 media (Polysciences Inc., Warrington, Pennsylvania). This tissue was sectioned at 4 μm with a JB-4 microtome (Du Pont-Sorvall Inc.) and stained with toluidene blue. The kidney tissue was determined to be well preserved as noted by the appearance of open tubular lumens and cellular integrity (fig. 1 a). Ten cross sections of afferent arterioles (fig. 1 a, inset) per kidney were photographed with a Zeiss photomicroscope onto Kodak Panatomic X film and projected onto a digitizing pad (supergrid, Summagraphics Corporation, Fairfield, Connecticut) at a constant magnification. Luminal area (A₁) and total vessel area (Aᵥ) were also determined. The wall thickness/radius was determined as described elsewhere with one modification, the luminal radius (r) was calculated from A₁ rather than luminal perimeter. Luminal radius was determined by:

\[
r = \sqrt{\frac{A_1}{\pi}}.
\]

Wall thickness was determined by:

\[
\sqrt{\frac{A_v}{\pi}} - r.
\]

These data were analyzed by the Student’s t test.

To confirm that the vessel diameters from the vascular casts were consistent with their functional state, we prepared freeze-substituted specimens from additional groups of 12-week-old rats. The 12-week-old SHR and WKY rats were anesthetized with pentobarbital, and a laparatomy was performed. The left kidney was gently dissected free of its perirenal fat and set in a

![Representative micrograph showing good fixation of an afferent arteriole (A), glomerulus (G), and kidney tubules (T) from a 12-week-old WKY animal by perfusion fixation (a) and freeze substitution (b). Inset shows a typical cross section of afferent arteriole that was used in determining the wall thickness/luminal radius ratio. The renal capsule (C) is seen in the freeze substitution kidney (b). a. ×340 (inset ×540). b. ×250.](http://hyper.ahajournals.org/doi/fig/10.1161/01.HYP.5.1.10)
plastic trough. Initially, isopentane (cooled by liquid nitrogen) was poured on the in situ kidney followed by liquid nitrogen. The hilus was fractured, allowing removal of the kidney. The kidney was immediately placed in liquid nitrogen, and segments of the anterior surface of the kidney were cryofractured and placed in 2% OsO₄-100% ethanol solution at -90°C for 10 days, then for 2 days at -25°C in absolute ethanol, followed by 1 day each at -4°C and 4°C in absolute ethanol. The tissue was brought up to room temperature, placed in two changes of propylene oxide, and infiltrated with Epon 812. Each sample was embedded in Epon 812 and cured at 60°C for 24 to 48 hours. The outer cortical region (outer 300-500 μm of cortex) from these blocks was sectioned (1.5 μm thick), stained with methylene blue-azure II, and cross sections of afferent arterioles (within 20 μm of a glomerulus) photographed with a Zeiss photomicroscope HI at constant magnification onto Kodak Panatomic X 35 mm film.

The negative of the arterioles were projected (from below) onto a digitizing pad (Supergrid, Summagraphics Corporation). The lumina of the afferent arterioles were traced and data collected and analyzed by a computer (Cromemco, Inc., Mountain View, California) which was programmed with image analysis software (Quantigraph, Norvus Instruments, Inc., Indianapolis, Indiana). The image of a calibrated stage micrometer was projected at the same magnification as the arterioles, and thus served as a standard for these measurements. The mean luminal diameter (Dₘ) was determined from the luminal area (Aₑ) as traced on the digitizing pad. The following formula was used, assuming the traced area to be a circle:

\[ Dₘ = 2 \times \sqrt{\frac{Aₑ}{\pi}} \]

The value for the mean diameter was determined from the cross section of at least 10 outer cortical afferents per animal. Only those areas that showed open tubules with well-preserved cellular integrity were used. Such tissue is seen in figure 1 b. Wall thickness/radius ratios were also determined from these same freeze substituted afferent arterioles as described earlier.

The data from both the PAD and DAD afferent arteriolar measurements and the afferent tapering were analyzed by three-way analysis of variance to consider rat strain, age, and cortical group. Multiple comparisons were made by Student’s t test for unpaired data. The 95% limit of probability was considered significant. The data are expressed as means ± standard deviation (SD).

