Increased medial smooth muscle cell length is responsible for vascular hypertrophy in young hypertensive rats

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Dickhout, J. G., and R. M. K. W. Lee. Increased medial smooth muscle cell length is responsible for vascular hypertrophy in young hypertensive rats. Am J Physiol Heart Circ Physiol 279: H2085–H2094, 2000.—Large mesenteric arteries from 3- to 4-wk-old spontaneously hypertensive rats (SHR) showed medial hypertrophy and an increased contractile response to various agonists before significant blood pressure increase. Here we determined the cellular nature of this vascular hypertrophy. Large mesenteric arteries from SHR and Wistar-Kyoto (WKY) rats were fixed at maximal relaxation either with an in situ perfusion fixation or an in vitro fixation method. With the use of morphometric protocols and confocal microscopy, the volume of the medial wall and lumen, numerical density of smooth muscle cell nuclei in the medial layer, and smooth muscle cell and nuclear length were measured. Both methods of fixation yielded similar results, showing significant medial volume expansion in SHR than WKY without lumen change. Numerical density of medial smooth muscle cells was significantly less in SHR than WKY, and their total number per 100 μm length were similar between the strains. Average smooth muscle nuclear and cell length from SHR was significantly longer than that of WKY. Regression analysis showed that the increase in smooth muscle cell length explained 80% of the medial volume increase. We concluded that increased smooth muscle cell length in prehypertensive SHR is responsible for increased medial volume in the mesenteric arteries.

ESSENTIAL HYPERTENSION is a disease of increased total peripheral resistance. This is true in animal models of hypertension, such as spontaneously hypertensive rats (SHR) (7), Dahl salt-sensitive rats (4), and in human essential hypertension (19). This increase in total peripheral resistance is associated with structural change in the blood vessels of the animal models (13–15) as well as in humans (22). The exact nature of the structural change may vary between animal models, vessel types, and at different age points in the disease (12).

Structural change of the resistance blood vessels may be an important factor in the genesis of hypertension in the SHR. We have found that at 4 wks of age, systolic blood pressure variation between the SHR and its normotensive control, the Wistar-Kyoto (WKY) rats, occurs mainly between inbreeding lines within each strain and not between the strains (6). Thus 4 weeks is an important age to investigate structural change in the SHR because inbreeding lines of the strains have been obtained where SHR do not show a statistically significant blood pressure increase over WKY. Thus we can rule out increased blood pressure as a factor in generating the structural change. We have found an increased medial volume in the small muscular artery of 4-wk-old SHR as compared with age-matched WKY (5). This is accompanied by a greater contractile response of these structurally modified vessels to various agonist and electrical field stimulation (5).

These results support our hypothesis that increased medial smooth muscle volume in small muscular arteries of the SHR is an important causative factor leading to increased total peripheral resistance and hypertension in the SHR.

The main purpose of this study was to test the hypothesis that medial volume hypertrophy in the large mesenteric arteries of the 4-wk-old SHR was due to an increase in smooth muscle cell number. To achieve this goal, two fixation methods, in vitro and in situ, were applied to the mesenteric blood vessels of SHR and its normotensive control, the WKY. This was done to determine whether preparation method had any influence on our results. Then the volume of the medial layer and the lumen area at midlongitudinal section were quantified for either method of fixation. The numerical density of smooth muscle cell nuclei was assessed by a disector method (8), as well as by exhaustive serial sectioning. From these results, the number of smooth muscle cells per unit length of vessels was calculated. The average length of smooth muscle cell nuclei and smooth muscle cell length in the in situ fixed arteries were also calculated. This information was correlated with the medial volume to determine whether the increase in smooth muscle cell length was responsible for medial volume expansion in the SHR.

MATERIALS AND METHODS

The care and handling of the rats were done in accordance with the guidelines set by the Canadian Council on Animal Care.

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Experimental groups. Male SHR and WKY rats (4 wk) were used for medial smooth muscle cell quantification and measurement. The SHR colony was originally obtained from Ayerst Laboratory (Montreal, PQ, Canada) and the WKY from Canadian Breeding Farms (Montreal, PQ, Canada). Both colonies were derived from the Charles River (Wilmington, MA) strains, and we have maintained these colonies in our institute by continuous full-sib inbreeding for over 20 generations.

