Mechanics and Composition of Cerebral Arterioles in Renal and Spontaneously Hypertensive Rats

Gary L. Baumbach and Michael A. Hajdu

The purpose of this study was to examine effects of hypertension on mechanics of cerebral arterioles in nongenetic and genetic models of chronic hypertension. Pressure (servo null) and diameter were measured in pial arterioles of anesthetized renal hypertensive rats (one-kidney, one clip), uninephrectomized nonhypertensive rats, spontaneously hypertensive rats, and nonhypertensive Wistar-Kyoto rats. During maximal dilatation with EDTA, external diameter of pial arterioles at 70 mm Hg pial arteriolar pressure was not significantly different in renal hypertensive and normotensive rats (86±5 [mean±SEM] versus 84±4 μm) but was less in spontaneously hypertensive rats than in Wistar-Kyoto rats (81±3 versus 92±3 μm; p<0.05). Cross-sectional area of the arteriolar wall (histological) was greater in renal hypertensive than in normotensive rats (1,360±131 versus 952±89 μm²; p<0.05) and in spontaneously hypertensive rats than in Wistar-Kyoto rats (1,294±97 versus 817±86 μm²; p<0.05). The stress-strain relation obtained from pressure–diameter data during maximal dilatation with EDTA indicated that distensibility of pial arterioles, when fully relaxed, was greater in renal hypertensive and spontaneously hypertensive rats than in normotensive and Wistar-Kyoto rats. We used point-counting stereology to quantitate composition of pial arterioles in renal hypertensive rats. Cross-sectional area of smooth muscle and elastin was significantly greater in renal hypertensive than in normotensive rats (smooth muscle, 947±108 versus 620±62 μm²; elastin, 101±11 versus 55±6 μm²; p<0.05), whereas cross-sectional area of collagen and basement membrane was not significantly different in the two groups (collagen, 6±1 versus 5±1 μm²; basement membrane, 120±12 versus 104±8 μm²). Thus, we conclude that 1) cerebral arterioles undergo hypertrophy in both renal hypertensive and spontaneously hypertensive rats; 2) external diameter is reduced in cerebral arterioles of SHRSP during maximal dilatation, whereas external diameter is smaller in spontaneously hypertensive than in Wistar-Kyoto rats; 3) distensibility of cerebral arterioles, when fully relaxed, is increased in renal hypertensive rats and is greater in spontaneously hypertensive than in Wistar-Kyoto rats and 4) the distensible components of the arteriolar wall are increased disproportionately in cerebral arterioles of renal hypertensive rats, which may contribute to increases in arteriolar distensibility. (Hypertension 1993;1:816–826)

KEY WORDS • arterioles • vascular resistance • hypertension, renovascular

Chronic hypertension alters structure and mechanics of cerebral arterioles in stroke-prone spontaneously hypertensive rats (SHRSP). We have found that distensibility of cerebral arterioles, when the arterioles are fully relaxed, is paradoxically increased in SHRSP, despite hypertrophy of the arteriolar wall. Increases in distensibility are accompanied by a disproportionate increase of distensible components in the arteriolar wall. We also have shown that external diameter is reduced in cerebral arterioles of SHRSP, even during maximal dilatation. Based on this finding, we have proposed that cerebral arterioles in SHRSP undergo remodeling of the arteriolar wall with a reduction of external diameter and encroachment on the vascular lumen, thus implying an increased wall-to-lumen ratio.

In this study, we have considered the possibility that the cerebral vascular changes that occur in SHRSP may not be observed in other forms of chronic hypertension. If, for example, increases in distensibility and remodeling of cerebral arterioles depend on genetic factors, these changes would not be expected to occur in a nongenetic model of chronic hypertension. Alternatively, these changes may depend on humoral factors, such as the renin-angiotensin system. Angiotensin has trophic effects on vascular smooth muscle, and activity of the renin-angiotensin system is increased in vessels of spontaneously hypertensive rats (SHR). Therefore, if increased distensibility and remodeling of cerebral arterioles are dependent on the renin-angio-
tension system, these changes might not occur in models of chronic hypertension in which activity of the renin-angiotensin remains normal.

Our goal in this study was to examine mechanics and composition of cerebral arterioles in rats with one kidney, one clip renal hypertension and in SHR. We chose one-kidney, one clip renal hypertension because 1) it is a nongenetic model of chronic hypertension, and 2) activity of the renin-angiotensin system is not elevated. Our hypothesis was that if increases in distensibility or remodeling of cerebral arterioles are dependent on genetic factors or increased activity of the renin-angiotensin system, these changes would occur in cerebral arterioles of SHR but not in those of rats with one kidney, one clip renal hypertension.

**Methods**

We studied normotensive and renal hypertensive Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, Ind.). To produce renal hypertension in 1-month-old Sprague-Dawley rats, we anesthetized the animals with sodium pentobarbital (25 mg·kg⁻¹ body wt i.p.), removed the right kidney, and placed a clip on the left renal artery. Clips with a gap size of 0.3 mm were made from 2-mm strips of silver sheet. Normotensive, uninephrectomized Sprague-Dawley rats were used for controls. We also studied age-matched, normotensive Wistar-Kyoto (WKY) rats and SHR (Harlan Sprague Dawley). Procedures followed in this study were in accordance with the institutional guidelines set forth by the University of Iowa.

