tive in preventing SMC hypertrophy and hyperploidy than hyperplasia\(^6\) suggest that the signals for SMC hypertrophy are different from those for hyperplasia. However, the specific factors that stimulate aortic SMC hypertrophy and hyperploidy in the SHR are unclear. Three lines of indirect evidence indicate that SMC hypertrophy represents a response to increased blood pressure or wall stress: 1) Our laboratory\(^2, 6, 8, 9\) and others\(^6\) have consistently observed a high degree of correlation between the level of blood pressure and the frequency of polyploidy in a variety of hypertensive models. 2) Development of SMC hypertrophy and hyperploidy in aortas of SHR occurs predominantly after blood pressure has increased to its maximal level.\(^2, 6, 3\) 3) Normalization of blood pressure in SHR with antihypertensive drug treatment is effective in preventing further development of SMC hypertrophy and hyperploidy,\(^4\) as well as in reversing some of the hypertrophic changes that have already occurred.

Results of our drug treatment studies\(^4\) support a role for elevated blood pressure in the hypertrophic response of SMCs. However, this evidence is not compelling, since treatment was with a combination of three drugs (reserpine, chlorothiazide, and hydralazine), and it is not clear whether the effects of drugs were the direct result of lowering of blood pressure or due to some specific effect of one or more of the drugs on cellular growth. Thus, in the present investigation, we explored the role of elevated blood pressure on the development of SMC hypertrophy and hyperploidy in the SHR by examining the effects of various single antihypertensive drugs that have different primary mechanisms of action. We used captopril, a converting enzyme inhibitor\(^11\); hydralazine, a direct smooth muscle relaxant\(^12\); and propranolol, a \(\beta\)-adrenergic blocker.\(^13\) Our rationale was that if each of these drugs influences hypertrophy in proportion to its blood pressure-lowering effects, additional support would be provided for the hypothesis that hypertrophic vascular changes were secondary to elevated pressure. If, however, blood pressure effects and indices of hypertrophy were dissociated, the studies would suggest that factors other than or in addition to blood pressure were important and perhaps provide insight as to what those factors might be.

**Materials and Methods**

Male SHR and Wistar-Kyoto rats (WKY) used in this study were obtained from Charles River Breeding Laboratories (Wilmington, MA, USA) at 5 weeks of age; 40 SHR and 37 WKY were used. Food and water were administered ad libitum. All animal use procedures have been approved by the University of Virginia Animal Use and Care Committee.

**Assessment of Hypertension**

Systolic blood pressure and heart rates were determined in conscious rats by a photoelectric tail cuff pulse detector (ITC, Landing, NJ, USA). Ambient temperature was maintained at 27°C. Animals were conditioned to the restraining cages before blood pressure measurements were attempted. Rats were identified by number, and the blood pressure technician was not aware of experimental groupings. A minimum of two blood pressure measurements were made before initiation of drug treatment experiments. Pressure measurements were made at weekly or biweekly intervals during the treatment period (i.e., 8–20 weeks of age).

At death, hearts were excised, perfused briefly with Hanks buffer (pH 7.4) to remove blood, and fat and connective tissue were excised. Hearts were then dried for 48 hours at room temperature and weighed.

**Drug Treatment**

The following antihypertensive agents were added to the drinking water of drug-treated rats: captopril (courtesy of E.R. Squibb & Sons, Princeton, NJ, USA), 375 mg/L; hydralazine (Apresoline HCl, courtesy of CIBA-Geigy, Summit, NJ, USA), 40 mg/L; and propranolol (Sigma Chemical, St. Louis, MO, USA), 1.5 g/L. An attempt was made to administer drugs at a dose that produced maximal blood pressure lowering effect. Doses were chosen based on 1) published reports of others\(^14–18\) and using estimates of daily water intake, and 2) short-term pilot studies in which blood pressure was measured in 3-month-old SHR and WKY treated with several different doses of each drug. Drug-containing water was changed daily and was placed in opaque bottles to minimize light-sensitive drug degradation.