Perfusion fixation. The rats were weighed and their systolic blood pressures measured using a tail cuff occlusion method (model PE300, Narco Bio-Systems, Houston, TX) in conscious animals. The rats were anesthetized intraperitoneally with 65 mg/kg pentobarbital sodium to allow fixation of the arteries for morphometric studies. We used ileal arteries, the primary branches of the superior mesenteric artery, as in our previous study (5). Mesenteric vessels were cleared of blood by perfusion as follows. An infusion cannula was placed in the abdominal aorta, distal to the origin of the superior mesenteric artery. The aorta was clamped just below the diaphragm proximal to the origin of the superior mesenteric artery. An exit for the perfusate was cut into the portal vein. This allowed the perfusate, oxygenated Hank's basic salt solution (BSS) containing 1 μmol/l sodium nitroprusside, to clear the vasculature in the abdominal viscera. The arteries were perfused at a flow rate of 1 ml·min⁻¹·100 g body wt⁻¹ for 5 min, resulting in maximal relaxation of the arteries.

Fixation of arteries. Arteries prepared by the in vitro fixation method were dissected out, cleared of fat by dissection with fine forceps, placed on micropipettes at 70 mmHg pressure, and fixed as in our previous study (5). The fixative consisted of 3.5% formaldehyde and 0.75% glacial acetic acid in 0.05 mol/l phosphate buffer at pH 7.4.

Arteries prepared by the in situ fixation method remained in the animal after perfusion with sodium nitroprusside. The fixative was introduced by perfusion at 1 ml·min⁻¹·100 g body wt⁻¹. This allowed the arteries length to be fixed while attached to the connective tissues of the mesentery. In this case, changes in pressure and artery diameter were monitored throughout fixation to determine whether our procedure altered the vessel from its original state. This was accomplished by attachment of a pressure transducer to the perfusion cannula and coupling it to the Digi-Med BP analyzer (Micro-Med, Louisville, KY) attached to a microcomputer allowing sampling of mean pressure at 3 kHz. Additionally, by positioning a video microscope over the blood vessels, changes in lumen diameter were monitored before and during perfusion. Vessels were fixed for 1 h in the same fixative as above and then removed from the mesentery, cleared of fat by dissection with fine forceps, and prepared for confocal microscopy.

Confocal microscopy. Fixed vessels were stained with either reduced ethidium bromide for overall morphometric measurements or with acridine orange for high-resolution nuclear observations to determine smooth muscle cell numerical density and nuclear length. Staining first involved reducing free aldehyde groups by addition of 1 mg/ml of sodium borohydride in Hank's BSS. Half of the vessels sampled were stained with reduced ethidium bromide as described earlier (5), and the remainder were stained with 50 μg/ml acridine orange (Sigma-Aldrich, Oakville, ON, Canada) in Hank's BSS. All arteries were mounted in 100% glycerol containing 2.5% 1,4-diazabicyclo[2.2.2]octane (DABCO, Sigma-Aldrich) as an antifade agent.

An LSM 10 system (Carl Zeiss, Don Mills, ON, Canada) was used for confocal microscopy. The system was equipped with an external argon laser with emission lines at 488 nm and 514 nm and an internal HeNe laser with an emission line at 543 nm. The spectral line at 488 nm produced the best excitation for both the ethidium and acridine dyes with the least nonspecific fluorescence, resulting in an optimal signal-to-noise ratio. The 515 to 565-band-pass filter was used to collect signal from the acridine dye and the 575 to 640-band-pass filter was used to collect signal from the ethidium dye. An 8 s/frame dwell time and 16-line averaging were used to improve the image quality in specimens stained with the ethidium dye. The images were collected with the ×20 objective and a ×20 zoom factor to yield a total magnification of ×400. Optical sectioning began at a random distance above the artery, each section was separated by 10 μm, and sectioning continued until the artery was completely traversed. Figure 1A shows a three-dimensional representation of the artery where optical sections are shown to be taken longitudinally through its volume. The volumes of the medial smooth muscle layer were calculated as the sum (Σ) of the measured area on each optical section over the 1 to N optical sections multiplied by the distance between each slice, dT (Fig. 1A). Figure 1B illustrates a three-dimensional reconstruction of the optical sections obtained from a WKY artery stained with the ethidium dye where the total volume of the medial layer was integrated as above.