Mechanics of first-order pial arterioles were examined in normotensive and renal hypertensive rats approximately 5 months after unilateral nephrectomy and clipping of the left renal artery and in WKY rats and SHR at 6 months of age. We have shown previously in WKY rats and SHRSP that first-order pial arterioles correspond to the arteriolar segment immediately distal to the fourth-order branching point of the middle cerebral artery, where local mean pial arteriolar pressure is approximately 70 and 110 mm Hg in renal hypertensive rats, 65 mm Hg in WKY rats, 85 mm Hg in SHRSP, respectively. Animals were anesthetized with sodium pentobarbital (50 mg·kg⁻¹ body wt i.p.) and mechanically ventilated with room air supplemented with oxygen. Paralysis of skeletal muscle was obtained with gallamine triethiodide (20 mg·kg⁻¹ i.v.). Because the animals were paralyzed, we evaluated them frequently for adequacy of anesthesia. Additional anesthesia was administered when pressure to a paw evoked a change in blood pressure or heart rate.

A catheter was inserted into a femoral vein for infusion of drugs and fluids. A catheter was inserted into a femoral artery to record systemic arterial pressure and to obtain blood samples, and a catheter was inserted into the other femoral artery to withdraw blood to produce hypotension. A blood gas analyzer (model 213, Instrumentation Laboratories Inc., Lexington, Mass.) was used to measure pH, Pco₂, and Po₂ in samples of arterial blood and artificial cerebrospinal fluid (CSF).

**Measurement of Pial Arteriolar Pressure and Diameter**

Pressure and diameter were measured in first-order pial arterioles using an open-skull preparation described in detail previously. After the animal was placed in a head holder, the skull was exposed through a 1-cm incision in the skin, the skin edges were retracted with sutures, and ports were placed for inflow and outflow of artificial CSF. A craniotomy was made over the parietal cortex of the left cerebral hemisphere with an air-cooled dental drill. The dura was incised to expose pial vessels. The craniotomy over the exposed cerebrum was suffused continuously with artificial CSF, warmed to 37°C, and equilibrated with a gas mixture of 5% CO₂-95% N₂. The composition of the CSF was (mM) KCl 3.0, MgCl 2 0.6, CaCl 2 1.5, NaCl 131.9, NaHCO₃ 24.6, urea 6.7, and dextrose 3.7. The CSF sampled from the craniotomy had a pH of 7.24±0.02 (mean±SEM), Pco₂ of 46±3 mm Hg, and Po₂ of 61±2 mm Hg.

Pressure was measured continuously in pial arterioles using a micropipette coupled to a servo-null pressure-measuring device (model 5, Instrumentation for Physiology & Medicine, Inc., San Diego, Calif.). Pipettes were sharpened to a beveled tip of 2-4 μm, filled with 1.5 M NaCl, and inserted into the lumen of a pial arteriole using a micromanipulator.

Pial vessels were monitored through an Olympus microscope (×10 objective) (Lake Success, N.Y.) connected to a closed-circuit video system with a final magnification of ×358. Internal diameter of pial arterioles was measured from videotapes using a Bioquant image-analyzing system (R&M Biometrics, Inc., Nashville, Tenn.). The precision of the Bioquant system is 0.4-0.6 μm.

**Experimental Protocol**

Approximately 30 minutes after surgery was completed, pressure and internal diameter were measured in pial arterioles at prevailing levels of systemic arterial pressure. Vascular smooth muscle of pial arterioles then was deactivated with ethylenediaminetetraacetic acid (EDTA) in the suffusate (25 mg·mL⁻¹). We have shown previously that this concentration of EDTA produces maximal dilatation of pial arterioles.

Pressure—diameter relationships were obtained in deactivated pial arterioles. In normotensive and renal hypertensive rats, blood was withdrawn from a femoral artery to reduce pial arteriolar pressure in steps of 10 mm Hg from 70 to 10 mm Hg. In WKY rats and SHR, pial arteriolar pressure was reduced in steps of 10 mm Hg from 70 to 30 mm Hg and in steps of 5 mm Hg from 30 to 5 mm Hg. Pial arteriolar diameter stabilized within 15 seconds, and internal diameter was measured 35-45 seconds later. After pressure was reduced to 10 or 5 mm Hg, blood was reinfused to restore pressure to baseline. Maximally dilated arterioles were fixed in vivo by suffusing arterioles with glutaraldehyde fixative (2.25% glutaraldehyde in 0.10 M cacodylate buffer) while pial arteriolar pressure was maintained at prevailing levels (approximately 70 mm Hg in normotensive Sprague-Dawley rats, 65 mm Hg in WKY rats, 85 mm Hg in renal hypertensive rats, and 105 mm Hg in SHR). We have shown previously that this method of tissue fixation does not produce significant changes in pial arteriolar diameter.

After the animal was killed, the arteriolar segment used for measurements of pressure and diameter was removed with a microsurgical knife. Fixed arterioles...
were postfixed in osmium tetroxide (1%), dehydrated, stained en bloc with uranyl acetate (0.5%), and embedded in Spurr's medium.

Cross-sectional area of the arteriolar wall was measured histologically from 1-μm sections using a light microscope interfaced with the Bioquant image-analyzing system described above. Luminal and total (lumen plus arteriolar wall) cross-sectional areas of the arteriole were measured with a digitizing pad by tracing the inner and outer edges of the arteriolar wall. The inner and outer edges of the arteriolar wall were defined by the luminal surface of endothelium and the abluminal surface of the tunica media, respectively. Cross-sectional area of the arteriolar wall was calculated by subtracting luminal cross-sectional area from total cross-sectional area.