**Aortic Preparation**

Animals were killed at either 8 weeks (pretreatment groups) or 20 weeks of age by CO\(_2\) asphyxia, and the chest cavity was opened. The thoracic aorta was measured at its in situ length between the diaphragm and the descending arch of the aorta. This same segment and the heart were excised, perfused with Hanks solution, and placed in fresh Hanks solution. After the length of the aorta was measured in vitro, 2-mm segments were removed from each end and fixed in 2% glutaraldehyde, 1% paraformaldehyde in Hanks buffer (pH 7.40) for morphometric evaluations. An intima-medial preparation of the remaining aortic segment was made by removing the adventitia and intercostal arteries under a dissecting microscope and by scraping the intima with a piece of Teflon. Resultant medial preparations were weighed (wet weight), and portions were used for isolation of SMCs for flow cytometric evaluation of cellular ploidy and for evaluation of aortic medial DNA content and cell number.

**Evaluation of Aortic Medial Hypertrophy**

Aortic medial smooth muscle content and mass were measured to provide a quantitative estimate of the effects of various drugs on aortic hypertrophy. Aortic medial smooth muscle content was determined as previously described.\(^8\) In brief, medial cross-sectional area measurements were determined by planimetry on 1-µm toluidine blue-stained sections of the aortic segments removed from each end of the thoracic aorta at
Influence of Blood Pressure on Development of Aortic Medial Smooth Muscle Hypertrophy in Spontaneously Hypertensive Rats

GARY K. OWENS

SUMMARY The hypothesis that a primary stimulus for aortic medial hypertrophy in spontaneously hypertensive rats (SHR) is increased blood pressure was tested by determining whether development of smooth muscle cell hypertrophy and hyperploidy in SHR could be dissociated from blood pressure levels in rats treated with various antihypertensive drugs with different mechanisms of action. Wistar-Kyoto rats (WKY) and SHR were treated between 2 and 5 months of age with captopril (375 mg/L), hydralazine (40 mg/L), or propranolol (1.5 mg/L) administered in their drinking water. Smooth muscle hypertrophy and hyperploidy were analyzed by morphometric evaluation of medial smooth muscle content, flow cytometric analysis of the frequency of polyploid smooth muscle cells, and biochemical estimates of smooth muscle cell number. All drugs significantly lowered blood pressure in SHR compared with untreated controls (order of efficacy: captopril > hydralazine > propranolol). Captopril also was most effective at changing blood pressure in WKY, while propranolol and hydralazine had similar blood pressure–lowering effects. The efficacy of drugs in preventing the development of smooth muscle cell polyploidism and medial hypertrophy in SHR was the same as their efficacy in lowering blood pressure, although propranolol had no effect on medial smooth muscle hypertrophy despite lowering blood pressure by 26 mm Hg. Regression analyses showed a high degree of correlation between blood pressure and the frequency of polyploid smooth muscle cells and medial smooth muscle content. These results are consistent with the hypothesis that aortic medial hypertrophy may be, in part, a response to increased blood pressure or wall stress. However, analysis of covariance and two-stage multiple regression analyses indicated that captopril had an effect over and above that predicted by its blood pressure–lowering effect. Furthermore, propranolol lowered blood pressure but did not affect medial hypertrophy. These results suggest that smooth muscle hypertrophy is not simply a response to increased blood pressure, but that other factors, such as angiotensin II, may be important in modulating aortic medial hypertrophy in SHR. (Hypertension 9: 178–187, 1987)

KEY WORDS • smooth muscle cell hypertrophy • spontaneously hypertensive rat • angiotensin II • captopril • hypertension