For the quantification of smooth muscle cell numerical density, the acridine orange dye was used because it provided better nuclear detail. However, it suffered from faster photobleaching than the ethidium dye. In this case, a 2 s/frame dwell time and 4-line averaging were used to reduce the photobleaching effect. Sections were taken with the ×40 objective at a zoom factor of ×50 to produce an overall magnification of ×2,000. A reference volume composed of a depth of 20 μm above the midlongitudinal section was used to calculate the numerical density of smooth muscle cells by the dissector method (8). In each artery, for each fixation method, two such reference volumes were randomly chosen providing replicates of the measure. The optical sections taken through these volumes were separated by 5 μm. Figure 2A shows an optical section obtained with this method through a SHR artery where the smooth muscle cell nuclei can be clearly seen. This method allowed smooth muscle cell nuclei to be followed through the medial layer to determine whether they were, or were not, shared by adjacent optical sections (Fig. 2B). Smooth muscle cell nuclei not shared by adjacent optical sections were counted while moving first up, then down, through the stack. This procedure is analogous to counting either the tops or bottoms of smooth muscle cell nuclei. Because the nuclei of smooth muscle cells have only one unique top or bottom, this count represents the number of smooth muscle cell nuclei in the given reference volume. Assuming that each smooth muscle cell has only one nucleus, this type of measurement gives us the number of smooth muscle cells in a given reference volume. This is a reasonable assumption because in mesenteric arteries from SHR and WKY, the incidence of polyplody smooth muscle cell was found to be very low (2–4%) and there was no difference between SHR and WKY (3, 21). Thus the numerical density of smooth muscle cells in the medial layer for either strain, for either method of fixation was calculated. Furthermore, in the in situ fixed arteries, exhaustive optical sectioning was performed to recalculate the numerical density of smooth muscle cells in the medial layer by using optical sections.
separated by 0.5 μm to test the accuracy of our 5-μm sampling distance.

Analysis of confocal microscopy data. Images were saved to electronic media and transferred to a personal computer using a Scion port (Frederick, MD) to the ×86 architecture of the NIH Image (National Institutes of Health, Bethesda, MD) for measurement of medial volumes, midsection lumen area, and calculation of smooth muscle cell numerical density. The number of smooth muscle cells per unit length of an artery was obtained as the product of the volume of the medial layer and the smooth muscle cell numerical density. The number of smooth muscle cells per unit length of an artery was obtained as the product of the volume of the medial layer and the smooth muscle cell numerical density (Fig. 1A). The average length of the smooth muscle cell nuclei was calculated from the in situ fixed arteries where exhaustive optical sectioning was performed. The length of the smooth muscle cells was calculated from the optical sections obtained for volume integration by following 10 smooth muscle cells per artery through the blood vessel wall. Length was calculated by measuring the distance the cell extended in the depth of the artery wall (dz), the distance the cell extended laterally within the wall (dx), and the diameter (D) of the arc within the blood vessel wall that they circumscribed. The diameter was measured by fitting the best circle to the blood vessel wall when the volume stack of optical sections was resliced in the NIH image package along the z-axis. Thus the length of a unique arc (dL) representing the length of the smooth muscle cell was calculated, as shown in Eq. 1.

\[
dL = \pi D \times \frac{2 \sin^{-1}\left(\frac{dx^2 + dz^2}{D}\right)}{360}
\]

Fig. 1. A: model of a blood vessel illustrating how optical sections were cut longitudinally through the arteries and the volume of the media layer and cell number calculated. See text for definitions of equation variables. B: large mesenteric artery from Wistar-Kyoto (WKY) rats reconstructed from optical sections used to produce the medial volume measurements. dT = distance between each slice.
number of smooth muscle cell nuclei per 100 \( \mu m \) length of artery. Smooth muscle cell nuclei length and smooth muscle cell length were compared with the strains by mixed model ANOVA, where replicated length measures were continuous variables nested within the strain’s fixed effects. Regression analysis was performed to determine whether the increase in SHR smooth muscle cell length could explain the increase in SHR medial volume.

RESULTS

The physiological characteristics collected from the rats used for morphometric analysis are listed in Table 1. There was no significant difference in age, body weight, or systolic blood pressure as measured by tail-cuff occlusion method between the strains for the 4-wk-old rats used in this study. The medial volume per unit length of blood vessel for either method of fixation is shown in Fig. 3, A and B, for SHR and WKY. ANOVA revealed that the mean medial volume was significantly larger in SHR (in vitro: 16,790 ± 945 \( \mu m^3/\mu m \), in situ: 15,490 ± 1,160 \( \mu m^3/\mu m \)) than WKY (in vitro: 11,250 ± 673 \( \mu m^3/\mu m \), in situ: 10,190 ± 671 \( \mu m^3/\mu m \)) for both methods of fixation. However, the medial vol-