**Calculation of Vascular Mechanics**

Incremental distensibility was calculated from internal pial arteriolar diameter (PAD) and pial arteriolar pressure (PAP):

\[ \text{Incremental distensibility} = \frac{\Delta \text{PAD}}{(\text{PAD} \times \Delta \text{PAP}) \times 100} \]

where \( \Delta \text{PAD} \) is the change in internal pial arteriolar diameter for each change of pial arteriolar pressure (\( \Delta \text{PAP} \)). The units of incremental distensibility are percent change in pial arteriolar diameter per millimeter of mercury change in pial arteriolar pressure (%/mm Hg).

Circumferential stress (\( \sigma \)) was calculated from pial arteriolar pressure, internal pial arteriolar diameter, and wall thickness (WT):

\[ \sigma = \frac{(\text{PAP} \times \text{PAD})}{(2 \times \text{WT})} \]

Pial arteriolar pressure was converted from millimeters of mercury to newtons per square meter (1 mm Hg = 1.334 × 10^5 N · m^-2). Based on the assumption that volume of the vessel wall does not change with changes in vessel diameter and pressure,11,12 we calculated wall thickness from cross-sectional area of the arteriolar wall (CSA) and internal pial arteriolar diameter:

\[ \text{WT} = \frac{\left(\frac{4 \times \text{CSA}}{\pi} + \text{PAD}_i\right)^{1/2} - \text{PAD}_i}{2} \]

External diameter of pial arterioles (PAD) was calculated as:

\[ \text{PAD}_e = \text{PAD}_i + 2 \times \text{WT} \]

Histological determinations of cross-sectional area were used in all calculations of wall thickness, circumferential stress, and external diameter.

Circumferential strain (\( \epsilon \)) was estimated as:

\[ \epsilon = \frac{(\text{PAD}_i - \text{PAD}_\text{org})}{\text{PAD}_\text{org}} \]

where \( \text{PAD}_\text{org} \) is original diameter. Original diameter is defined as diameter at 0 mm Hg or very low pressure with the vessel extended to its in situ length.12,13 Reliable measurements of pial arteriolar diameter could not be obtained at 0 mm Hg, because blood flow stops during reduction of pressure to 0 mm Hg. Blood flow through pial arterioles at 5–10 mm Hg of pial arteriolar pressure was adequate to maintain an intact red cell column. Thus, we estimated strain in normotensive and renal hypertensive rats using internal diameter measured at 10 mm Hg and in WKY rats and SHR using internal diameter measured at 5 mm Hg.

To obtain tangential elastic modulus, we fitted the stress–strain data from each animal to an exponential curve (\( y = ae^{\beta x} \)) using least-squares analysis:

\[ \sigma = \sigma_{\text{org}} e^{\beta \epsilon} \]

where \( \sigma_{\text{org}} \) is stress at original internal diameter and \( \beta \) is a constant related to the rate of increase of the stress–strain curve. Tangential elastic modulus (\( E_t \)) was estimated at several different values of stress from the derivative of the exponential curve:

\[ E_t = \frac{d\sigma}{d\epsilon} = \beta \sigma_{\text{org}} e^{\beta \epsilon} \]

**Determination of Wall Composition**

Volume density of smooth muscle, elastin, collagen, basement membrane, and endothelium was quantitated in pial arterioles of normotensive and renal hypertensive rats from electron micrographs of the arteriolar wall using a method described previously.2 Ultrathin sections of the arteriolar wall were cut on a Reichert Ultracut microtome and stained with phosphotungstic acid (0.25%). Sections were examined with a Hitachi 7000 electron microscope. Electron micrographs were taken at a standard magnification of ×9,000 and enlarged by a factor of 2.8 for a final magnification of ×25,200. To ensure uniform sampling, we divided the arteriolar wall into four quadrants of equal size. Two or three electron micrographs were taken randomly in each quadrant for a total of nine or 10 electron micrographs per section.

A standard point-counting grid (double-square lattice test system D16)14 was used to count the number of points contained within profiles of smooth muscle, elastin, collagen, basement membrane, and endothelium. Volume density (\( V_v \)) of each component was calculated from the number of points in each component (\( P_v \)) and the total number of points contained within the arteriolar wall (\( P_T \)):

\[ V_v = \frac{P_v}{P_T} \]