There is considerable interest in the cellular mechanisms responsible for the accelerated smooth muscle growth in arteries from hypertensive patients and animals. This interest derives from suggestions 1) that the accelerated smooth muscle growth associated with hypertension contributes to atherogenesis and 2) that medial smooth muscle hypertrophy in resistance vessels may play a role in the etiology of hypertension. Previous studies in this laboratory and others have demonstrated that the increased mass of smooth muscle in aortas of several models of chronic hypertension, such as spontaneously hypertensive rats (SHR) or a two-kidney Goldblatt hypertensive model, compared with normotensive controls was due principally to smooth muscle cell (SMC) hypertrophy rather than hyperplasia and that SMC hypertrophy was accompanied by the development of polyploidy. In contrast, induction of acute severe hypertension in rats by aortic coarctation resulted in SMC proliferation without accompanying polyploidy. Thus, the growth response of SMCs in a given blood vessel can be quite different in different models of experimental hypertension. These cited observations, and our observations that antihypertensive drug treatment is much more effec-
Flow Cytometric Analysis of Smooth Muscle Cell Ploidy
The SMCs were dissociated enzymatically from medial preparations of thoracic aortas, and nuclei were extracted and stained for their DNA content with diaminodinoxyphenylindole as previously described.\(^\text{17}\) Measurement, acquisition, and analysis of cellular DNA content was done on a FACS IV fluorescent activated cell sorter (Becton-Dickinson, Sunnyvale, CA, USA). Since there were no background counts or cells in S phase, curve-fitting algorithms were not needed and estimates of the diploid, tetraploid, and octaploid populations were made simply by determining the fraction of cells present in each population. We have previously demonstrated that results of this flow cytometric assay are identical to those obtained by Feulgen-DNA microdensitometric analysis of aortic sections and provide a valid measure of the frequency of polyploid cells in the intact aorta.\(^\text{17}\)

Determination of Medical DNA Content and Smooth Muscle Cell Number
The DNA content of thoracic aortic medial preparations was measured using the fluorometric assay of Labarca and Peigen.\(^\text{18}\) The DNA content was expressed on the basis of vessel length (in vitro) as well as on a per aorta basis. Total aortic medial DNA content was calculated as the product of vessel length × DNA/length. Aortic medial SMC number was determined by dividing medial DNA content by the average DNA content per SMC determined by flow cytometry. Calculations were made assuming 6.64 pg of DNA per diploid nucleus.

Statistical Analysis
Data for each parameter from all the experimental groups were first examined for overall effects of strain, age, drug groups, and strain–drug group interactions, using an analysis of variance calculated from a general linear models procedure (Statistical Analysis System, SAS Institute, Cary, NC, USA). A \(t\) statistic was then performed on least-square means from the general linear model for specific intergroup comparisons. This analysis permitted examination of individual group-by-group differences in the context of a multiple comparison test and used the pooled variability obtained by analysis of variance.

Least-squares methods were used for curve fitting in regression analyses. The significance of the slope and intercept (i.e., testing the null hypothesis that they equaled 0) was evaluated using a \(t\) statistic. A \(p\) level of 0.05 was considered significant. To address whether blood pressure effects could account for or predict the effects of drugs on indices of hypertrophy (e.g., SMC ploidy, SMC content), the following analyses were also performed: 1) analysis of covariance using a general linear model procedure (SAS Institute) incorporating rat strain, blood pressure, and drug treatment as predictors and 2) two-stage regression analysis where-by the variance attributed to blood pressure effects was accounted for first, followed by determination as to whether any residual variance could be accounted for by inclusion of the specific drug group as a predictor. This latter type of analysis is more appropriate than a multiple regression analysis because of the possible interaction of the predictor terms (e.g., blood pressure and drug type).

Results

Blood Pressure
Mean systolic blood pressures of untreated SHR were significantly greater than pressures of WKY at 7 weeks of age and older (Figure 1). Blood pressures of each of the drug-treated groups were significantly reduced when compared with the pressures of the corresponding untreated controls (Table 1; see Figure 1). There were significant differences in the effectiveness of the different drugs, and all drugs had proportionately greater effects in SHR than in WKY. In SHR, captopril was the most effective drug in reducing blood pressure, followed by hydralazine and then by propranolol. Captopril was also most effective in WKY, while hydralazine and propranolol had similar blood pressure–lowering effects.

Heart Weight
Heart weights were significantly reduced from control values in captopril-treated and propranolol-treated SHR and WKY at 20 weeks of age, while no differences from control values were evident in hydralazine-treated groups (see Table 1). Body weight was also significantly reduced in propranolol-treated groups such that the heart weight/body weight ratio in these groups was not different from that of their respective untreated controls, indicating that reductions in heart weight in animals treated with a \(\beta\)-blocker may be secondary to weight reduction, although, as expected, heart rates were significantly reduced from control levels in both SHR and WKY given propranolol (data not shown). Captopril caused only a modest reduction in body weight in SHR but substantially reduced body weight in WKY. In contrast to other groups, the heart weight/body weight ratio of captopril-treated SHR was significantly less than that of controls, indicating that the effect of this drug on heart weight was not secondary to weight loss.

