Mechanics of Cerebral Arterioles in Hypertensive Rats

Gary L. Baumbach, Philip B. Dobrin, Michael N. Hart, and Donald D. Heistad

Chronic hypertension is associated with hypertrophy of cerebral blood vessels. Previous studies of the mechanical properties of cerebral vessels in chronic hypertension have examined large cerebral arteries. The goals of this study were first to develop a method to examine vascular mechanics of cerebral arterioles in vivo and second to determine whether the stiffness of cerebral arterioles is altered in the presence of chronic hypertension. We calculated circumferential stress and strain of pial arterioles in age-matched, anesthetized stroke-prone spontaneously hypertensive rats (SHRSP) and in Wistar Kyoto rats (WKY) from measurements of pial arteriolar pressure, inner diameter, and wall thickness. Pial arteriolar pressure was measured with a servonull system. Smooth muscle of pial arterioles was deactivated with ethylenediaminetetraacetic acid (EDTA), and pressure-diameter relations were examined during step-wise reductions in pressure. Prior to deactivation of smooth muscle in 3-4-month-old rats, pial arteriolar pressure was greater in SHRSP than in WKY (110 ± 4 versus 75 ± 2 mm Hg [mean ± SE]; p<0.05). Pial arteriolar diameter, which was measured at prevailing levels of pial arteriolar pressure, was less in SHRSP than in WKY (52 ± 5 versus 63 ± 3 μm; p<0.05). Following deactivation of smooth muscle, diameter of pial arterioles at 70 mm Hg of pial arteriolar pressure was similar in the two groups: 104 ± 6 μm in SHRSP and 109 ± 3 μm in WKY (p>0.05). Wall thickness was 4.5 ± 0.2 μm in SHRSP and 4.1 ± 0.1 μm in WKY (p>0.05). The stress-strain relation in deactivated pial arterioles was shifted to the right in SHRSP, which indicates that circumferential stiffness of pial arterioles is decreased in young SHRSP. To determine whether hypertrophy of pial arterioles, which occurs with maturation, is associated with increases in arteriolar stiffness, we examined stress-strain characteristics in 6-8-month-old SHRSP and WKY. In older rats, diameter of both active and deactivated pial arterioles was less in SHRSP than in WKY. Wall thickness was significantly greater in SHRSP than in WKY (5.8 ± 0.5 versus 3.8 ± 0.2 μm; p<0.05). The stress-strain relation, however, was shifted even further to the right in 6-8-month-old SHRSP with respect to WKY. We conclude that the stiffness of cerebral arterioles is decreased in SHRSP with established hypertension despite pronounced vascular hypertrophy. (Circulation Research 1988;62:56-64)
Materials and Methods

We studied age-matched, male SHRSP and WKY rats. Two different age groups were used: 3–4 months and 6–8 months. The animals were anesthetized with sodium pentobarbital (50 mg/100 g body wt i.p.), intubated, and mechanically ventilated with room air and supplemental O₂. Paralysis of skeletal muscle was obtained with gallamine triethiodide (20 mg/kg i.v.).

A catheter was inserted into a femoral vein for injection of drugs and fluids. A catheter was inserted into a femoral artery to record systemic arterial pressure and to obtain blood samples for measurement of arterial blood gases, and a catheter was inserted into the other femoral artery to withdraw blood to produce hypotension.

Measurement of Pial Arteriolar Pressure and Diameter

We measured pressure and diameter of first order (1A) pial arterioles through an open skull preparation. The head was placed in an adjustable head holder, and a 1-cm incision was made in the skin to expose the skull. The skin edges were retracted with sutures, and ports were placed for inflow and outflow of artificial cerebrospinal fluid (CSF). A craniotomy was performed over the left parietal cortex, and the dura was incised to expose cerebral vessels. The exposed brain was continuously suffused with artificial CSF, warmed to 37–38°C and equilibrated with a gas mixture of 5% CO₂–95% N₂. The composition of the CSF was (in mM) KCl 3.0, MgCl₂ 0.6, CaCl₂ 1.5, NaCl 131.9, NaHCO₃ 24.6, urea 6.7, and dextrose 3.7. The CSF pH was 7.24 ± 0.02, PCO₂ was 47 ± 1 mm Hg, and PO₂ was 61 ± 2 mm Hg.