The total number of counts per electron micrograph was 323 ± 9 in normotensive rats and 370 ± 10 in renal hypertensive rats. The total number of counts per vessel was 2,873 ± 188 in normotensive rats and 3,490 ± 284 in renal hypertensive rats. The total number of arterioles used for point counting was 11 in normotensive rats and 10 in renal hypertensive rats (one vessel per rat). To determine the precision of counting, the relative standard error (SE\( _R \)) of mean volume density was calculated for each component. The relative standard errors for smooth muscle, elastin, basement membrane, and endothelium were <10%. The relative standard errors for collagen ranged from 7% to 13%. Thus, the number of points counted achieved a precision (100–SE\( _R \)) of at least 90% for smooth muscle, elastin, basement membrane, and endothelium. The precision obtained for collagen, which was the component with the smallest volume density, was between 87% and 93%. Cross-sectional area of individual wall components (CSA\( _c \)) was calculated from \( V_v \) of each component and total cross-sectional area (CSA\( _T \)):

\[ \text{CSA}_c = \text{CSA}_T \times V_v \]
Statistical Analysis

Comparison of relations of pressure–diameter, incremental distensibility, and stress–strain was performed using a univariate repeated-measures analysis of variance. The sources of variance were groups (normotensive and hypertensive rats); subjects within groups; and pressure, strain, or stress. Measurements of baseline pressure and diameter (obtained at prevailing levels of arterial pressure before deactivation with EDTA), coefficients of the stress–strain relation ($\beta_0$ and $\beta_1$), cross-sectional area (total, luminal, and arteriolar wall), wall thickness, volume density, and cross-sectional area of individual components as well as ratios of nondistensible-to-distensible components were compared using an unpaired $t$ test.

Results

Renal Hypertensive Rats

Baseline measurements. Internal diameter of pial arterioles was similar in normotensive and renal hypertensive rats both before and during deactivation of vascular smooth muscle with EDTA (Table 1). External diameter of deactivated arterioles was similar in the two groups. Thus, 5 months of one-kidney, one clip renal hypertensive rats resulted primarily from increases in the more distensible components of the arteriolar wall, smooth muscle and elastin, whereas the stiffer components, collagen and basement membrane, contributed little to the increase in wall mass.

Wall thickness, cross-sectional area of the arteriolar wall, and wall-to-lumen ratio were significantly greater in renal hypertensive than normotensive rats (Table 1). Thus, despite significant hypertrophy of the arteriolar wall and an increase in wall-to-lumen ratio, lumen diameter was preserved.

Vascular mechanics. Internal and external diameters of deactivated pial arterioles decreased passively during pressure reductions in renal hypertensive and normotensive rats (Figure 1). At all levels of pial arteriolar pressure between 70 and 10 mm Hg, internal and external diameters were similar in the two groups. Incremental distensibility was significantly greater ($p<0.05$) in renal hypertensive than in normotensive rats for pial arteriolar pressures between 30 and 70 mm Hg (left panel, Figure 2). The stress–strain curve in hypertensive rats was shifted to the right of the curve in normotensive rats (right panel, Figure 2). The slope of tangential elastic modulus versus stress ($\beta$) was significantly less in the hypertensive than the normotensive group (4.3±0.2 versus 5.0±0.2; $p<0.05$). These findings suggest that arteriolar distensibility was increased over a major portion of the pressure–diameter curve in renal hypertensive rats, even though maximal dilatation was not altered.

Composition. The composition of pial arterioles was qualitatively similar in normotensive and renal hypertensive rats (Figure 3). Arterioles in both groups had one or two layers of smooth muscle. Elastin was confined primarily to the internal elastica, although small amounts of elastin were observed occasionally between smooth muscle cells. Other components in the arteriolar wall included basement membrane, which lined endothelial and smooth muscle cells, and collagen fibrils, which were found between smooth muscle cells.

Most of the hypertrophy that occurred in pial arterioles of renal hypertensive rats resulted from increases in cross-sectional area of smooth muscle and elastin, whereas cross-sectional area of collagen, basement membrane, and endothelium did not increase significantly (Table 2). Thus, hypertrophy of pial arterioles in one-kidney, one clip renal hypertensive rats resulted primarily from increases in the more distensible components of the arteriolar wall, smooth muscle and elastin, whereas the stiffer components, collagen and basement membrane, contributed little to the increase in wall mass.

Spontaneously Hypertensive Rats

Baseline measurements. Before deactivation of smooth muscle, internal diameter of pial arterioles was less in SHR than in WKY rats (Table 3). During maximal
dilatation produced by EDTA, both internal and external diameters of pial arterioles were less in SHR than in WKY rats. Wall thickness, cross-sectional area of the arteriolar wall, and wall-to-lumen ratio were significantly greater in SHR than in WKY rats (Table 3).

**Vascular mechanics.** Internal and external diameters of deactivated pial arterioles decreased passively during pressure reductions in both WKY rats and SHR (Figure 4). At all levels of pial arteriolar pressure between 70 and 5 mm Hg, both internal and external diameters were less in SHR than in WKY rats. Incremental distensibility was significantly greater (*p*<0.05) in SHR than in WKY rats for pial arteriolar pressures between 50 and 60 mm Hg and between 5 and 25 mm Hg (left panel, Figure 5). The stress–strain curve in SHR was shifted to the right of the curve in WKY rats (right panel, Figure 5). The slope of tangential elastic modulus versus stress (β) was significantly less in SHR than in WKY rats (4.0±0.2 versus 6.1±0.3; *p*<0.05). These findings suggest that maximal dilatation was reduced in SHR, and arteriolar distensibility was increased over a large portion of the pressure–diameter curve.

**Discussion**

There are four major findings in this study. First, cerebral arterioles undergo hypertrophy in rats with one-kidney, one clip renal hypertension as well as in SHR. Second, external and internal diameters of cerebral arterioles during maximal dilatation are reduced in SHR but not in one-kidney, one clip renal hypertensive rats. These findings suggest that renal hypertension, in contrast to SHR and SHRSP, does not result in remodeling of cerebral arterioles. Third, distensibility of fully relaxed cerebral arterioles is increased in one-kidney, one clip renal hypertensive rats and SHR, despite hypertrophy of the arteriolar wall. Fourth, the distensible components of the arteriolar wall are increased disproportionately in cerebral arterioles in rats with one-kidney, one clip renal hypertension. The increase in distensible components may contribute to increases in passive distensibility of pial arterioles in renal hypertensive rats.