Drug Intake
Water intake was monitored for each treatment group to estimate the approximate amount of drugs...
Evaluation of Aortic Medial Hypertrophy

The effect of drug treatments on aortic medial hypertrophy is shown in Table 3 and Figure 2. Medial hypertrophy was evaluated by 1) measurement of SMC weight per thoracic aorta and 2) SMC volume per centimeter of aortic length. Our initial analysis of variance indicated an overall effect of rat strain (i.e., SHR vs WKY), age, and drug treatment on SMC weight per aorta or SMC content (mm²/mm length; see Figure 2), but no significant overall differences between rat strains in the response to drugs. No differences in smooth muscle weight per thoracic aorta were observed between 7- to 8-week-old SHR and WKY, indicating that medial hypertrophy was not present in SHR at the initiation of drug treatment (see Table 3). Captopril was more effective than hydralazine in preventing increases in aortic medial SMC content and weight in SHR between 8 and 20 weeks of age, while propranolol had no significant effect. Only captopril reduced the increases in thoracic aortic medial SMC content and weight that occurred in WKY between 8 and 20 weeks of age.

The findings that captopril was more effective than hydralazine in preventing aortic medial hypertrophy, even though both drugs lowered blood pressures to a similar extent (see Figure 1 and Table 1), and that treatment with propranolol did not prevent the increases in aortic SMC content despite lowering blood pressure by 26 mm Hg suggest that factors other than blood pressure per se may be involved in the hypertrophic response. Two types of statistical analyses were performed to further assess the interrelationship between pressure, drug treatment, and medial smooth muscle hypertrophy: analysis of covariance (pressure effects and drug effects) and a two-stage regression analysis, in which we first assessed what portion of the variance could be accounted for by pressure effects and then determined whether inclusion of the drug treatment could account for any additional variance. Results demonstrated a significant effect of blood pressure on aortic medial SMC content ($p < 0.0001$). However, the results also demonstrated that captopril had an additional effect over and above that predicted by its blood pressure-lowering effect ($p < 0.002$).

Analysis of Smooth Muscle Cell Polyploidy

Untreated 20-week-old SHR had a higher frequency of both tetraploid and octaploid SMC than all other groups (Figure 3; Table 4). The order of effectiveness of drugs in preventing development of polyploidy in SHR between 8 and 20 weeks of age was the same as their potency in lowering blood pressure (i.e., captopril was most effective, followed by hydralazine and then by propranolol). In WKY, only captopril significantly reduced the frequency of polyploidy compared with that in untreated WKY.

Although mean values for polyploidy for treated SHR were similar to those of the corresponding group of WKY, the percentage reduction in tetraploidy from control was greater for SHR than for WKY. However, there did not appear to be an overall difference in the nature of the response in the two strains based on analysis of covariance (i.e., strain-drug interactions).

A highly significant linear relationship was observed between the frequency of tetraploid SMC and the level of blood pressure (Figure 4). This finding indicates that lowering blood pressure of SHR to a level similar to WKY results in a parallel reduction in polyploidy. Further analysis of the interrelationship of pressure, drug treatment, and polyploidy by two-
TABLE 1. Heart Weight, Body Weight, Heart Weight to Body Weight Ratios, and Blood Pressure of Drug-Treated and Untreated (Control) SHR and WKY