Fig. 2. A: acridine orange labeling of smooth muscle nuclei in medial layer of spontaneously hypertensive rat (SHR) large mesenteric artery. B: numerical density of medial smooth muscle cells was determined by the disector method of nuclear counting where optical sections separated by a known distance, in this case 5 \( \mu m \), were used to form a reference volume. Nuclei shared by both frames were not counted but unshared nuclei were counted. The numerical density of smooth muscle cells was so determined, then multiplied by the volume of the medial layer in a given length of artery to produce the total number of cells in that artery length.
volume did not statistically differ between the different methods of fixation for SHR or WKY. Figure 3, C and D, shows the mean lumen area at midlongitudinal section for SHR (in vitro: 95,060 ± 3,036 μm²/μm, in situ: 102,860 ± 3,686 μm²/μm) and WKY (in vitro: 95,450 ± 6,103 μm²/μm, in situ: 113,660 ± 7,183 μm²/μm) in vessels prepared by either method of fixation. In this case, ANOVA found no statistically significant difference between the strains or methods of fixation.

Table 1. Physiological characteristics of rats used for the study

<table>
<thead>
<tr>
<th>Strain</th>
<th>In Vitro Fixation</th>
<th>In Situ Fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age, days</td>
<td>Body Wt, g</td>
</tr>
<tr>
<td>SHR</td>
<td>28 ± 0.1</td>
<td>73.4 ± 5.0</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>WKY</td>
<td>28 ± 0.2</td>
<td>66.3 ± 3.4</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats in each group. Physiological characteristics were compared between the strains by ANOVA. Significance was tested at the 5% level. There was no significant differences between the strains.

Figure 4, A and B, shows the changes in infusion pressure as well as the changes in lumen diameter for the blood vessels being studied during in situ fixation for SHR and WKY. From Fig. 4 it is clear that lumen diameter increased with perfusion pressure. The initial perfusion pressure was far below the physiological pressure in these vessels causing a decrease in lumen diameter. However, the addition of fixative began to increase perfusion pressures to physiological levels. The lumen diameter was increased to the levels found...
in the intact vessels before perfusion (85% in SHR, and 91% in WKY of their in vivo lumen diameter).

The values for smooth muscle cell numerical density from SHR and WKY arteries fixed by either method are listed in Table 2. It was found by ANOVA that SHR had a significantly smaller smooth muscle cell numerical density than WKY. When these data were combined with the volume data for a 100-μm length of artery, it was found that the number of smooth muscle cells per unit length of artery did not differ between SHR and WKY, regardless of the method of fixation (Table 2).

In the in situ fixed arteries, exhaustive optical sectioning was performed to test the reliability of our numerical density sampling method, which was based on optical sections separated by 5 μm. The exhaustively sectioned volume allowed us to count every nuclear top or bottom in the reference volume. These results were compared with the results obtained by our sampling method and found to differ by <5%. A three-dimensional reconstruction from the exhaustive sectioned material over 10 μm of the reference volume is illustrated in Fig. 5, showing the appearance of the smooth muscle cell nuclei within the vessel wall from above (Fig. 5A) and rotated away from the viewer at an angle of 60° (Fig. 5B). The exhaustive optical sections were also used to measure the mean length of smooth muscle cell nuclei. It was found that SHR had significantly longer smooth muscle cell nuclei than WKY (Table 2). The smooth muscle cell length was also

![Fig. 4. Changes in perfusion pressure during the course of in situ fixation and the corresponding changes in lumen diameter of the blood vessels before and during perfusion fixation. A: SHR; B: WKY. BSS, basic salt solution.](image)

Table 2. *Medial smooth muscle cell number and characteristics*

<table>
<thead>
<tr>
<th>Strain</th>
<th>SHR</th>
<th>WKY</th>
<th>P Level SHR vs. WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In vitro</td>
<td>In situ</td>
<td>In vitro</td>
</tr>
<tr>
<td>Numerical density</td>
<td>0.64 ± 0.08</td>
<td>0.74 ± 0.06</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Cell number</td>
<td>1,110 ± 120</td>
<td>1,050 ± 110</td>
<td>1,370 ± 120</td>
</tr>
<tr>
<td>Nuclei length</td>
<td>24.8 ± 1.9</td>
<td>17.6 ± 0.3</td>
<td>57 ± 0.9</td>
</tr>
<tr>
<td>Cell length</td>
<td>70 ± 1.1</td>
<td>57 ± 0.9</td>
<td>5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats in each group. Numerical density is expressed as no. of cells/μm³. Cell number was the product of numerical density and the medial volume of a 100-μm segment of artery producing the number of cells in the 100-μm segment. All lengths are expressed in microns. Differences between the strains were tested by ANOVA at the 5% level. NS, not significant.
found to be significantly longer in SHR than WKY (Table 2).