**Consideration of Methods**

The method we used to examine mechanics of pial arterioles takes into account several factors that could compromise our calculations of stress, strain, and tangential elastic modulus. These factors, which include plasma skimming, effectiveness of smooth muscle deactivation, compressibility of the wall, and definition of original diameter in the determination of strain, have been considered in detail previously and are unlikely to significantly affect the accuracy of our measurements.
An important consideration in this study is the use of equations based on simplified concepts of continuum mechanics to estimate passive mechanics of cerebral arterioles. The concepts assume that the vessel wall is composed of a homogeneous, isotropic material in a definable state of strain. The validity of applying these assumptions to large arteries has been discussed previously. Large arteries, such as femoral and carotid artery in dog, consist of several layers of smooth muscle and relatively large amounts of collagen and elastin. Cerebral arterioles, on the other hand, consist of one to two layers of smooth muscle (Figure 3) and relatively small amounts of collagen and elastin (Table 2). Based on these differences in wall composition, one might conclude that cerebral arterioles are more heterogeneous than large arteries. Thus, application of the concepts of continuum mechanics may be less valid in cerebral arterioles than in large arteries. We would emphasize, however, that the possibility of increased distensibility in cerebral arterioles of renal hypertensive rats and SHR was supported in this study by the finding of an upward shift in incremental distensibility (left panel, Figures 2 and 5), as well as a rightward shift in the stress-strain relation (right panel, Figures 2 and 5). In contrast to calculations of wall stress, calculations of incremental distensibility do not include wall thickness and thus do not rely on the assumption that the arteriolar wall is homogeneous.

With respect to the application of point-counting methods to cerebral arterioles, we considered three factors that could compromise our estimates of composition of the arteriolar wall. First, the large disparity in volume density of the various components in the pial arteriolar wall requires that the criteria for determining optimal point density are closely observed. Second, random sampling of the arteriolar wall may be impeded by heterogeneous distribution of the components within the arteriolar wall. Third, the arachnoid layer adjacent to the outer surface of pial vessels was not included in our calculations of volume density. We have considered these factors in detail previously.

**TABLE 2. Composition of Vessel Wall in Normotensive and Renal Hypertensive Rats**

<table>
<thead>
<tr>
<th>Individual component</th>
<th>Normotension</th>
<th>Renal hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-sectional area (μm²)</td>
<td>Smooth muscle</td>
<td>620±62</td>
</tr>
<tr>
<td>Elastin</td>
<td>55±6</td>
<td>101±11*</td>
</tr>
<tr>
<td>Collagen</td>
<td>4.8±0.6</td>
<td>5.6±0.9</td>
</tr>
<tr>
<td>Basement membrane</td>
<td>104±8</td>
<td>120±12</td>
</tr>
<tr>
<td>Endothelium</td>
<td>169±18</td>
<td>186±15</td>
</tr>
<tr>
<td>Volume density (%)</td>
<td>Smooth muscle</td>
<td>65±1.0</td>
</tr>
<tr>
<td>Elastin</td>
<td>6±0.4</td>
<td>8±0.6*</td>
</tr>
<tr>
<td>Collagen</td>
<td>0.5±0.04</td>
<td>0.4±0.05</td>
</tr>
<tr>
<td>Basement membrane</td>
<td>11±0.4</td>
<td>9±0.5*</td>
</tr>
<tr>
<td>Endothelium</td>
<td>18±0.8</td>
<td>14±1.0*</td>
</tr>
</tbody>
</table>

Components are of arterioles used for in vivo determination of vascular mechanics. Values are mean±SEM in 11 normotensive and 10 renal hypertensive rats. *P<0.05 vs. normotensive rats.

**Determinants of Hypertrophy**

Hypertrophy of the vessel wall in cerebral arterioles occurs in several experimental models of chronic hypertension, including SHR, SHRSP, and two-kidney, one clip renal hypertension. In addition to confirming the previous finding of hypertrophy in cerebral arterioles of SHR, this study indicates that hypertrophy of cerebral arterioles also occurs in one-kidney, one clip renal hypertension. Some of the determinants of vascular hypertrophy during chronic hypertension include intra-vascular pressure, neural stimuli, humoral agents, and genetic factors.

With respect to humoral agents, it has been suggested that angiotensin may have important trophic effects on the vascular wall. This possibility is based on the findings that angiotensin stimulates hyperplasia of vascular smooth muscle cells in culture, and treatment of
hypertension with an inhibitor of the angiotensin converting enzyme may prevent hypertrophy of noncerebral vessels even in the absence of reductions in arterial pressure. These findings suggest that angiotensin may stimulate hypertrophy in other vascular beds independently of its pressor effect.