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Heart weight (g, dry)</th>
<th>Heart weight (\times 10^{-4}) body weight ratio (g)</th>
<th>Blood pressure* (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>Control</td>
<td>327 ± 9 (B)</td>
<td>0.265 ± 0.006 (B)</td>
<td>8.1 ± 0.2 (B)</td>
<td>166 ± 2</td>
</tr>
<tr>
<td>SHR</td>
<td>Captopril</td>
<td>308 ± 9 (A)</td>
<td>0.210 ± 0.007 (A)</td>
<td>6.8 ± 0.1 (A)</td>
<td>121 ± 2 (A)</td>
</tr>
<tr>
<td>SHR</td>
<td>Hydralazine</td>
<td>317 ± 5 (A, B)</td>
<td>0.259 ± 0.005 (B)</td>
<td>8.2 ± 0.2 (B)</td>
<td>128 ± 3 (B)</td>
</tr>
<tr>
<td>SHR</td>
<td>Propranolol</td>
<td>257 ± 6 (C)</td>
<td>0.216 ± 0.007 (A, C)</td>
<td>8.4 ± 0.2 (B)</td>
<td>140 ± 2</td>
</tr>
<tr>
<td>WKY</td>
<td>Control</td>
<td>363 ± 6 (D)</td>
<td>0.245 ± 0.009 (B)</td>
<td>6.9 ± 0.2 (A)</td>
<td>127 ± 2 (B)</td>
</tr>
<tr>
<td>WKY</td>
<td>Captopril</td>
<td>308 ± 12 (A, B)</td>
<td>0.193 ± 0.019 (A)</td>
<td>6.2 ± 0.5 (A)</td>
<td>112 ± 2</td>
</tr>
<tr>
<td>WKY</td>
<td>Hydralazine</td>
<td>350 ± 5 (D)</td>
<td>0.241 ± 0.014 (A)</td>
<td>6.9 ± 0.4 (A)</td>
<td>119 ± 2</td>
</tr>
<tr>
<td>WKY</td>
<td>Propranolol</td>
<td>274 ± 6 (C)</td>
<td>0.188 ± 0.005 (A, C)</td>
<td>6.9 ± 0.2 (A)</td>
<td>120 ± 2</td>
</tr>
<tr>
<td>SHR</td>
<td>Pretreatment</td>
<td>199 ± 6 (E)</td>
<td>0.152 ± 0.003</td>
<td>7.6 ± 0.2 (B)</td>
<td>129 ± 2</td>
</tr>
<tr>
<td>WKY</td>
<td>Pretreatment</td>
<td>186 ± 7 (E)</td>
<td>0.132 ± 0.006</td>
<td>7.1 ± 0.2 (A)</td>
<td>120 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Means with the same letter (in parentheses) are not significantly different \((p > 0.05\), analysis of variance; general linear models procedure, SAS Institute).

*Average systolic blood pressure during the drug treatment period \(\text{(i.e., 8–20 weeks of age)}.\)

stage regression modeling and analysis of covariance indicated that 1) there was a significant interaction of blood pressure and aortic polyploidism \((p < 0.0001)\); 2) once we accounted for pressure differences between SHR and WKY, inclusion of rat strain into the model did not account for any additional variance in tetraploidy \((p = 0.8)\); and 3) there was a significant effect of captopril on the percentage of tetraploidy that could not be accounted for by the drug’s antihypertensive effect \((p < 0.03)\).

Analysis of Smooth Muscle Cell Number and Volume

As reported previously, no differences in aortic SMC number were observed between control SHR and WKY at 5 months of age \(\text{(see Table 4)}\). No differences in cell number were observed between drug-treated SHR and WKY and their respective untreated controls for any of the treatment groups. These same results were seen when data were expressed on a per aorta or per centimeter of length basis. With the exception of the hydralazine-treated WKY, no differences in SMC number were apparent between treated SHR and their respective untreated controls. With the exception of the hydralazine-treated WKY, no differences in SMC number were apparent between treated SHR and their respective WKY. When expressed on a per aorta basis, hydralazine-treated WKY had a significantly lower cell number than did all groups of SHR and appeared to be somewhat lower than the control and captopril-treated groups of WKY, although these differences were significant only at a level less than 0.18. Smooth muscle content was also reduced in hydralazine-treated WKY compared with untreated WKY. Cell numbers were not different between pretreatment SHR and WKY on either a per aorta or a per length basis.

