Biochemical and Mechanical Properties of Resistance Arteries from Normotensive and Hypertensive Rats

JOSEPH E. BRAYDEN, PH.D., WILLIAM HALPERN, PH.D., AND LINDA R. BRANN, PH.D.

SUMMARY Microchemical techniques were employed to measure the DNA, contractile protein, and connective tissue protein composition of 150 μg samples of mesenteric and cerebral resistance arteries taken from 25-week-old spontaneously hypertensive (SHR) and control Wistar-Kyoto (WKY) rats. The active and passive mechanical properties of intact resistance arteries also were determined. The DNA content of branches of the posterior cerebral and mesenteric arteries (170 μm I.D.) were elevated by nearly 30% in the SHR compared to the WKY. The amounts of actin and myosin when normalized to DNA content were unchanged in SHR mesenteric arteries compared to control, whereas these amounts were decreased by 25% and 49%, respectively, in the SHR cerebral arteries vs control. The functional implications of these contractile protein measurements agreed with determinations of active smooth muscle cell stress-generating capabilities, which were found unchanged in the mesenteric arteries and depressed in the SHR cerebral arteries. Neither the absolute amounts and concentrations (relative to tissue mass) of elastin in mesenteric and cerebral arteries, nor the absolute amounts and concentrations of collagen in the mesenteric artery, were changed in the SHR. However, cerebral artery total collagen was elevated by 31% in the SHR, with no change in collagen concentration between the two strains. Under conditions where the smooth muscle cells were fully relaxed, the internal radii of SHR brain and SHR mesenteric arteries were smaller at all pressures with respect to the WKY. However, only the SHR cerebral arteries were actually less distensible than controls. Thus, it is apparent that hypertension-associated changes in the chemical and mechanical properties of the resistance artery wall vary considerably depending upon which vascular bed is examined. The measurements made in this study suggest that these changes are more pronounced in brain arteries. This finding could be of significance regarding the autoregulatory capability of, and blood pressure distribution within, brain vessels of hypertensive animals. (Hypertension 5: 17-25, 1983)

KEY WORDS  •  actin • myosin • DNA • smooth muscle mechanics • collagen • elastin • arterial distensibility

In both large and small arteries of the rat, hypertension usually is accompanied by an arterial wall thickening caused by hyperplasia or hypertrophy of the vascular smooth muscle cells. However, the maximum contractile response of these tissues seems to vary from the large elastic and muscular arteries to small resistance arteries. In many cases these effects have been reported to be decreased in large arteries whereas they are increased in resistance vessels. In large arteries, the decrease in active force generation is not accompanied by a change in cellular contractile protein content. Prior to this study, comparisons of maximum contractile response and contractile protein content have not been made in resistance vessels.

The passive mechanical properties of the arterial wall also are altered in hypertensive animals, with the most common observation being that the wall is less distensible. In some cases this is concurrent with an increase in collagen and elastin content, which alone could cause the decreased distensibility. In this series of experiments we have examined the contractile and connective tissue protein composition of resistance arteries of about 170 μm lumen diameter from the mesenteric and cerebral circulation of the spontaneously hypertensive rat (SHR) and normotensive Wistar-Kyoto controls (WKY), and correlated these measures with intact vessel mechanical properties. These studies disclose some fundamental differences between the two vascular beds with regard to these parameters and emphasize the need for care in extrapolation of results from studies of one type or size vessel to another.
Methods

Male 25-week-old SHR and WKY rats were obtained from a colony of animals that has been maintained at the University of Vermont for the past 8 years. Animal weights and blood pressures, the latter obtained by tail cuff plethysmography, were measured at various ages, as well as on the day prior to use. Before removal of tissues, rats were anesthetized with ether and then decapitated.

Methods for isolation of mesenteric and cerebral blood vessels for studies of the resistance vasculature have been described. In brief, for mesenteric vessels, a section of duodenum located approximately 5 cm distal to the pylorus and its arcade of supplying blood vessels were removed and placed in calcium-free physiological salt solution (PSS-EGTA). Artery segments of approximately 170 μm internal diameter were then freed of adhering adipose tissue and sections of about 3 mm in length were taken from the artery and placed in cold PSS-EGTA. For isolation of cerebral vessels, the intact brain was removed and placed in cold PSS-EGTA. The entire extraparenchymal portions of left and right posterior cerebral arteries were then dissected from the surface of the brain and adhering arachnoid tissue was gently removed. Small branches of these arteries located just proximal to entry into the parenchyma were then used. Biochemical or passive mechanical experiments usually were initiated within 30 minutes of tissue dissection.