Pial arteriolar pressure was measured continuously with a micropipette connected to a servonull pressure measuring device (model 4A, Instruments for Medicine and Physiology, Inc., San Diego, Calif.). Pipettes were sharpened to a beveled tip of 3–5 μm in diameter, filled with 1.5 M NaCl, and inserted into the lumen of a 1A pial arteriole with a micromanipulator. The presence of the pipette tip in the vessel wall had no discernible effect on most pial arterioles. When hemorrhage, persistent constriction, or dilatation occurred at the insertion point, pial arteriolar pressure was measured in another 1A arteriole distant from the original site, or the experiment was terminated.

Pial vessels were monitored through a Leitz microscope (NP1 10 × objective) attached to a closed circuit video system consisting of a television camera, a time-date generator, a videotape recorder, and a video monitor. Final magnification of the video image was 354 × . Pial arteriolar diameter was measured on the video monitor during an experiment and from videotapes using a Bioquant image analyzing system (R&M Biometrics, Inc., Nashville, Tenn.). The Bioquant system consists of an Apple IIe computer, a video-board, a digitizing pad, and software. To determine the precision of this system, lengths of 10, 50, and 100 μm were measured 5 times each from a stage micrometer. The averages and standard deviations of these measurements were 9.8 ± 0.4, 50 ± 0.5, and 100 ± 0.6 μm. Therefore, the precision of the Bioquant system ranged from 0.4 to 0.6 μm.

Measurement of inner pial arteriolar diameter in vivo included the width of the red cell column and excluded the cell-free layer created by plasma skimming. To determine whether plasma skimming caused significant underestimation of inner pial arteriolar diameter, we measured inner diameter just before and immediately after intravenous injection of Evans blue dye (1.25%) under two conditions: 1) in active pial arterioles at control levels of systemic arterial pressure, and 2) in deactivated pial arterioles at a pial arteriolar pressure of 10 mm Hg. Because Evans blue dye is bound to serum albumin, measurement of diameter in arterioles that contain Evans blue dye eliminates underestimation produced by the plasma layer. In active pial arterioles, the difference in diameter before and after Evans blue dye injection was 1.0 ± 0.5 μm (mean ± SE) in WKY (n = 4) and 1.0 ± 0.4 μm in SHRSP (n = 4). The difference in diameter in deactivated arterioles was 1.2 ± 0.7 and 1.3 ± 0.6 μm in WKY (n = 3) and SHRSP (n = 3), respectively. Thus, the error produced by plasma skimming was small.

To determine whether first order pial arterioles in WKY and SHRSP were from similar levels in the vascular tree, we traced the branching order of the middle cerebral artery in 4 WKY and 4 SHRSP. In all rats, the arteriolar segment examined in vivo was immediately distal to the fourth order branching point of the middle cerebral artery.

Deactivation of Pial Arterioles

Ethylenediaminetetraacetic acid (EDTA) has been shown to produce maximal dilatation of pial arterioles in cat. To determine whether EDTA produces maximal dilatation of pial arterioles in WKY and SHRSP, we performed several preliminary experiments.

First, the concentration of EDTA required to produce maximal dilatation was determined in 7 WKY and 6 SHRSP. We examined the response of 1A pial arterioles to 10, 25, and 50 mg/ml of EDTA. Maximal effect of EDTA was obtained at a concentration of 25 mg/ml (Figure 1, left panel). Pial arterioles were dilated maximally within 5 minutes after beginning suffusion of EDTA. Therefore, 25 mg/ml EDTA was used throughout this study to produce deactivation of vascular smooth muscle.

Second, to determine whether EDTA dilates 1A pial arterioles as effectively as another cerebral vascular dilator, we compared the dilator response to EDTA and adenosine. In WKY rats (n = 3) and SHRSP (n = 3), pial arteriolar diameter was significantly greater during suffusion of pial vessels with EDTA (25 mg/ml) than with adenosine (10⁻³ M) (Figure 1, right panel).

Third, we examined the effect of intravascular injection of EDTA and systemic hypercapnia on pial arterioles already dilated by suffusion of EDTA. EDTA (50 mg/ml of normal saline) was injected into a 1A pial arteriole through a micropipette (tip diameter 8–10 μm) for 5 minutes before and during suffusion of pial arterioles with EDTA. To produce hypercapnia, rats
were ventilated with 10% CO₂ and supplemental oxygen, and CSF was equilibrated at a pH of 7.04 ± 0.04, P<sub>CO₂</sub> of 95 ± 10 mm Hg, and P<sub>O₂</sub> of 59 ± 6 mm Hg. Pial arteriolar diameter in WKY (n = 3) and SHRSP (n = 3) was no greater during suffusion of EDTA in combination with intravascular injection of EDTA or systemic hypercapnia than during suffusion of EDTA alone (Figure 2). Mean arterial pressure did not change during intravascular injection of EDTA and hypercapnia.