We have considered the role of the renin-angiotensin system in the development of vascular hypertrophy in renal hypertension. Plasma renin activity is markedly elevated in rats with two-kidney, one clip renal hypertension and remains normal in rats with one-kidney, one clip renal hypertension. Blockade of angiotensin II by a competitive inhibitor or by an antibody to angiotensin II has no effect on arterial pressure in one-kidney, one clip renal hypertension but normalizes arterial pressure in two-kidney, one clip renal hypertension. If the renin-angiotensin system plays a predominant role in the development of cerebral vascular hypertrophy in renal hypertension, therefore, one would anticipate that hypertrophy of cerebral arterioles would occur in the two-kidney, one clip model of renal hypertension but not in the one-kidney, one clip model. As indicated by findings in this and a previous study, however, hypertrophy of cerebral arterioles is a prominent feature in both the two-kidney, one clip and one-kidney, one clip models of renal hypertension. Thus, the findings suggest that vascular hypertrophy can occur in the absence of stimulation of the renin-angiotensin system.

A finding that surprised us is that vascular hypertrophy did not significantly alter internal and external diameters of fully relaxed pial arterioles in normotensive and renal hypertensive rats. Hypertrophy of the arteriolar wall would be expected to result in either a reduction in internal diameter or an increase in external diameter of the arterioles. These findings, therefore, suggest that the renin-angiotensin system plays a predominant role in the development of cerebral vascular hypertrophy in renal hypertension.

### Table 3. Baseline Values in Wistar-Kyoto Rats and Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before deactivation of smooth muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic arterial mean pressure (mm Hg)</td>
<td>115±4</td>
<td>173±6*</td>
</tr>
<tr>
<td>Pial arteriolar mean pressure (mm Hg)</td>
<td>64±4</td>
<td>105±5*</td>
</tr>
<tr>
<td>Internal pial arteriolar diameter (μm)</td>
<td>45±2</td>
<td>36±4*</td>
</tr>
<tr>
<td>Arterial blood gases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCO₂ (mm Hg)</td>
<td>35±1</td>
<td>38±1</td>
</tr>
<tr>
<td>pH</td>
<td>7.36±0.01</td>
<td>7.37±0.01</td>
</tr>
<tr>
<td>PO₂ (mm Hg)</td>
<td>114±2</td>
<td>117±3</td>
</tr>
<tr>
<td>After deactivation of smooth muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal pial arteriolar diameter (μm)</td>
<td>86±3</td>
<td>71±3*</td>
</tr>
<tr>
<td>External pial arteriolar diameter (μm)</td>
<td>92±3</td>
<td>81±3*</td>
</tr>
<tr>
<td>Wall:lumen ratio</td>
<td>0.03±0.003</td>
<td>0.08±0.004*</td>
</tr>
<tr>
<td>Wall thickness (μm)</td>
<td>2.9±0.3</td>
<td>5.4±0.3*</td>
</tr>
<tr>
<td>Cross-sectional area of vessel wall (μm²)</td>
<td>817±86</td>
<td>1,294±97*</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats. Measurements of internal diameter before deactivation of smooth muscle were obtained at prevailing levels of arterial pressure. Measurements of internal diameter after deactivation of smooth muscle were made at a pial arteriolar pressure of 70 mm Hg. Values of external diameter and wall thickness after deactivation of smooth muscle were calculated from measurements of internal diameter at 70 mm Hg pial arteriolar pressure and histological measurements of cross-sectional area of the vessel wall. Values are mean±SEM in seven WKY rats and six SHR.

*P<0.05 vs. WKY rats.

**Figure 4.** Line graphs show relation of pressure to internal (left panel) and external (right panel) diameter in deactivated pial arterioles of seven Wistar-Kyoto (WKY) rats and six spontaneously hypertensive rats (SHR). Values are mean±SEM; *P<0.05 vs. WKY rats.
diameter. We considered two possible explanations for this apparent discrepancy. One explanation is related to possible differences in the sensitivity of the methods used to determine dimensional characteristics of pial arterioles. For example, if the histological method used for measuring cross-sectional area of the arteriolar wall was more sensitive than the in vivo method used for measuring internal diameter, it is possible that small differences in internal or external diameter of pial arterioles would have been more readily detected with the histological method. To examine this possibility, we calculated internal and external diameters of pial arterioles from histological measurements of luminal (CSAL) and total (CSAT) cross-sectional area:

$$P_{AD, L} = \frac{\text{CSAL}}{\pi \times 2}; \quad P_{AD, T} = \frac{\text{CSAT}}{\pi \times 2}$$

Internal and external diameters of pial arterioles calculated from histological measurements were not significantly different in normotensive and hypertensive rats (Table 4). Thus, failure to find a significant change in the diameter of pial arterioles of hypertensive rats cannot be explained on the basis of methodology. Another possibility for the failure to find a significant change in the diameter of pial arterioles of hypertensive rats is related to the large disparity between wall thickness and vessel diameter. The ratio of wall thickness to vessel diameter is small in pial arterioles of both normotensive (<5%) and hypertensive (<7%) rats (Table 1). Thus, a 43% increase in wall thickness (1.6 μm) will result in only a 3–4% change in diameter (3.2 μm) of pial arterioles (Table 1). In other words, even though the arteriolar wall is significantly thicker in hypertensive rats, the effect of vascular hypertrophy on internal and external diameters is minimal at full relaxation and therefore may be more difficult to detect. During activation of smooth muscle, on the other hand, the increase in wall-to-lumen ratio would be expected to exert its “amplifying effect” on the lumen for any given level of activation.