The mass of each vessel sample used in the biochemical studies was estimated as follows: after vessel dissection, outside and inside diameters were measured using a Vickers image shearing eyepiece and a Nikon SLE microscope at a magnification of 60 X. Vessel length was measured using an eyepiece micrometer. Tissue mass was calculated by the formula:

\[ M = 1.05 \pi (r_o^3 - r_i^3)g, \]

where \( r_o \) = outside radius, \( r_i \) = inside radius, \( g \) = vessel length, and 1.05 equals the tissue specific gravity.5

Biochemical Measurements

Actin and myosin heavy chains were quantified using a sodium dodecyl sulfate (SDS) microslab polyacrylamide gel electrophoresis technique.4 Artery samples, 2–3 mm in length, were homogenized at 4°C in 10 μl of an extraction solution containing 40 mM pyrophosphate, 4 mM EGTA, 3 mM MgCl₂, and 3 mM ATP (pH 7.0) using a motor driven microhomogenizer (50 μl capacity). SDS was then added to a final concentration of 2% (wt/vol), samples were heated at 90°C for 5 minutes, and then centrifuged at 5000 x g for 15 minutes. Then 2 μl of a sample buffer containing 50% glycerol, 1% mercaptoethanol, 5% SDS, 10 mM tris-HCl (pH 6.8), and bromphenol blue tracking dye were mixed with 8 μl of supernatant containing the solubilized proteins. Samples (5 μl) of the above mixture containing approximately 1.5 μg total protein were subjected to slab gel electrophoresis. The gel consisted of a 5% acrylamide — 0.15% bis-acrylamide, 0.1% SDS stacker gel, and a 7.5% acrylamide — 0.2% bis-acrylamide, 0.1% SDS running gel. Sample wells were 2 mm wide and gel dimensions were 8 x 10 x 0.05 mm.

Actin standards were purified from rabbit skeletal muscle by the method of Rees and Young4 and myosin heavy chain standards from bovine aorta by the method of Megerman and Murphy.10 Protein concentrations of purified standards were determined by the method of Lowry et al.,11 and, in every experiment, various quantities of standards ranging from 100 to 1000 ng protein were subjected to electrophoresis in the same gels used for analysis of tissue extract samples. Electrophoresis was carried out at 150 V (constant voltage) for about 1 hour. The gels were then stained for several hours in 0.025% Coomassie brilliant blue, 25% isopropanol, and 10% acetic acid. Following destaining for 1 hour in 10% acetic acid, densitometer scans of actin and myosin heavy chain bands were obtained using a highly focused laser beam densitometer.12 Quantitative analyses of actin and myosin heavy chain were completed by measuring the areas under the actin and myosin peaks and estimating the quantity of protein represented by this area by comparison with their respective standard curves. A photograph of a representative polyacrylamide gel sample is shown in figure 1.

The DNA content of various tissue types was measured using the micro method of Boer,13 which is based on an ethidium bromide fluorescence enhancement of DNA. Vessel samples were homogenized in 10 μl of phosphate-buffered saline (PBS) pH 7.5, and duplicate 3 μl samples were incubated with RNAase and pronase

![Figure 1. Representative microslab gel. From left to right, the sample wells contained 5 μl SHR mesenteric artery extract; 5 μl SHR cerebral artery extract; aortic myosin heavy chains 175, 215, 260, 300, and 325 ng, respectively; rabbit skeletal muscle actin 425, 650, 875, and 1000 ng, respectively.](image-url)
CHEMISTRY AND MECHANICS OF RESISTANCE ARTERIES/Brayden et al. 19

to destroy RNA and increase fluorescence enhancement by ethidium bromide. Sample fluorescence (excitation wavelength = 360 nm, emission wavelength = 590 nm) was then measured in an Amicon spectrofluorometer before and after addition of ethidium bromide. Standard curves were prepared using calf thymus DNA.