Results

At 6 weeks of age, the mean blood pressures of the SHR and WKY rats were not significantly different (SHR 134 ± 9 mm Hg, WKY 121 ± 12 mm Hg, p > 0.05). By 12 weeks, the blood pressures of SHR (157 ± 9 mm Hg) were significantly greater (p < 0.05) than those of WKY rats (119 ± 3 mm Hg).

Figure 2 illustrates vascular casts of glomeruli from juxtamedullary and inner cortical regions (inner glomeruli) as well as from mid- and outer cortical regions (outer glomeruli). Figures 3 and 4 were obtained from glomerular casts of 6- and 12-week-old SHRs respectively. The afferent arteriolar dimension with its sequential narrowing as it enters the glomerular tuft can be clearly seen. Figures 5 and 6 display casts from a 6- and 12-week-old WKY rat. Histologic examination of the contralateral kidney shows that the renal parenchyma and vasculature were well fixed prior to casting (fig. 1 a).

The afferent arteriolar diameters are presented in table 1. Three-way analysis of variance indicated that the diameters of the vessels were smaller in SHR than WKY rats at both the PAD and DAD locations (p < 0.001). Likewise, arterioles of 6-week-old SHRs were smaller than those of the 12-week-old animals at both PAD and DAD locations (p < 0.001). Analysis revealed a two-way interaction between rat strain and age. At 6 weeks, the arteriolar diameter in the WKY was slightly larger (about 22% for PAD, 11% for DAD) than in the SHR, but by 12 weeks the WKY’s arterioles were considerably larger than SHR (32% for PAD, 38% for DAD). This interaction was more pronounced in the DAD (p < 0.001) than PAD locations (p < 0.01). Analysis also indicates that the diameters of arterioles in the inner glomerular group were larger than in the outer glomerular group at both measurement locations (p < 0.05). Another two-way interaction was identified between age and glomerular location (p < 0.01). The diameters of the inner glomerular group had a much larger afferent arteriolar dimension than the outer group at 6 weeks; by 12 weeks, however, there was less of a size differential between the inner and outer groups.

Utilizing the differences between the PAD and DAD we calculated the degree of reduction or tapering along the lengths of the individual afferent arterioles. These values are expressed as percent tapering (table 1). Three-way analysis of variance of these values showed a difference depending on location (with the tapering increased in the inner group, p < 0.001). There were no overall differences based on rat strain (p > 0.05) but there was a significant two-way interaction between strain and age (p < 0.001). This interaction indicates that, between 6 and 12 weeks, the afferent arteriole in the WKY became relatively less tapered, while in the SHR, it became more tapered. This increased tapering, which was more prominent in the SHR’s outer glomerular than in the inner group, was demonstrated by specifically comparing arteriolar tapering of the 12-week-old SHR with that of the 6-week-old SHR (both inner, p < 0.05; and outer group, p < 0.001) or with 12-week-old WKY (outer, p < 0.001). While examining the specimens in the scanning electron microscopes, it was apparent that the more tapered afferent arterioles occurred in small spa-
FIGURE 2. Low magnification scanning electron micrograph showing typical renal vascular cast. The segment of renal arterial vasculature from arcuate artery to glomerulus can be demonstrated. The inner and outer cortical glomerular groups can also be seen; inner below dashed line, outer above. Arcuate artery (A), Interlobular artery (I), Afferent arteriole (arrows); glomerulus (arrowhead). × 120

TABLE 1. Afferent Arteriolar Diameters and Percent Tapering (means ± so)