**Remodeling**

We found in a previous study that external, as well as internal, diameter of cerebral arterioles is reduced in SHRSP. Based on that finding, we suggested that cerebral arterioles in SHRSP undergo “remodeling” that results in a reduction of external diameter and encroachment on the vascular lumen. It was anticipated, therefore, that remodeling of cerebral arterioles might also occur in renal hypertensive rats and SHR. Although we found in this study that external diameter of cerebral arterioles is reduced in SHR, it was not reduced in one-kidney, one clip renal hypertension. Thus, cerebral arterioles undergo remodeling in SHR but not in rats with one-kidney, one clip renal hypertension. These findings suggest that the factors which lead to remodeling of cerebral arterioles in SHRSP and SHR are absent in one-kidney, one clip renal hypertension. One of the possibilities that may account for remodeling of cerebral arterioles in SHRSP and SHR but not Sprague-Dawley rats with one-kidney, one clip renal hypertension is that these models of hypertension differ with respect to the contribution of genetic factors. Genetic factors play a major role in the development of hypertension in SHRSP and SHR but not in one-kidney, one clip renal hypertension.

Another possibility is that remodeling may require increased activity of the renin-angiotensin system. If remodeling of cerebral arterioles results from increased activity of the renin-angiotensin system, the absence of remodeling in one-kidney, one clip renal hypertensive rats would not be surprising, because activity of the renin-angiotensin system remains normal in the one-kidney, one clip model of renal hypertension. Remodeling of the hind limb vasculature in rats with two-kidney, one clip renal hypertension and cerebral arterioles in SHRSP and SHR (the present study), on the other hand, would be anticipated, because activity of the renin-angiotensin system is increased in two-

---

**TABLE 4. Histological Values in Pial Arterioles of Normotensive and Renal Hypertensive Rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotension</th>
<th>Renal hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-sectional area (μm²)</td>
<td>5,394±464</td>
<td>5,654±570</td>
</tr>
<tr>
<td>Lumen</td>
<td>4,41±409</td>
<td>4,295±479</td>
</tr>
<tr>
<td>Pial arteriolar diameter (μm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal</td>
<td>74.2±3.5</td>
<td>72.8±4.3</td>
</tr>
<tr>
<td>External</td>
<td>82.1±3.5</td>
<td>83.7±4.5</td>
</tr>
</tbody>
</table>

Values are mean±SEM in 11 normotensive and 10 renal hypertensive rats.
kidney, one clip renal hypertension and SHR. This possibility also is supported by our recent finding that treatment with an angiotensin converting enzyme inhibitor but not hydralazine prevents remodeling of pial arterioles in SHRSP. A third possibility that might be considered to play a role in remodeling of cerebral arterioles in SHRSP and SHR but not in rats with one-kidney, one clip renal hypertension is that these models of hypertension may differ with respect to the contribution of the sympathetic nervous system. However, we think that this possibility is unlikely. In a previous study, we found that unilateral sympathectomy results in a further reduction, rather than an increase, in internal diameter of pial arterioles in SHRSP. This finding indicates that sympathetic nerves do not contribute to remodeling of cerebral arterioles in SHRSP.

An apparent paradox in this study is that internal diameter of pial arterioles before deactivation of vascular smooth muscle was similar in normotensive and renal hypertensive rats. We had anticipated that internal diameter would be reduced in renal hypertensive rats because of the assumption that first-order pial arterioles are fully representative of cerebral resistance vessels and thus would contribute to the increases in vascular resistance that typically accompany chronic hypertension in other vascular beds, such as hindquarter vessels. However, we think that this possibility is unlikely. In a previous study, we found that unilateral sympathectomy results in a further reduction, rather than an increase, in internal diameter of pial arterioles in SHRSP. This finding indicates that sympathetic nerves do not contribute to remodeling of cerebral arterioles in SHRSP.

A factor in this study that may have contributed to the absence of a significant reduction in internal diameter before deactivation of smooth muscle is that pial arterioles did not undergo remodeling during one-kidney, one clip renal hypertension. We have proposed previously that remodeling may play an important role in increased vascular reactivity of cerebral arterioles in SHRSP. Another factor that may have contributed to the absence of a significant reduction in internal diameter before deactivation is rarefaction. Rarefaction of resistance vessels is a mechanism, in addition to reduction of internal diameter, by which increases in vascular resistance may be sustained during chronic hypertension. Although vascular rarefaction has been demonstrated convincingly in gracilis and cremaster muscle in several models of chronic hypertension, rarefaction of cerebral resistance vessels remains controversial. Sokolova et al have reported that there is a reduction in number of pial arterioles in rats with renal hypertension and deoxycorticosterone acetate-saline hypertension. In contrast, Werber et al were unable to demonstrate a reduction in surface area of pial arterioles in SHR, Dahl salt-sensitive rats, or rats with two-kidney, one clip renal hypertension.

A third factor in this study that may have contributed to the absence of a significant reduction in pial arteriolar diameter before smooth muscle deactivation is that renal hypertensive rats were slightly hypercapnic relative to normotensive rats (Table 1). Hypercapnia produces dilatation of pial arterioles. It is possible, therefore, that differences in arterial blood gases may have masked differences of pial arteriolar diameter in renal hypertensive and normotensive rats.