Elastin was estimated by measurement of tissue desmosine content and multiplication of that value by 71.4, a factor that is based on a measured elastin desmosine content of 1.4% by weight. 14 Samples of tissue (30–60 μg) were placed in micro test tubes, and 15 μl 12N HCl was added to each. Samples were heated at 80° C for 20 minutes to dissolve the tissues. An equal volume of water was then added and the sample tubes were sealed under nitrogen and placed in a 110° C oven for 48 hours. Following acid hydrolysis, total desmosine content was measured using the radioimmunoassay for desmosine developed by King et al. 14

Collagen content was estimated by measurement of tissue hydroxyproline content and multiplication by a factor of 7.43, which is based on the measured collagen hydroxyproline content of 13.46% by weight. 15 Amounts of tissue used and tissue treatment prior to hydroxyproline analysis were the same as for the elastin measurements. Following acid hydrolysis, total hydroxyproline was measured using the ultramicro method of amino acid analysis of Airhart et al. 16 Hydroxyproline was quantified as a function of its double labeled dansyl-hydroxyproline was then analyzed using standard liquid scintillation counting techniques. The quantity of hydroxyproline in the hydrolysate was determined by comparing the 14 C/3H ratio of a dansylated sample of a known amount of 'H-labeled hydroxyproline were allowed to react with 14 C-labeled dansyl chloride, and the resultant dansylated hydroxyproline was separated from other dansylated amino acids by two dimensional thin layer chromatography on polyamide sheets. The double labeled dansyl-hydroxyproline was then analyzed using standard liquid scintillation counting techniques. The quantity of hydroxyproline in the hydrolysate was determined by comparing the 14 C/H ratio of the hydrolysate/3H-hydroxyproline mixture to the 14 C/3H ratio of a dansylated sample of a known amount of non-diluted 3H-hydroxyproline standard.

Mammalian elastin contains approximately 1.3% hydroxyproline by weight, 15 and the amount of hydroxyproline arising from tissue elastin (measured as described above) was subtracted from the dansyl chloride determined total hydroxyproline measurements before calculation of collagen contents.

Mechanical Measurements

Passive mechanical properties were determined as described previously. 1 Briefly, 3 mm vessel segments were cannulated at one end, clamped at the other, and mounted in a chamber that could be attached to the stage of a microscope having Nomarski interference contrast optics. Filar micrometer measurements of wall thickness and internal radius were then made at various transmural pressures while the vessel was filled with, and bathed in, PSS-EGTA. This relaxing solution was maintained at 37° C and continuously bubbled with a 95% O2, 5% CO2 gas mixture to maintain pH at 7.4. In each experiment, the pressure-internal radius data were fitted to a third-order polynomial equation, and radii at 10 mm Hg pressure intervals were predicted from the data fit. The average radius from all experiments at each pressure interval was then plotted against pressure to generate the pressure-radius curves. Distension ratio was calculated as r/rp where r equals the predicted radius at any transmural pressure and rp equals the predicted radius at zero pressure. Pressure-distension ratio curves were then prepared to provide a measure of vessel wall distensibility.

Circumferential wall tensions were calculated using a generalized tension equation. 17 This equation, which takes into account the finite thickness of the vessel wall, is: T = pr - 101.3 h, where p is transmural pressure (mN/m2), 101.3 is standard atmospheric pressure (assumed constant, mN/m2), r, p, and h are actual measured internal radius and wall thickness, respectively (mm). Using this formula, wall tension values are negative until pressures are reached such that the term (pr) becomes greater than 101.3 h (negative wall tension implies wall compression). Positive tension values generally were not obtained until transmural pressures of 70–100 mm Hg were reached. Mean wall stress was calculated as σ = T/h where h equals the wall thickness at T = 0 (positive stress indicates tensile stress; negative stress indicates compressive stress). Similarly, wall strain was defined as: e = (r - r0)/r0, where r0 equals the vessel radius at T = 0. Incremental elastic moduli were then obtained in the accepted manner 18 from tangents drawn to stress-strain diagrams at particular stresses for each vessel to provide data for the the SHR and WKY passive stress-elastic modulus curves.