Finally, to determine whether pial arterioles could respond to a constrictor stimulus after dilatation by EDTA, we examined the response of LA arterioles to arginine vasopressin before and during suffusion with EDTA. Prior to dilatation, suffusion of pial arterioles with vasopressin (10⁻⁷ M) produced a reduction in diameter of 26 ± 6% in WKY (57 ± 9 to 42 ± 3 μm; n = 3) and 31 ± 4% in SHRSP (42 ± 1 to 29 ± 1 μm; n = 3). After dilatation by EDTA, however, vasopressin had minimal effects on diameter in WKY (116 ± 6 to 121 ± 6 μm) and SHRSP (95 ± 4 to 96 ± 6 μm).

**Experimental Protocol**

Twenty to thirty minutes after completion of surgery, measurements were obtained under baseline conditions in active pial vessels. Vascular smooth muscle then was deactivated by suffusion of pial vessels with artificial CSF containing EDTA (25 mg/ml). To obtain pressure-diameter relations in deactivated pial arterioles, controlled hemorrhage was used to reduce pial arteriolar pressure in decrements of 10 mm Hg at pressures between 70 and 10 mm Hg. After each pressure step, arteriolar diameter achieved a steady state within 15 seconds. Inner diameter was measured approximately 30 seconds later.

After the last pressure step, blood was reinfused to restore pial arteriolar pressure to control levels. The outer edges of the arteriole were visualized with a combination of direct and indirect lighting so that both outer and inner diameters could be measured. Suffusion of pial vessels with artificial CSF containing EDTA was stopped, and the maximally dilated arterioles were fixed at physiologic pressure in vivo by suffusion of vessels with glutaraldehyde fixative (2.25% glutaraldehyde in 0.10 M cacodylate buffer) while maintaining pial arteriolar pressure at 60–80 mm Hg. Arterioles were considered to be adequately fixed when blood flow through the arteriole had ceased. To determine whether the fixation process altered vessel diameter, external diameter of pial arterioles was monitored during fixation. External diameter before and after fixation was 114 ± 3 and 115 ± 2 μm in 17 WKY and 105 ± 3 and 106 ± 4 μm in 12 SHRSP (p > 0.05).

After the animal was killed, the arteriolar segment used for pressure-diameter measurements was removed with a microsurgical knife. Fixed arterioles were processed, embedded in Spurr's media while cross-sectional orientation was maintained, and sectioned at 1 μm. Sections were examined with a light microscope attached to the Bioquant image analyzing system described previously. Luminal and total (lumen plus vessel wall) cross-sectional areas of the arteriole were measured with the digitizing pad by tracing the inner and outer edges of the vessel wall. Subtraction of luminal cross-sectional area from total cross-sectional area yielded cross-sectional area of the arteriolar

**Figure 1.** Response of pial arterioles to suffusion with different concentrations of EDTA in 7 WKY and 6 SHRSP (left panel), and to suffusion with EDTA (25 mg/ml) or adenosine (10⁻⁴ M) in 3 WKY and 3 SHRSP (right panel). Values are mean ± SE.
wall. We also calculated cross-sectional area (CSA) of the arteriolar wall from in vivo measurements of external (PAD) and internal (PA0) pial arteriolar diameter: CSA = \(\pi(PAD^2 - PA0^2)/4\). The percent difference in cross-sectional area determined histologically and in vivo was 1.2 ± 3.3%.

**Calculation of Mechanical Characteristics**

Incremental distensibility, which gives the relative change in pial arteriolar diameter due to alterations in intravascular pressure, was calculated from \(PAD\) and pial arteriolar pressure (PAP): incremental distensibility = \(\Delta PAD/(PAD \cdot \Delta PAP)\cdot 100\), where \(\Delta PAD\) is the change in inner pial arteriolar diameter for each decremental reduction of pial arteriolar pressure (\(\Delta PAP\)). The units of incremental distensibility are percent change in pial arteriolar diameter per millimeters mercury change in pial arteriolar pressure (\%/mm Hg).