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>No.</th>
<th>PAD (μm)</th>
<th>DAD (μm)</th>
<th>Tapering (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY -</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 weeks old</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inner</td>
<td>4</td>
<td>15.6 ± 1.5</td>
<td>10.5 ± 1.6</td>
<td>30.9 ± 2.6</td>
</tr>
<tr>
<td>Outer</td>
<td>4</td>
<td>10.6 ± 0.8</td>
<td>8.0 ± 0.8</td>
<td>23.3 ± 3.0</td>
</tr>
<tr>
<td>12 weeks old</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inner</td>
<td>3</td>
<td>21.6 ± 1.0</td>
<td>15.9 ± 1.4</td>
<td>25.0 ± 3.8</td>
</tr>
<tr>
<td>Outer</td>
<td>5</td>
<td>20.3 ± 0.7</td>
<td>15.4 ± 0.4</td>
<td>22.2 ± 2.2</td>
</tr>
<tr>
<td>SHR -</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 weeks old</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inner</td>
<td>3</td>
<td>12.0 ± 1.2</td>
<td>9.1 ± 0.4</td>
<td>23.1 ± 3.4</td>
</tr>
<tr>
<td>Outer</td>
<td>5</td>
<td>9.3 ± 0.9</td>
<td>7.5 ± 0.8</td>
<td>17.9 ± 3.3</td>
</tr>
<tr>
<td>12 weeks old</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inner</td>
<td>3</td>
<td>15.2 ± 1.7</td>
<td>11.1 ± 0.9</td>
<td>30.7 ± 2.7</td>
</tr>
<tr>
<td>Outer</td>
<td>4</td>
<td>16.8 ± 1.3</td>
<td>11.6 ± 0.9</td>
<td>30.2 ± 0.9</td>
</tr>
</tbody>
</table>

*Parenthetical values for afferent diameters from outer cortex of freeze substitution specimens.

*P < 0.05 for differences between 6- and 12-week-old animals.

*P < 0.001 for proximal and distal afferent diameter difference between SHR and WKY animals.
FIGURE 3. Scanning electron micrograph of vascular cast from outer cortical region of 6-week-old SHR showing afferent arteriole (arrow), glomerulus and efferent arteriole (arrowhead). X450.

FIGURE 4. Scanning electron micrograph of vascular cast from outer cortical region of 12-week-old SHR showing afferent arteriole (arrow), glomerulus and efferent arteriole (arrowhead). X450.

FIGURE 5. Scanning electron micrograph of vascular cast from outer cortical region of 6-week-old WKY rat showing afferent arteriole (arrow), glomerulus and efferent arteriole (arrowhead). X450.

FIGURE 6. Scanning electron micrograph of vascular cast from outer cortical region of 12-week-old WKY rat showing afferent arteriole (arrow), and glomerulus. X450.


### Table 2. Wall Thickness/Luminal Radius Ratio (means ± so)

<table>
<thead>
<tr>
<th>Age (week)</th>
<th>No.</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>3</td>
<td>0.806 ± 0.147</td>
<td>0.807 ± 0.131</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>0.572 ± 0.093</td>
<td>0.629 ± 0.166</td>
</tr>
<tr>
<td></td>
<td>(3,4)</td>
<td>(0.517 ± 0.045)*</td>
<td>(0.535 ± 0.097)*</td>
</tr>
</tbody>
</table>

*p > 0.05 for difference between 12-week-old animals perfusion fixed or frozen in situ and freeze substitution processed (parenthetical values).

### Discussion

This study demonstrated a vascular difference between the SHR and WKY rat strains. At both 6 and 12 weeks of age, the diameter of the SHR's afferent arteriolar was smaller than in age-matched WKY controls. This diameter difference was greater in the 12-week-old (hypertensive) SHR than in the 6-week-old SHR. Using multiple comparisons of the diameters of the 6 week animals, significance (p < 0.05) was seen for the PAD values but not for the DAD values, suggesting that the quantitative methodology does not allow differentiation of subtle differences in vessels smaller than 9 μm in diameter. In the 12-week-old hypertensive SHR there was also a significantly greater arteriolar tapering (which might represent preglomerular constriction) when compared to the 6-week-old SHR or 12-week-old WKY (outer cortical group). Therefore, this study has shown a predisposing vascular difference between the SHR and WKY (the difference in the afferent arteriolar diameter) as well as an adaptive change in the hypertensive SHR (an increased arteriolar tapering).