Active wall force measurements for the cerebral arteries were made using 32 arteries obtained from 16 WKY and 16 SHR rats. The procedure and myograph technique for these tests was the same as previously used in this laboratory for mesenteric arterial experiments. 1 Dissected vessels were threaded onto two 32 μm diameter wires attached to separate stainless steel blocks. One block was connected to a force transducer and the second block to a micrometer so that the vessel circumference could be adjusted. The mounted vessel was about 0.8 mm in axial length; this length and media thickness were measured at ×500 using Nomarski optics and a filar micrometer eyepiece. Force determinations were made with the vessels set to a standardized internal circumference, Ls, as described previously. 1 Vessels were activated in a solution (PSS-K), which was identical to the PSS-EGTA described above, but lacked EGTA and contained KCl in an equimolar substitution for NaCl, and 5 mM CaCl2. Active wall tension was designated as half the maximal force elicited, divided by the axial length. The active smooth muscle cell stress was calculated as the active wall tension divided by the product of media thickness and the fraction of media occupied by smooth muscle cells. The proportion of media occupied by smooth muscle cells was determined from point grid counting.
of low power electron micrographs of fixed vessels according to techniques described previously.\(^1,19\)

**Statistics**

Data are expressed as means ± standard error of the mean. Two-way analysis of variance (ANOVA) was used for comparison of the pressure-internal radius, pressure-distension ratio, and stress-elastic modulus curves within the separate tissue types. All other variables were compared using Student's \(t\) test for non-paired data.

**Results**

The physical and hemodynamic characteristics of the rats used in these studies are shown in table 1. SHR systolic blood pressures were significantly elevated over those of WKY controls, and SHR body weights were less than control. Heart rates tended to be elevated in the SHR.

### Table 1. Animal Physical and Hemodynamic Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WKY (n=18)</th>
<th>SHR (n=12)</th>
<th>WKY (n=16)</th>
<th>SHR (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (wks)</td>
<td>25±1</td>
<td>26±1</td>
<td>24±1</td>
<td>24±1</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>374±8</td>
<td>334±9*</td>
<td>406±9</td>
<td>346±7†</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>129±2</td>
<td>188±3†</td>
<td>119±3</td>
<td>187±3†</td>
</tr>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>381±17</td>
<td>437±16</td>
<td>354±6</td>
<td>442±18*</td>
</tr>
</tbody>
</table>

Parenthetical numbers indicate the number of vessels. Data are means ± se.

\*Significantly different from control \((p < 0.05)\).

\†Significantly different from control \((p < 0.01)\).

**DNA and Contractile Protein Measurements**

The DNA content of SHR mesenteric samples was elevated by 29% relative to control and cerebral samples were elevated 26% (table 2). The conditions necessary to assure accuracy of the contractile protein ratio and content determination using the micro slab polyacrylamide gel technique\(^20\) were met in this study. Tissue extract actin and myosin peaks were clearly resolved and identified by co-electrophoresis of purified actin and myosin. Extraction of actin and myosin was apparently nearly complete as evidenced by no detectable amounts of actin or myosin in re-extracts of the pellet remaining after the 5000 \(\times\) g centrifugation (system detection limit was about 20 ng protein).

The high actin-to-myosin weight ratio (> 2:1) characteristic of arterial smooth muscle\(^20\) was observed in this study of resistance arteries (table 2). The actin-to-myosin ratios for WKY and SHR mesenteric vessels

### Table 2. DNA and Contractile Protein Content of SHR and WKY Resistance Arteries

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tissue wet weight (µg)</th>
<th>DNA content (ng/µg tissue)</th>
<th>Actin:myosin weight ratio</th>
<th>Actin content</th>
<th>Myosin content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total extract (µg)</td>
<td>Per µg tissue (ng)</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>WKY (n=6) 181±17</td>
<td>1.64±0.14</td>
<td>2.1±0.2</td>
<td>1.54±0.09</td>
<td>8.5±0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.3±0.1</td>
<td>11.9±1.1*</td>
</tr>
<tr>
<td></td>
<td>SHR (n=6) 195±16</td>
<td>2.11±0.05*</td>
<td>2.3±0.1</td>
<td>2.32±0.16*</td>
<td>14.4±1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.0±0.1</td>
<td>13.6±1.0</td>
</tr>
<tr>
<td>Cerebral</td>
<td>WKY (n=5) 136±8</td>
<td>3.09±0.19</td>
<td>2.1±0.1</td>
<td>1.98±0.22</td>
<td>14.4±1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.0±0.1</td>
<td>13.6±1.0</td>
</tr>
<tr>
<td></td>
<td>SHR (n=5) 140±13</td>
<td>3.89±0.19</td>
<td>3.0±0.1</td>
<td>1.89±0.18</td>
<td>13.6±1.0</td>
</tr>
</tbody>
</table>

\*Significantly different from control \((p < 0.05)\).