Regression analysis of smooth muscle cell length on medial volume found a significant relationship ($P = 0.0012$) and showed that increase in smooth muscle cell length accounted for 80% of the increase in medial volume (Fig. 5C).

**DISCUSSION**

At an age when blood pressure did not significantly differ between SHR and WKY, there was a significant increase in medial volume without a decrease in midsection lumen area. This increase in medial volume in the SHR was accompanied by a decrease in numerical density of the smooth muscle cells within that tissue layer as determined by optical sectioning with confocal microscopy and the application of the disector method (8). This measurement allowed the calculation of the number of smooth muscle cells per unit length of a large mesenteric artery, and this value was not found to significantly differ between SHR and WKY. These findings were mirrored in both of our in vitro-fixed arteries, where the vessels were pressurized on micropipettes to their in vivo length, and by our in situ fixation method, where the vessels were perfusion-fixed as they remained attached to the connective tissues, thus preventing distortions in our measurements that may be caused by vessel shortening on excision. Furthermore, in the in situ-fixed material, exhaustive optically sectioning was performed to verify our numerical density measurements derived from the disector method based on optical sections separated by 5 μm. These results confirmed our findings showing that our sampling
method produced results that differed from direct measurements by <5%.

These results suggest that smooth muscle cell hypertrophy was responsible for the increase in medial volume we measured in the SHR. However, our previous studies have determined that a significantly greater number of smooth muscle cell layers was present in the SHR compared with WKY at this age (5). This result and other analysis we have done with scanning electron microscopy (10) precluded the possibility of a general hypertrophy of the SHR smooth muscle cell. Specifically, cell width appears to be similar between SHR and WKY at this age. We therefore investigated whether smooth muscle cell lengthening might explain the greater medial volume and the similar number of smooth muscle cells we have observed in these vessels. Nuclear length in SHR was found to be significantly greater than that in WKY as calculated from dissected sample material. Furthermore, direct calculation of smooth muscle cell length by three-dimensional reconstruction (Eq. 1) revealed a significantly greater smooth muscle cell length in SHR than WKY. Regression analysis revealed that smooth muscle cell lengthening explains 80% of the medial volume expansion in SHR.

Previously, we have consistently found that there was an increase in the number of smooth muscle cell layers in the larger mesenteric arteries from hypertensive rats compared with their normotensive controls (12－14). To understand how an artery from SHR, with an equal number but longer smooth muscle cells compared with an artery from WKY, can have more smooth muscle cell layers, we have constructed a model as shown in Fig. 6. In this model, the dimensions of the vessel size and cell size are in the same ratio as those found in 4-wk-old rats. Both vessels are composed of the same number of smooth muscle cells (10), which wrap around a lumen of the same radius 1. However, the smooth muscle cells in the SHR are longer (2.25) than those from the WKY (1.8). As a result, there are more smooth muscle cell layers in the SHR (3.2) than WKY (2.4), and a greater medial cross-sectional area in the SHR (3.5) than WKY (3.0). Longer smooth muscle cells in an artery may increase the contractility of the artery by being able to shorten to a greater degree. Smooth muscle cells that have been induced to lengthen in cell culture by serum deprivation have shown greater contractile ability compared with freshly isolated cells (17). This would provide a structural correlate to the increased contractile response of the mesenteric arteries we have observed in the SHR compared with WKY in this age group (5).

This finding differs from our previous study (12), where we concluded that the increase in medial volume in the mesenteric arteries from SHR was mainly due to hyperplasia of the smooth muscle cells. In our previous work, the method used to determine cell hypertrophy or hyperplasia was based on the measurement of smooth muscle cell volume-to-surface ratio and the number of smooth muscle cell layers. This type of measurement could be preformed on randomly ori-

![Fig. 6. Models of arteries from SHR and WKY, showing a SHR artery with the same lumen size (Radius, R = 1) and the same number of smooth muscle cells (10) as a WKY artery. However, SHR smooth muscle cells are longer (cell length = 2.25 U) than WKY smooth muscle cells (cell length = 1.8 U) that contributed to the increased in the number of smooth muscle cell layers (cell layers = 3.2) and medial area (medial area = 3.5 U) compared with the WKY artery (cell layers = 2.4, medial area = 3.0 U).](http://ajpheart.physiology.org/)