### Vascular Casting Technique

The technique of vascular casting utilized in this study used a partially polymerized methylmethacrylate (Batson's No. 17, Polysciences). There are several previously published vascular cast studies of the kidney dealing mainly with the glomerulus. However, Bielke et al. describe a lack of reproducibility in renal vascular casts that does not reflect the physiologic vascular condition. A major difference between these earlier studies and ours is that the investigators casted unfixed kidneys while we perfusion-fixed the kidneys before casting. Prior fixation allows the vessels to stabilize before introduction of the plastic. A glutaraldehyde fixative stabilizes the vessel prior to vasocostriction. In addition, intravascular perfusion is considered an accurate way of preserving vessels in their functional state without constriction, dilatation, or distortion. Once this vasculature is fixed, the plastic casting material merely fills these vessels, not altering their in vivo-like state. We do not know what effect unpolymerized plastic has on living vasculature, thereby limiting our ability to interpret results form the prior vascular cast studies of the kidney.

The consistency of the results in this study indicates the superiority of perfusion fixed-cast tissue. This advantage clearly outweighs the need for longer digestion times with more concentrated KOH maceration solutions, as described by Nowell and Lohse. Using perfusion fixed kidneys also allows a slightly higher perfusion pressure during casting with a hand syringe, which seems to give superior filling results. We did not encounter the inability to completely fill the efferent vasculature with Batson's plastic, as cautioned by Gannon. Clearly, the technique utilized in this study, perfusion-fixing kidneys prior to vascular casting, allows reproducibility of casting so that morphometric measurements (as in this study) or structural distortions can be determined with confidence.

The validity of our results from the vascular casts is suggested by: 1) the uniformity of mean diameters between rats within each group even though diameters showed some variability within each kidney; 2) values obtained by our technique are consistent with values obtained by the microsphere techniques; and 3) the luminal diameter (table 1) and wall/radius ratio (table 2) from our freeze-substituted kidneys (parenthetical values in the tables) suggest little, if any, alteration of the afferent by perfusion fixation. This last point is especially important since freezing the kidney in situ is probably the only adequate control to represent the functioning kidney. Since the luminal diameters (table 1) are between the values for the PAD and DAD for the outer cortical group, of their respective strains, these data support our casting values. The fact that the wall/radius area ratios are not statistically different between the 12-week freeze-substituted and perfusion-fixed animals further supports the reliability of the perfusion fixation to stabilize the vasculature in its functional state. Our casting technique does not reverse vasoconstriction of the afferent arteriole as produced in the glycerol-induced acute renal failure model, which indicates that the fixation-casting method does not reverse functionally constricted vasculature.

While all animals were treated similarly, nevertheless, it is possible that extraneous stimuli (anesthesia, surgery, etc.) may provoke more vasoconstriction in the SHR than in the WKY. This difference, if present, could be a function of the disease, since the diameter differences obtained tend to be supported by data from microsphere and freeze substitution afferent diameters. The values we obtained for luminal diameter from both the casting and freeze substitution methods may be slightly smaller than their functional in vivo values due to slight cast and tissue shrinkage during preparation.

### Afferent Arteriolar Findings

To our knowledge, this is the first study to directly determine the diameters of afferent arterioles from 6-
and 12-week-old SHR and WKY rats. Most vascular studies of the SHR have dealt with larger caliber vessels rather than the true resistance vessels in the kidney, the afferent arteriole. It is difficult to do morphometric analysis of the afferent arteriolar diameter using conventional histological techniques because of: 1) inconsistencies in obtaining perfect cross sections; and 2) difficulties in determining the location along the length of the arteriole. However, using microspheres, Hsu and Slavicek 23 found the afferent arteriole to be smaller in the SHR than WKY at 8 and 12 weeks of age. The diameters they gave for 12-week-old animals were not significantly different from our values for PAD at this age and therefore confirm our data. Similarly, Morkrid et al. 23 studied the afferent arterioles of rats with DOCA-salt hypertension by microspheres and found smaller afferent arterioles in the hypertensive animals compared to the controls.