Circumferential stress (\(\sigma\)) was calculated from pial arteriolar pressure, inner pial arteriolar diameter, and wall thickness (WT): \(\sigma = (PAP \cdot PAD)/(2WT)\). Pial arteriolar pressure was converted from millimeters mercury to dynes per centimeter squared (1 mm Hg = 1.334 × 10^3 dynes/cm^2). Because it has been shown that volume of the vessel wall does not change with changes in intravascular pressure,23 we assumed that cross-sectional area of the vessel wall remains constant with changes in arteriolar diameter. Thus, wall thickness can be calculated from cross-sectional area and inner pial arteriolar diameter: \(WT = [(4CSA/\pi + PAD^2) - PA0^2]/2\). Histologic determinations of cross-sectional area were used in all calculations of wall thickness and circumferential stress.

Circumferential strain (\(\varepsilon\)) was calculated as \(\varepsilon = (PAD - PA0)/PA0\), where \(PA0\) is original diameter. Original diameter has been defined as the diameter at very low or 0 mm Hg pressure with the vessel extended to its full length.24,25 Because reduction of pressure to 0 mm Hg stops blood flow and because passive vascular collapse is likely at 0 mm Hg, it was not possible to obtain reliable measurements of inner diameter of pial arterioles at 0 mm Hg. Blood flow through pial arterioles at 10 mm Hg of pial arteriolar pressure was adequate to maintain an intact red cell column. Therefore, we calculated strain using two different original diameters: diameter measured at 10 mm Hg, and diameter estimated at 0 mm Hg. An estimate of diameter at 0 mm Hg was obtained by fitting the pressure-diameter data for each animal to a third order polynomial equation.22

The stress-strain data from each animal were fitted to an exponential curve \((y = ae^{bt})\) using least-squares analysis: \(\sigma = \sigma_0 e^{\beta \psi}\), where \(\sigma_0\) is stress at original diameter, and \(\beta\) is a constant that is related to the rate of increase of the stress-strain curve.

**Statistical Analysis**

Comparison of the relations of pressure-diameter, incremental distensibility, and stress-strain was performed using analysis of variance. The sources of variance were groups, subjects within groups, and pressure or strain. Baseline measurements and coefficients (\(\sigma_0\) and \(\beta\)) from analysis of stress-strain relations were compared with an unpaired \(t\) test.

**Results**

**Baseline Measurements**

Prior to deactivation of vascular smooth muscle with EDTA, pial arteriolar diameter was significantly smaller in young and older SHRSP than in age-matched WKY (Table 1). Following deactivation of smooth muscle, pial arteriolar diameter was similar in young SHRSP and WKY (Table 2). Furthermore, wall thickness and cross-sectional area of the vessel wall were not significantly different in young SHRSP and WKY. Thus, cerebral vascular hypertrophy was minimal or absent in 3–4-month-old SHRSP.

**Table 1. Baseline Measurements Prior to Deactivation of Smooth Muscle**

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<th>3–4 months old</th>
<th>6–8 months old</th>
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<tr>
<td></td>
<td>WKY</td>
<td>SHRSP</td>
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<tr>
<td></td>
<td>WKY</td>
<td>SHRSP</td>
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<tr>
<td>Systemic arterial</td>
<td></td>
<td></td>
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<tr>
<td>pressure (mm Hg)</td>
<td>119 ± 7</td>
<td>186 ± 3*</td>
</tr>
<tr>
<td></td>
<td>109 ± 4</td>
<td>182 ± 7*</td>
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<tr>
<td>Pial arteriolar</td>
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<tr>
<td>pressure (mm Hg)</td>
<td>75 ± 2</td>
<td>110 ± 4*</td>
</tr>
<tr>
<td></td>
<td>72 ± 3</td>
<td>112 ± 5*</td>
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<tr>
<td>Pial arteriolar</td>
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<tr>
<td>diameter (µm)</td>
<td>63 ± 3</td>
<td>52 ± 5*</td>
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<td></td>
<td>56 ± 4</td>
<td>43 ± 2*</td>
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Measurements of pial arteriolar diameter were obtained at baseline levels of pial arteriolar pressure. Values are mean ± SEM; * \(p<0.05\) for SHRSP vs. age-matched WKY.