The preceding cell number data suggest that the reduction in aortic SMC content \(\text{(see Figure 2)}\) and weight \(\text{(see Table 3)}\) observed in captopril-treated SHR...
TABLE 3. Evaluation of Thoracic Aortic Medial Hypertrophy in Drug-Treated and Untreated (Control) SHR and WKY

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Aortic medial cross-sectional area (mm²)</th>
<th>Volume density (mm²/mm length)</th>
<th>Content (mm³/mm length)</th>
<th>Weight (mg/thoracic aorta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>Control</td>
<td>0.480 ± 0.010 (A) (n = 6)</td>
<td>0.347 ± 0.007 (A, B, C) (n = 6)</td>
<td>0.166 ± 0.005 (A) (n = 6)</td>
<td>8.69 ± 0.25 (A) (n = 6)</td>
</tr>
<tr>
<td>SHR</td>
<td>Captopril</td>
<td>0.380 ± 0.012 (B, C) (n = 5)</td>
<td>0.338 ± 0.006 (A, B, C) (n = 7)</td>
<td>0.128 ± 0.003 (B, E) (n = 6)</td>
<td>6.19 ± 0.30 (B, D, E) (n = 7)</td>
</tr>
<tr>
<td>SHR</td>
<td>Hydralazine</td>
<td>0.458 ± 0.018 (A) (n = 5)</td>
<td>0.319 ± 0.007 (B) (n = 6)</td>
<td>0.146 ± 0.007 (C) (n = 5)</td>
<td>7.30 ± 0.41 (B, C)* (n = 6)</td>
</tr>
<tr>
<td>SHR</td>
<td>Propranolol</td>
<td>0.476 ± 0.009 (A) (n = 6)</td>
<td>0.344 ± 0.011 (A, B, C) (n = 6)</td>
<td>0.164 ± 0.005 (A) (n = 6)</td>
<td>7.43 ± 0.44 (A, C, D)†, ‡ (n = 6)</td>
</tr>
<tr>
<td>WKY</td>
<td>Control</td>
<td>0.398 ± 0.015 (B) (n = 5)</td>
<td>0.353 ± 0.014 (A, B, C) (n = 6)</td>
<td>0.142 ± 0.010 (B, C) (n = 5)</td>
<td>7.10 ± 0.64 (B, C, D) (n = 6)</td>
</tr>
<tr>
<td>WKY</td>
<td>Captopril</td>
<td>0.330 ± 0.011 (D) (n = 6)</td>
<td>0.327 ± 0.011 (B) (n = 6)</td>
<td>0.108 ± 0.005 (D) (n = 6)</td>
<td>5.40 ± 0.27 (E) (n = 6)</td>
</tr>
<tr>
<td>WKY</td>
<td>Hydralazine</td>
<td>0.352 ± 0.011 (C, D) (n = 6)</td>
<td>0.335 ± 0.018 (A, B, C) (n = 6)</td>
<td>0.117 ± 0.005 (B, D) (n = 6)</td>
<td>6.60 ± 0.53 (B, D, E) (n = 6)</td>
</tr>
<tr>
<td>WKY</td>
<td>Propranolol</td>
<td>0.368 ± 0.012 (B, C) (n = 6)</td>
<td>0.367 ± 0.019 (C) (n = 6)</td>
<td>0.135 ± 0.008 (C, E) (n = 6)</td>
<td>7.57 ± 0.82 (A, C, F) (n = 6)</td>
</tr>
<tr>
<td>SHR</td>
<td>Pretreatment</td>
<td>—</td>
<td>0.282 ± 0.015 (D) (n = 6)</td>
<td>—</td>
<td>3.94 ± 0.24 (G) (n = 6)</td>
</tr>
<tr>
<td>WKY</td>
<td>Pretreatment</td>
<td>—</td>
<td>0.306 ± 0.014 (D) (n = 6)</td>
<td>—</td>
<td>3.82 ± 0.20 (G) (n = 6)</td>
</tr>
</tbody>
</table>

Results are means ± SE. Means with the same letter (in parentheses) are not significantly different (p > 0.05) by analysis of variance and Duncan’s multiple range test (SAS Institute).