**Distensibility and Composition**

In a previous study, we found that distensibility of fully relaxed cerebral arterioles is increased paradoxically in SHRSP despite hypertrophy of the arteriolar wall. This study indicates that distensibility of cerebral arterioles is increased in rats with one-kidney, one clip renal hypertension and in SHR as well as in SHRSP. Thus, increases in distensibility of relaxed cerebral arterioles occur in nongenetic as well as in genetic forms of experimental hypertension.

The findings in this study also provide support for the concept that passive distensibility of blood vessels is dependent, at least in part, on proportional composition of the vessel wall. To relate alterations in structure of pial arterioles to alterations in distensibility, we calculated the ratio of nondistensible-to-distensible components in the arteriolar wall, as discussed previously. Basement membrane was included with the relatively nondistensible element, collagen, because basement membrane contains significant amounts of type IV collagen. Smooth muscle, on the other hand, was included with the relatively distensible elements because its elastic modulus is similar to that of elastin. Endothelium was included with the distensible elements because its elastic modulus is assumed to be equal to or less than the elastic modulus of smooth muscle.

When basement membrane (BSM) was combined with collagen (C), and smooth muscle (SM) and endothelium (Endo) were combined with elastin (E), the ratio of nondistensible-to-distensible components ((C+BSM)/(E+SM+Endo)) was less in renal hypertensive than in normotensive arterioles (0.10±0.006 versus 0.13±0.006; p<0.05). Thus, when all of the major components of the arteriolar wall are taken into account, hypertrophy of pial arterioles in rats with one-kidney, one clip renal hypertension is accompanied by a relative increase in the more compliant components of the arteriolar wall. These findings, together with findings from our previous studies, suggest that chronic hypertension may produce alterations in proportional composition of cerebral arterioles, which, in turn, contribute to increases in arteriolar distensibility. The association of alterations in composition and mechanics of cerebral vessels, however, may be coincidental and therefore must be interpreted with caution. The relation of vascular structure and distensibility is complex and undoubtedly depends on factors in addition to proportional composition, including orientation of wall components with respect to vascular circumference and interconnections among the various components. In some vessels, alterations in composition may not be predictive of alterations in vascular mechanics. For example, distensibility of the internal carotid artery is decreased in SHR despite a reduction in the ratio of collagen to elastin.

Another consideration in this study is related to the effects of smooth muscle activation on vascular distensibility. Folkow and Karlström found that, when compared at equal levels of smooth muscle activation, distensibility of resistance vessels was reduced in hindquarter of SHR. Although we did not examine effects of smooth muscle activation on vascular distensibility in...
this study, it is reasonable to think that, during activation of smooth muscle, distensibility of pial arterioles may have been reduced in renal hypertensive rats and SHR, even though passive distensibility was increased. This possibility is based on the finding that activation of smooth muscle alters distensibility characteristics of the vessel wall.12,59 We would emphasize here that under most conditions, smooth muscle in resistance vessels is more or less contracted rather than fully relaxed.

**Implications**

We conclude that cerebral arterioles in rats with one-kidney, one clip renal hypertension undergo hypertrophy, but not remodeling, of the arteriolar wall. In contrast, cerebral arterioles in SHR undergo both hypertrophy and remodeling. Furthermore, hypertrophy of the wall is accompanied by an increase in passive distensibility of cerebral arterioles in both renal hypertensive rats and SHR. These findings may have important implications with respect to vascular hypertrophy and remodeling and their effects on impairment of cerebral vascular dilatation in chronic hypertension.

The primary mechanism that has been proposed to account for impairment of maximal dilatation by chronic hypertension is hypertrophy of the vessel wall with a reduction of internal diameter by encroachment on the vascular lumen.46,54 Based on earlier findings in rats55 and humans54 and more recent findings in SHRSP,3 SHR (the present study), and humans,59 we have proposed an alternative mechanism.3 During chronic hypertension, cerebral arterioles may undergo remodeling with a reduction in external diameter. We have estimated that reduction in external diameter contributes more than hypertrophy to encroachment on the vascular lumen and impairment of maximal dilatation of cerebral arterioles in SHRSP.3 The finding in this study that pronounced hypertrophy in the absence of remodeling did not impair maximal dilatation of cerebral arterioles in rats with one-kidney, one clip renal hypertension supports the hypothesis that impairment of maximal vasodilatation in chronic hypertension may be related primarily to remodeling, rather than to vascular hypertrophy per se, with encroachment on the lumen. The hypothesis also is supported by the finding that remodeling rather than hypertrophy accounted for the majority of impaired maximal dilatation of cerebral arterioles in SHR. Furthermore, even though hypertrophy and remodeling both result in impaired maximal dilatation, the finding in this study that vascular hypertrophy can occur without remodeling indicates that hypertrophy and remodeling are responses to different stimuli.

**Acknowledgments**

We thank Jay Siems and Shams Ghoneim for technical assistance, and Dr. Donald Heistad for critical review of the manuscript.

**References**

52. Folkow B, Karlstrom G: Age- and pressure-dependent changes of systemic resistance vessels concerning the relationships between geometric design, wall distensibility, vascular reactivity and smooth muscle sensitivity. *Acta Physiol Scand* 1984;122:17–33
Mechanics and composition of cerebral arterioles in renal and spontaneously hypertensive rats.

G L Baumbach and M A Hajdu

Hypertension. 1993;21:816-826
doi: 10.1161/01.HYP.21.6.816

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 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/21/6_Pt_1/816

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/