**Table 2. Baseline Measurements After Deactivation of Smooth Muscle**

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<th>3–4 months old</th>
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<tr>
<td></td>
<td>WKY</td>
<td>SHRSP</td>
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<tr>
<td></td>
<td>WKY</td>
<td>SHRSP</td>
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<tr>
<td>Inner diameter of</td>
<td>109 ± 3</td>
<td>104 ± 6</td>
</tr>
<tr>
<td>pial arterioles (µm)</td>
<td>105 ± 4</td>
<td>94 ± 5*</td>
</tr>
<tr>
<td>Original diameter of</td>
<td>77 ± 6</td>
<td>69 ± 4</td>
</tr>
<tr>
<td>pial arterioles (µm)</td>
<td>75 ± 2</td>
<td>62 ± 4*</td>
</tr>
<tr>
<td>Wall thickness (µm)</td>
<td>4.1 ± 0.1</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>Wall:lumen ratio</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Cross-sectional area</td>
<td>1,458 ± 103</td>
<td>1,507 ± 176</td>
</tr>
<tr>
<td>of arterioles (µm²)</td>
<td>1,355 ± 80</td>
<td>1,748 ± 64*</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>9</td>
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Measurements of diameter and wall thickness were made at a pial arteriolar pressure of 70 mm Hg. "Original diameter" of pial arterioles is defined as diameter at a pial arteriolar pressure of 10 mm Hg.

Values are mean ± SEM; * \(p<0.05\) for SHRSP vs. age-matched WKY.
In older rats, pial arteriolar diameter was less and wall thickness and cross-sectional area of the vessel wall were greater in SHRSP than in WKY after deactivation of smooth muscle (Table 2). Thus, with prolonged hypertension, there was significant hypertrophy of the arteriolar wall and a reduction of diameter. These findings suggest that the reduction in diameter of pial arterioles in older SHRSP is due in part to encroachment of the thickened wall on the vascular lumen because the diameter was reduced in SHRSP even in deactivated vessels.

Vascular Mechanics

Diameter of deactivated pial arterioles decreased passively during reductions of pressure in both 3–4-month- and 6–8-month-old SHRSP and WKY (Figure 3). At all levels of pial arteriolar pressure between 70 and 10 mm Hg, diameter tended to be smaller in young SHRSP than in young WKY (left panel) and was significantly smaller (p < 0.05) in older SHRSP than in older WKY (right panel). However, the shape of the pressure-diameter curves was similar in both young and old SHRSP and WKY. Furthermore, incremental distensibility was not significantly different in young SHRSP and WKY (Figure 4, left panel) and was significantly greater (p < 0.05) in older SHRSP than in WKY for pial arteriolar pressures between 30 and 70 mm Hg (Figure 4, right panel). Thus, although maximal dilatation was decreased in pial arterioles of SHRSP, arteriolar distensibility was increased over a major portion of the pressure-diameter curve.

Figure 5 presents stress-strain curves calculated from original diameters measured at 10 mm Hg of pial arteriolar pressure. The curve for young SHRSP demonstrated a rightward shift relative to the curve in WKY (left panel). The curve was shifted even further to the right in older SHRSP (right panel). The stress-strain curves closely approximated an exponential curve in both young and old SHRSP (R² = 0.98 ± 0.01 and 0.99 ± 0.01) and WKY (R² = 0.96 ± 0.01 and 0.99 ± 0.01). At minimal strain (ε = 0), stress (σ) was similar in young SHRSP and WKY (0.09 ± 0.01 and 0.10 ± 0.01 dynes/cm² × 10¹⁶); stress was significantly less in older SHRSP than in WKY (0.06 ± 0.01 and 0.10 ± 0.004 dynes/cm² × 10¹⁶; p < 0.05). The rate of rise in the stress-strain curve, as defined by the β coefficient, tended to be less in young SHRSP than in WKY (5.37 ± 0.43 and 5.95 ± 0.53; 0.10 > p > 0.05) and was significantly less in older SHRSP than in WKY (4.78 ± 0.40 and 6.04 ± 0.20; p < 0.05). Because the slope of the stress-strain curve is proportional to elastic modulus, these findings suggest that circumferential stiffness of pial arterioles was reduced, and not increased, by prolonged chronic hypertension.