\†Significantly different from control \((p < 0.01)\).

Data are means ± se.
were the same. However, the SHR cerebral artery actin-to-myosin ratio was significantly greater than control.

Actin and myosin contents of vessel samples were expressed in three different ways. Since there was no significant difference in the amounts of tissue used for biochemical analyses (average tissue mass equaled 188 μg for WKY and SHR mesenteric arteries, 138 μg for all cerebral samples), contractile protein content, expressed in terms of absolute amounts in the total tissue extract, could be compared directly between the two animal strains. In addition, values were expressed on a concentration basis (ng/μg tissue), as well as normalized per nanogram DNA. The actin content of SHR mesenteric arteries was significantly elevated vs control with respect to absolute amount and concentration in the tissues (table 2). In addition, values were expressed on a concentration basis (ng/μg tissue), as well as normalized per nanogram DNA, however, was not different from control values. The same situation was found with regard to SHR mesenteric myosin content values.

The SHR cerebral actin content per nanogram DNA was significantly decreased compared to control values, whereas no differences in total actin content or actin per μg tissue were observed. SHR cerebral myosin content was significantly reduced versus control, regardless of how expressed (table 2).

Connective Tissue Content

There were no differences between SHR and WKY mesenteric vessels with regard to concentration or absolute amounts of collagen or elastin (table 3). Collagen concentrations were the same in SHR and WKY cerebral vessels. However, there was a 31% increase in the absolute amount of collagen in the SHR samples. SHR and WKY cerebral elastin concentrations and absolute amounts were not different.

Passive Mechanics

SHR mesenteric and cerebral vessel internal radii were less than their respective control values over the entire pressure range examined (fig. 2) and at the standardized circumference for the cerebral arteries used for the active mechanical experiments (table 4). The distension ratio at any given pressure was not different from control in SHR mesenteric vessels (fig. 3). However, the pressure-distension ratio curve for SHR cerebral vessels was lower than that of the WKY cerebral vessels at all positive transmural pressures. The SHR and WKY passive stress-elastic modulus curves were not different from each other for both mesenteric and cerebral vessels (figs. 4 and 5). Thus, at any given wall stress, the elastic modulus was the same for WKY and SHR vessel samples.

Cerebral Artery Active Mechanics

At the standardized circumference, L,, the mean active wall tensions were the same for the SHR and WKY vessels (table 4). Since the SHR media was significantly thicker than that of the WKY and the percentages of smooth muscle cells in the media were nearly alike, the mean active smooth muscle cell stress was 20% lower in the arteries from the SHR (table 4). Although the animals used for these studies were from a different group than those used for biochemical and passive mechanical analyses, their physical and hemodynamic characteristics were not markedly different (table 1).
Figure 2. Pressure-internal radius relationship for mesenteric and cerebral resistance arteries. SHR mesenteric and cerebral artery curves are significantly different from their respective controls at all transmural pressures (p < 0.001). Data means ± se; for mesenteric arteries n = 7; for cerebral arteries, n = 13 for WKY, and n = 9 for SHR.

Figure 3. Pressure-distension ratio curves for mesenteric and cerebral arteries. SHR cerebral curve is significantly different from control at all positive pressures (p < 0.001). Data are means ± se. The number of rat arteries are the same as for figure 2.