Between 6 and 12 weeks, the outer cortical afferents grow more than the inner ones. This reflects the normal maturation of renal function in the outer cortical region. 24 However, the diameter growth is greater in the WKY than the SHR. This is particularly true for the inner cortical glomeruli, which may predispose this group of glomeruli in the SHR to the more severe damage that they later show. 25 The smaller afferent arterioles of the SHR are a basis for the increased renal vascular resistance. Renal vascular resistance is increased in the SHR, possibly as a result of these smaller arterioles 26, 27 and, after the onset of hypertension, probably also as a consequence of a physiological autoregulation. The renal vascular resistance and diameter differences are not seen in 4-week-old SHR but are seen in 8- and 12-week-old animals. 28 Isolated kidneys from SHRs were found to have lower flows and higher resistances than those in WKYs, across a wide range of blood pressures even after maximal dilatation with papaverine. 21 In addition, a recent study showed reduced renal blood flow per unit mass of kidney tissue in the New Zealand SHR and a reduced distribution and flow to the outer cortical compartment. 29 The 6-week-old SHR has a lower renal blood flow and greater preglomerular pressure drop. 30, 31 These findings suggest a preglomerular vascular origin, which emphasize the possible role of the kidney in the pathogenesis of hypertension in the SHR. Arendshorst and Beierwaltes 2 suggest a preglomerular afferent arteriolar constriction in the hypertensive SHR based on physiologic evidence. These functional observations correspond to the structural changes we describe.

The renal vascular reactivity of the SHR is increased according to data obtained from isolated perfused kidney experiments. 32 In these experiments, the responses of the renal vessels to sympathetic nerve stimulation were no different in SHR and WKY rats. However, it has been shown that the SHR's vascular smooth muscle is more sensitive to norepinephrine than that of the WKY. 5, 33 This increased norepinephrine sensitivity is related to a humoral factor. 34 This sensitivity may be why the SHR's vascular smooth muscle (from portal vein) shows an increase in the frequency and magnitude of phasic contractions as well as an increased contractility and a decreased extensibility. 33 While the greater maximum contractile strength of the SHR renal vascular smooth muscle could be due to altered excitation contraction coupling, a structural vascular change would also provide a plausible explanation. The studies of SHR mesenteric resistance vessels favor a morphological explanation. 3 Mulvany et al. 4 describe an increase in the number of smooth muscle cells in mesenteric vessels, while smooth muscle cell hypertrophy was seen only after the hypertension was well established. 5 They also describe smaller mesenteric vessels in the SHR at all ages studied (6, 12, and 24 weeks), which agrees with our findings in renal afferent arterioles. The wall thickness/luminal radius ratios (table 2) at 6 and 12 weeks show no difference between SHR and WKY. There is no evidence to suggest an increased thickness of the tunica media (media hypertrophy) from these data. Instead, an increased state of vascular contraction (increased vascular tone) or hypoplasia would seem more plausible explanation. The increased vascular tone could be related to an increased norepinephrine sensitivity in the SHR. 3, 34

In conclusion, this study has demonstrated that the overall diameter of the major resistance vessels (the afferent arteriole) of the kidney — that organ which is central in the pathogenesis of hypertension in the SHR — is smaller in the SHR than WKY rats. This diameter difference is present at both 6 weeks of age (prior to a statistically elevated blood pressure) and 12 weeks (hypertensive phase). The afferent arterioles have a smaller caliber along their entire length. The smaller caliber vessel is consistent with the findings of vasculature in other systems of the SHR. This possibly indicates the animal's predisposition to increased vascular resistance. It may relate to an increased vascular tone, which may result from the norepinephrine sensitivity. However, underlying morphologic alteration of the vasculature cannot be eliminated. Initially, at 6 weeks the arteriolar tapering is greater in the WKY than in SHR. However, after the hypertension has become well established at 12 weeks, the tapering is greater in the SHR than in the 12-week-old WKY (outer cortical group) or the 6-week-old SHR. The development of this greater tapering or preglomerular constriction probably relates to autoregulatory control because of the increased pressure the afferent arteriole experiences.

Through the use of a vascular casting techniques, as described in this study, it was, for the first time, possible to obtain morphologic evidence that could be analyzed and, thereby, support functional studies in the spontaneously hypertensive rat.

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