Figure 6 presents stress-strain curves calculated from original diameters estimated at 0 mm Hg of pial arteriolar pressure. The estimated diameters were 49 ± 5 and 51 ± 5 μm in young SHRSP and WKY and 46 ± 4 and 54 ± 2 μm in old SHRSP and WKY. Substitution of diameter estimated at 0 mm Hg for diameter measured at 10 mm Hg affected the stress-strain relation in young animals only. The rightward shift of the stress-strain curve was eliminated in young SHRSP (left panel) but not older SHRSP (right panel). Thus, prolonged hypertension reduced circumferential stiffness of pial arterioles both when the calculation of circumferential strain was based on original diameter measured at 10 mm Hg of pial arteriolar pressure and
when original diameter was estimated at 0 mm Hg.

To determine whether smaller original diameters of the arterioles in SHRSP (Table 2) could account for the rightward shift of the stress-strain curves shown in Figure 5, we compared pressure-diameter and stress-strain relations in a subgroup of SHRSP and WKY with comparable original diameters selected from the group of older animals. These data are presented in Table 3. The pressure-diameter curves of this group were virtually identical in SHRSP and WKY (Figure 7, left panel). The stress-strain curve in SHRSP, however, was still shifted to the right of the curve in WKY (Figure 7, right panel). This finding indicates that the reduction in circumferential stress and stiffness of pial arterioles in SHRSP can be attributed in part to an increase in wall thickness as well as to a smaller original diameter.

Discussion

This study provides the first in vivo determination of stiffness characteristics of intracranial vessels. The major finding in this study is that circumferential stiffness of pial arterioles is reduced in SHRSP. In addition, the finding that incremental distensibility is greater in SHRSP than in WKY suggests that distensibility of pial arterioles is increased by prolonged chronic hypertension despite hypertrophy of the arteriolar wall. The discussion will focus on three areas: consideration of methods used to determine vascular mechanics in vivo, consideration of previous studies, and implications of this study.

Consideration of Methods

The method we used to examine mechanical characteristics in pial arterioles takes into consideration several factors that could compromise our calculations of stress and strain in the arteriolar wall. These factors include plasma skimming, effectiveness of deactivation of vascular smooth muscle, compressibility of the vessel wall during changes in pressure and diameter, and determination of diameter in pial arterioles at 0 mm Hg of pial arteriolar pressure.

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Plasma skimming results in a cell-free layer of plasma interposed between the red cell column and the inner surface of the vessel wall. Investigations of effects of shear stress on plasma skimming suggest that the width of the cell-free zone becomes greater as flow velocity increases. Because the cell-free zone cannot be visualized under ordinary conditions, we considered the possibility that plasma skimming might lead to significant underestimation of inner diameter of pial arterioles and thus affect our calculations of stress and strain. Our finding with Evans blue dye indicates that the error produced by plasma skimming was small in magnitude (about 1 μm) in the arterioles that we studied.

A valid comparison of distensibility of pial arterioles in WKY and SHRSP requires that vascular tone is at the same level in normotensive and hypertensive arterioles during determination of stress-strain characteristics. Vascular tone was equalized in this study by deactivation of smooth muscle with doses of EDTA that produce maximal dilatation. Furthermore, we have demonstrated that pial arterioles that are deactivated by EDTA do not respond to a vasodilator and vasoconstrictor stimuli.

Wall thickness changes when diameter of pial arterioles changes. Therefore, to calculate wall stress over a range of arteriolar diameters, we calculated wall stress (\( \sigma \)) and strain (\( \varepsilon \)) using the following equations:

\[
\sigma = E \varepsilon
\]

where \( E \) is the modulus of elasticity. The modulus of elasticity was calculated from the slope of the stress-strain curve at each pressure level.

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Substitution of original diameter estimated at 0 mm Hg for original diameter measured at 10 mm Hg of pressure in the pressure-diameter relation between 0 and 10 mm Hg of pial arteriolar pressure is not representative of the pressure-diameter relation between 0 and 10 mm Hg of pressure, however, may differ in SHR and WKY at the same level of systemic pressure. Second, interpretation of the pressure-diameter relations depends on the definition of distensibility. Based on the definition of distensibility, Harper and Bohlen proposed that distensibility of cerebral arterioles is reduced in chronic hypertension. SHR arterioles were dilated with adenosine. At normal levels of systemic arterial pressure, during which pial arteriolar pressure was significantly higher in SHR than in WKY, diameter was similar in the two strains. During hypotension, however, pial arteriolar diameter was less in SHR than in WKY. The authors suggested that, if distensibility of pial arterioles were not reduced in SHR, the diameter at low levels of pressure should have been similar in SHR and WKY, and the diameter at normal levels of systemic pressure should have been greater in SHR than in WKY. This conclusion may not be valid for at least two reasons. First, Harper and Bohlen based their assessment of pial arteriolar distensibility on the relation between pial arteriolar diameter and systemic arterial pressure. Pial arteriolar pressure, however, may differ in SHR and WKY at the same level of systemic pressure.