Figure 4. Mesenteric artery stress-elastic modulus relationships. Positive stress indicates tensile stress; negative stress indicates compressive stress. Data are means ± se; n = 7 for both groups.
Discussion

These studies represent the first direct measures of contractile proteins, DNA, collagen, and elastin in the resistance vasculature of normotensive and hypertensive animals. The results suggest several specific mechanisms that may be responsible for the altered vascular smooth muscle contractile capability often associated with the hypertensive process. The 29% increase in DNA content and increased absolute amounts of actin and myosin in SHR mesenteric vessels relative to the WKY are indicative of an increased smooth muscle cell mass in the SHR arteries. Seidel has made comparable measurements in aortic samples from SHR and WKY rats with similar results; i.e., DNA and contractile protein content are both elevated in the SHR.

Owens et al. presented evidence that about 10% of aortic smooth muscle cells of the WKY and about 20% of those from the SHR are tetraploid. Based on this and other evidence, they concluded that the increased wall mass of the SHR aorta could be accounted for entirely as a result of hypertrophy of the vascular smooth muscle cells. It is not known if tetraploidy exists in resistance artery smooth muscle cells, but if so, it could explain part of the increase in DNA content of SHR mesenteric arteries observed in the present study.

Previous studies in this laboratory showed that fully activated SHR mesenteric resistance arteries produce 30% more wall tension than control tissues. When these results are normalized to smooth muscle cell content, however, the intrinsic cellular stress-generating capabilities of WKY and SHR mesenteric vessels are the same. The demonstration in the present study that mesenteric artery contractile protein content normalized to DNA did not change in association with hypertension supports that finding.

The hypertension-associated changes in the posterior cerebral artery DNA and contractile protein measurements, although qualitatively quite different from those for the mesentery, also correlate well with morphologic and mechanical measurements. The DNA values suggest that there is an increased smooth muscle cell mass in the SHR cerebral vessels, and thus may explain the medial thickening measured in brain arteries in this study (table 4), and by others. There was not, however, a concomitant increase in contractile protein content, as was observed in mesenteric samples. In fact, when normalized on a DNA basis, actin and myosin content both actually decreased, a finding which implies that contractile force should be decreased. When actually measured, the intrinsic contractile ability (active smooth muscle cell stress) of the SHR brain arteries was significantly less than that measured for the WKY arteries (table 4).

The cause of the different response of SHR brain arteries compared with SHR mesenteric arteries with regard to protein composition cannot be determined from this study. Apparently, the signal inducing the increased smooth muscle cell mass, be it a circulating factor, nervous input, or the pressure elevation itself, reaches both vascular beds. Brain vessels, however, must be responsive to additional factors which alter contractile protein metabolism or cell division rates and which lead to a compromised contractile capability in these vessels.

Connective Tissue Proteins and Passive Mechanics — Cerebral Arteries

The cerebral vessel pressure-radius curves (fig. 2) suggest that, in the absence of smooth muscle cell activation, SHR arteries have a smaller internal radius at transmural pressures from 0 to 120 mm Hg. Thus, under activating conditions, the SHR vessels may be in position to exert a greater effect on flow resistance than are WKY vessels by virtue of their reduced internal radius prior to activation.
Cerebral arteries are primarily medial-intimal vessels, possessing little, if any, adventitia. Collagen is located nearly entirely in the extracellular space of the media of these arteries, and, in all vessels, is thought to be synthesized and secreted primarily by the vascular muscle cells. In the cerebral arteries considered in this study, the smooth muscle cells were found to occupy 72% of the media in both SHR and WKY (table 4). Thus, the 30% increase in absolute amount of collagen in the SHR cerebral vessels and an unchanged collagen concentration (table 3), would be predicted following medial thickening with a proportionate increase in the area of the extracellular matrix.

The decreased distensibility of SHR cerebral vessels (fig. 3) is expected as a result of the increased total collagen content of the vessels, assuming that an inverse relationship exists between connective tissue content and vessel distensibility. However, some investigators have found no direct relationship between absolute amounts of connective tissue protein and the mechanical properties of large vessels. In particular, Cox found that the collagen content of SHR tail and carotid arteries was reduced with respect to WKY, whereas passive stiffness of these vessels was increased. He suggested that other mechanisms, such as changes in connective tissue orientation, amino acid composition, or peptide cross-linking, could account for his results. Although these possibilities can not be ruled out in the present studies, a direct effect of increased collagen in the SHR vessel wall is the simplest explanation for the decreased distensibility characteristic of the SHR cerebral arteries.