Hydrodynamic results for...
estimated to represent a transmural pressure of 100 mmHg, and fixed for the study of smooth muscle cell number by the application of the disector method. A series of 1 \( \mu \)m-thick serial sections were used to produce a reference volume for the disector. They found that a greater number of smooth muscle cells in the SHR contributed to the medial volume increase compared with WKY. These results may differ from our study because the different age of the rats used, the different order of mesenteric artery branch used, or the different methods employed.

Other studies have revealed similar findings to our present work in different models of genetic hypertension. In mesenteric arteries from the genetically hypertensive New Zealand rat strain (11), and in stroke-prone SHR (1), media volume increase was accompanied by a decrease in the numerical density of medial smooth muscle cells compared with the normotensive controls. Furthermore, no change in the total number of smooth muscle cells per unit length of blood vessel was found in stroke-prone SHR (1). These studies, however, did not measure the size of the medial smooth muscle cells to determine what was responsible for the medial volume expansion.

Discussion of methods. Serial sectioning by mechanical means suffers from variable section thickness, lack of precision of measurement over small distances (<1 \( \mu \)m), and the exhaustive nature of the process. The ability of confocal microscopy to resolve structure more easily in three dimensions may be of particular importance in the analysis of arterial structure as it relates to hypertension (2). One major criticism, however, of the currently available confocal microscopes, is the lack of a transmission mode of operation (9). As such, dyes generally used for light microscopy, which increase the optical density of certain elements of the cell, usually the nuclei and structural proteins in the cytoplasm, are useless in the confocal microscope. The lack of a fluorescent dye, which provides a clear general histology, has seriously limited the use of this instrument, thereby forcing those who use it for image generation to resort to such devices as nonspecific fluorescence induced by gluteraldehyde fixation (2). We have found that reduced ethidium bromide is an ideal fluorescent stain to provide nuclear contrast in confocal microscopy (5). It is excited at 510 nm, in the range of argon lasers commonly used in confocal microscopes, and the chemical reduction of ethidium bromide greatly increased its utility as a nuclear fluorophore by increasing its permeability across the cellular membrane, allowing its use in whole mounted tissues. Moreover, the long laser exposures necessary to produce many optical sections for serial reconstructions were only possible because the low photobleaching properties of the ethidium dye.

Vessel shortening during excision is a significant problem faced by any investigator who attempts to quantify some feature per unit length of blood vessel, such as tissue volume or cell number. This shortening of the vessel makes the calculation of effective pressures in a wire myograph system prone to errors, because this apparatus applies only radial force, whereas true intramural pressure applies forces in all directions. This may produce systemic errors of measurement if the vessels from different strains of the rats shorten to different degrees on the wire mounted preparation. This type of difference between SHR and WKY vessels in the pressurized and wire-mounted apparatus has been previously noted (16). The measurement of lumen diameter using a wire myograph method has shown smaller effective lumen diameters in 6- and 50-wk-old SHR than WKY rats (23). This is in contrast with our measurements of mesenteric arteries using pressurized myographs where similar lumen size was found in maximally relaxed vessels.

The elastic modulus of the artery wall has been shown to be considerably less in the longitudinal directions. In canine femoral artery, it was found to be five
times less in the longitudinal direction, resulting in mostly lengthening of artery segments in vitro with increasing pressure (18). Effective tension in the artery wall is dependent on wall thickness, and thickness of the artery wall in turn will vary under the influence of different expanding pressures. In the wire myograph method, the effective pressure expanding the vessels in the longitudinal direction is zero. Therefore the arterial wall would be thicker under the radially applied tension of the wire mounts than it would be under in situ conditions. This appears to be the main difference in the methods applied by Mulvany et al. (20) and the methods applied in this study.

In conclusion, we have found that medial volume expansion occurs in prehypertensive SHR compared with their normotensive WKY controls. This medial volume expansion, which we have previously shown to be accompanied by a greater contractile response of the SHR smooth muscle cells. These are primary changes in the SHR small muscular arteries which we hypothesize may lead to the eventual development of hypertension in these rats through an increase in total peripheral resistance. Similar changes in other vessel types and vessels from other vascular beds in the SHR may also be critical for the development of hypertension.

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