### Consideration of Previous Studies

Several investigators have presented evidence, both direct and indirect, that distensibility of cerebral vessels is reduced in chronic hypertension. We and others have found that increases in cerebral blood flow during seizure or hypercapnia are less in the hypertensive than in normotensive cerebrovascular bed. In addition, we have shown in this study that maximal dilatation of first order pial arterioles with EDTA is less in 6–8-month-old SHRSP than in WKY. Based on findings such as these, it has been suggested that maximal dilator capacity is reduced during chronic hypertension by a reduction of vascular distensibility. Another explanation, proposed by Folkow, is that reduction in maximal dilatation of hypertensive vessels is caused, at least in part, by encroachment of the vessel wall on the vascular lumen.

Harper and Bohlen have suggested that distensibility is reduced in cerebral arterioles of SHR. Diameter was measured in pial arterioles of SHR and WKY that were dilated with adenosine. At normal levels of systemic arterial pressure, during which pial arteriolar pressure was significantly higher in SHR than in WKY, diameter was similar in the two strains. During hypotension, however, pial arteriolar diameter was less in SHR than in WKY. The authors suggested that, if distensibility of pial arterioles were not reduced in SHR, the diameter at low levels of pressure should have been similar in SHR and WKY, and the diameter at normal levels of systemic pressure should have been greater in SHR than in WKY. This conclusion may not be valid for at least two reasons. First, Harper and Bohlen based their assessment of pial arteriolar distensibility on the relation between pial arteriolar diameter and systemic arterial pressure. Pial arteriolar pressure, however, may differ in SHR and WKY at the same level of systemic pressure.

### Table 3. Baseline Measurements After Deactivation of Smooth Muscle in 6–8-Month-Old WKY and SHRSP with Matched Original Diameters

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHRSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner diameter of pial arterioles (μm)</td>
<td>105 ± 5</td>
<td>110 ± 4</td>
</tr>
<tr>
<td>Original diameter of pial arterioles (μm)</td>
<td>69 ± 3</td>
<td>68 ± 2</td>
</tr>
<tr>
<td>Wall thickness (μm)</td>
<td>3.9 ± 0.4</td>
<td>5.3 ± 0.4</td>
</tr>
<tr>
<td>Wall:lumen ratio</td>
<td>0.04 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Cross-sectional area (μm²)</td>
<td>1,301 ± 113</td>
<td>1,800 ± 78*</td>
</tr>
</tbody>
</table>

Measurements of inner diameter and wall thickness were made at a pial arteriolar pressure of 70 mm Hg. Values are mean ± SEM; * p < 0.05 for SHRSP vs. WKY.
distensibility as the relative change in vessel volume (or diameter) per unit change in pressure, the distensibility of different vessels cannot be compared simply by comparing vessel diameters at the same level of arterial pressure. Instead, distensibility characteristics must be based on the pressure-diameter relation over the same range of intravascular pressure.

Effects of chronic hypertension on cerebral vascular distensibility also have been examined previously in vitro. Winquist and Bohr and Toda et al concluded that distensibility of basilar artery in 3-5-month-old SHR and 5-7-month-old SHRS$^2_{10-26}$ is reduced relative to normotensive WKY. Brayden et al demonstrated that the distensibility of branches of posterior cerebral artery is less in 25-week-old SHR than in WKY. Thus, in contrast to our findings in small cerebral arteries, the distensibility of larger arteries in the brain is reduced by chronic hypertension.

We have considered several possible explanations for the different results. First, the distensibility of cerebral vessels was examined with different methods. In previous studies, in vitro methods were used to examine vascular mechanics. This study is the first to use in vivo methods to determine the effect of chronic hypertension on mechanical characteristics of cerebral blood vessels. Differences between in vitro and in vivo methods include 1) the ability to control vessel length during changes in pressure, and 2) the relation of pial vessels to the arachnoid layer. It is not clear, however, that differences in experimental approaches necessarily account for different findings.