The fact that the absolute amount of elastin contained in SHR cerebral vessels was not changed suggests that the balance between synthesis and degradation of elastin is not affected, or is altered proportionately by, the hypertensive process in these resistance vessels. This appears true for large arteries as well, since studies on the aorta, and carotid and tail arteries from the SHR indicated no change in elastin content of these vessels. In contrast, Wolinsky and Greenwald and Berry reported an increase in aortic elastin content of arteries taken from renal and DOCA salt hypertensive rats, respectively. This difference may be vessel-dependent, or model-dependent, with the increased elastin somehow associated with the more acute onset of hypertension in the renal and DOCA salt models. Although not significantly reduced, the average elastin concentration was somewhat less in the SHR, compared with control, as would be expected following a wall thickening with no change in the absolute amount of elastin.

The passive wall stress-elastic modulus characteristics describe the physical interrelationships between the constituent elements of the blood vessel wall. These measures include normalizations that take into account variations in wall thickness and lumen size, and thus are not influenced by the SHR wall thickening or decreased lumen diameter. However, if there were significant changes in SHR connective tissue protein concentration, structure, or orientation, then these would be expected to cause shifts in the stress-elastic modulus curve. Figure 5 demonstrates that this characteristic is the same for the two animal strains within the range of stresses examined. The SHR and WKY collagen and elastin concentration measures found in this study were in fact not significantly different and hence, support these mechanical findings.

Measurements of collagen and elastin content, in this study and others, were based on the assumption that the hydroxyproline content of collagen and the desmosine content of elastin from the different blood vessel types is invariant. Since little is known about this point, it should be mentioned that if this assumption is incorrect, then connective tissue protein estimates derived by this method could be in error.

Collagen, Elastin, and Passive Mechanics — Mesenteric Arteries

Mesenteric resistance arteries have a substantial adventitia containing large amounts of collagen. The collagen measurements in this study did not distinguish between adventitially and medially derived collagen. Because there is evidence that the media and adventitia may act differentially to influence passive wall mechanics, correlation of connective tissue content and passive mechanical properties was less clear-cut for mesenteric tissues than for cerebral tissues. However, in general, the mesenteric results do indicate an agreement between the collagen and elastin contents and passive mechanics. Specifically, the absolute amounts of collagen and elastin in the SHR and WKY vessels were not different and their distensibility characteristics were unaltered. Likewise, collagen and elastin concentrations were the same in SHR and WKY arteries as were the arterial stress-elastic modulus characteristics.

The physiological implications of these studies are different for the two vascular beds. In the mesentery of hypertensive rats, resistance vessels apparently undergo a medial thickening with a proportionate increase in contractile protein content, thus increasing their overall contractile capability. The connective tissue protein composition and distensibility of the mesenteric vessels also were not altered in the SHR. In the brain, although a medial thickening also occurred in arteries from hypertensive animals, the media of these vessels contained less contractile protein and developed less active smooth muscle cell stress relative to that of the WKY. Since active properties are necessary to maintain vessel tone and underlie the myogenic response of arteries in this vascular bed, the observed changes in contractile proteins could have significant effects on the autoregulatory capability of, and blood pressure distribution within brain arteries. In addition, these protein alterations might lower brain vessel resistance to damage caused by blood pressure elevation since active smooth muscle cell contractile activity would be required to oppose higher blood pressures. If true, this hypothesis could be a partial explanation for the reported increased incidence of stroke in the occipital lobe of the SHR, an area supplied by the posterior artery in the
rat. Conversely, the increased collagen content and decreased distensibility of the SHR cerebral arteries would tend to oppose the effects of the elevated blood pressure and hence, in that sense, may have a stabilizing influence on the vascular wall. All of the above factors and, no doubt, others not considered in this study must interact to determine the net effect of elevated blood pressure on the integrity of the blood vessel.

Acknowledgments
The authors thank Dr. David Counts for his advice and assistance regarding the elastin determinations and Stephen Mongeon and Daniel Root for their excellent technical assistance.

References
Biochemical and mechanical properties of resistance arteries from normotensive and hypertensive rats.
J E Brayden, W Halpern and L R Brann

_Hypertension_. 1983;5:17-25
doi: 10.1161/01.HYP.5.1.17

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 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/5/1/17

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/