Another possible explanation for different findings in these studies is that arteries from different regions of the brain may respond differently to chronic hypertension. Responses to acute$^27,28$ and chronic$^{29,30}$ hypertension differ in the cerebrum and brainstem. Autoregulation of blood flow is more effective in brainstem than in cerebrum during acute increases$^28$ and decreases$^27$ in systemic arterial pressure. Moreover, lesions in the central nervous system during chronic hypertension are found frequently in cerebrum but rarely in brainstem.$^{29,30}$ Thus, the effect of chronic hypertension on arterial distensibility may differ in basilar artery$^{10-12,26}$ and in branches of middle cerebral artery, as described in this study.

Another likely explanation for the difference in our findings and those of previous studies$^{10-12,26}$ is that the arteries examined in these studies were of different sizes. Diameter of the basilar artery was >200 μm in SHR$^{12}$, SHRS$^{10-26}$, and WKY.$^{10,12,26}$ Wall thickness of the basilar artery was >30 μm in SHRS and WKY.$^{10,26}$ In branches of posterior cerebral artery, diameter was 150-200 μm, and thickness of the tunica media was 15-20 μm.$^{11}$ In this study, much smaller vessels were studied. Composition of the arterial wall varies with vessel size,$^{12}$ and the effect of chronic hypertension on cerebral vessels may vary with vessel caliber. Also, pressure is not elevated as much in pial arterioles$^{24,25}$ as in large arteries. Thus, chronic hypertension may have different effects on the mechanical characteristics of small and large cerebral arteries.

**Implications**

We have shown that distensibility of cerebral arterioles in SHRS is increased by chronic hypertension and that stiffness of the arteriolar wall is not increased despite pronounced hypertrophy. In contrast, previous studies$^{10-12,26}$ have demonstrated that the distensibility of large cerebral arteries is reduced by chronic hypertension. Although our findings appear to be unique for chronic hypertension, similar findings$^{25}$ have been reported in fibrosclerotic human cerebral arteries. Hudetz et al$^{23}$ examined mechanical characteristics of anterior cerebral arteries considered to be fibrotic on the basis of histologic examination. They found that the incremental distensibility of fibrotic arteries was unchanged and that the incremental elastic modulus, an indicator of wall stiffness, was reduced.

An implication of the changes that we observed is that cerebral arterioles may manifest several positive aspects of hypertrophy while retaining important elements of normal vascular function. The advantages of hypertrophy are twofold. The first advantage is that hypertrophy may encroach on the vascular lumen,$^{24}$ even during maximal vasodilation, and may increase minimal vascular resistance. Encroachment may contribute to a rightward shift of the autoregulatory curve, which permits the cerebral circulation to autoregulate blood flow at a higher range of systemic blood pressure,$^{14}$ and attenuates increases in microvascular pressure of downstream vessels.$^{15}$ Our finding that wall thickness of pial arterioles was greater in older SHRS, even during maximal dilatation, supports this possibility. Encroachment on the lumen by hypertrophy, however, accounts only in part for the smaller diameter of pial arterioles in SHRS relative to WKY. Furthermore, our findings suggest that the smaller diameter of pial arterioles in SHRS is not the result of a reduction in distensibility. Thus, the explanation for the smaller diameter of cerebral arterioles in SHRS is not clear.

The second advantage of hypertrophy is that an increase in wall thickness presumably attenuates increases in wall stress that accompany increases in intravascular pressure. However, it must be emphasized that the computation of stress and elastic modulus (stiffness) depends on wall thickness and on the assumption that the load is borne uniformly by the entire wall thickness. If the hypertrophied wall is not entirely load-bearing, then the computed stress will underestimate the stresses on the portion of the wall that actually does bear load. This unknown factor applies to the findings in the present study and also may apply to the findings of Hudetz et al.$^{25}$ It may be noted that because of the methods of computation, a change in wall thickness will alter values of stress and elastic modulus, but the computation of distensibility is independent of wall thickness. Therefore, an increase in wall thickness will decrease the computed stress and elastic modulus even without a change in distensibility.

A potential disadvantage of hypertrophy of cerebral arteries is that an increase in wall thickness may increase circumferential wall stiffness and decrease distensibility.$^{10-12,26}$ As we have shown in this study,
however, hypertrophy of the vessel wall does not necessarily lead to an increase in wall stiffness. Thus, cerebral arterioles may adapt to hypertension with minimal impairment of mechanical characteristics.

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References

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G L Baumbach, P B Dobrin, M N Hart and D D Heistad

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