*p = 0.11, †p = 0.07, compared with captopril-treated SHR.
fp = 0.08, compared with control SHR.

and WKY and hydralazine-treated SHR could not be accounted for by a reduction in SMC number, suggesting that cell size must be reduced. To directly assess this possibility, two indices of cell size were calculated: 1) mean SMC volume (i.e., [SMC content/aorta]/[SMC number/aorta]) and 2) mean SMC weight (i.e., [SMC weight/aorta]/[SMC number/aorta]). The relatively large variances associated with these measurements limit their usefulness except in instances where differences between experimental groups are large. Results were similar with either method of assessing cell size, but only cell volume data are present-

![Figure 2](http://hyper.ahajournals.org/)

**FIGURE 2.** Morphometric evaluation of thoracic aortic medial smooth muscle hypertrophy in drug-treated and untreated SHR and WKY. Smooth muscle content was calculated from measurements of medial cross-sectional area and smooth muscle volume fractions. Smooth muscle content was significantly greater in untreated SHR than in WKY. Treatment with captopril was more effective than hydralazine in reducing aortic smooth muscle content in SHR, while propranolol had no effect. Only captopril significantly reduced aortic smooth muscle content in WKY. Values are means ± SEM. See Table 3 for additional statistical comparisons.

![Figure 3](http://hyper.ahajournals.org/)

**FIGURE 3.** Flow cytometric analyses of the frequency of tetraploid aortic smooth muscle cells (SMCs) in drug-treated and untreated SHR and WKY. Untreated SHR had the highest frequency of tetraploid SMCs. In SHR, all drugs were effective in reducing the frequency of tetraploid aortic SMCs. The order of effectiveness of drug treatment in lowering tetraploidy in SHR was captopril > hydralazine > propranolol. Only captopril was effective in reducing the frequency of tetraploid SMCs in WKY. Values are means ± SEM.
TABLE 4. Frequency of Tetraploid and Octaploid Smooth Muscle Cells and Smooth Muscle Cell Number and Volume* in Drug-Treated and Untreated (Control) SHR and WKY

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Tetraploid SMC (%)</th>
<th>Octaploid SMC (%)</th>
<th>SMC number (×10⁶)</th>
<th>Thoracic aorta cm Aorta</th>
<th>SMC volume (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>Control</td>
<td>14.7±0.8 (n = 7)</td>
<td>0.5±0.1 (n = 7)</td>
<td>4.83±0.39 (A)</td>
<td>1.85±0.17 (A, B)</td>
<td>907±59 (A, D)</td>
</tr>
<tr>
<td>SHR</td>
<td>Captopril</td>
<td>6.3±0.8 (A, B, C, D) (n = 7)</td>
<td>0 (A) (n = 7)</td>
<td>4.67±0.16 (A)</td>
<td>1.88±0.07 (A, B)</td>
<td>699±43 (B, E, F)</td>
</tr>
<tr>
<td>SHR</td>
<td>Hydralazine</td>
<td>8.3±0.9 (B) (n = 7)</td>
<td>0 (A) (n = 7)</td>
<td>4.72±0.25 (A)</td>
<td>1.76±0.08 (A, B)</td>
<td>867±87 (A, C, E)</td>
</tr>
<tr>
<td>SHR</td>
<td>Propranolol</td>
<td>10.6±1.2 (n = 8)</td>
<td>0.1±0.0 (n = 8)</td>
<td>4.63±0.21 (A)</td>
<td>1.75±0.07 (A, B)</td>
<td>961±43 (A)</td>
</tr>
<tr>
<td>WKY</td>
<td>Control</td>
<td>8.1±0.5 (A, B) (n = 5)</td>
<td>0 (A) (n = 5)</td>
<td>4.51±0.22 (A, B)</td>
<td>1.90±0.08 (A, B)</td>
<td>773±66 (B, C, E)</td>
</tr>
<tr>
<td>WKY</td>
<td>Captopril</td>
<td>5.3±0.6 (C) (n = 8)</td>
<td>0 (A) (n = 8)</td>
<td>4.41±0.14 (A, B)</td>
<td>1.93±0.07 (A)</td>
<td>567±41 (F)</td>
</tr>
<tr>
<td>WKY</td>
<td>Hydralazine</td>
<td>7.3±0.5 (A, B, C) (n = 7)</td>
<td>0 (A) (n = 7)</td>
<td>3.91±0.18 (B)</td>
<td>1.61±0.08 (B)</td>
<td>709±68 (B, C, F)</td>
</tr>
<tr>
<td>WKY</td>
<td>Propranolol</td>
<td>8.4±0.6 (A, B) (n = 7)</td>
<td>0 (A) (n = 7)</td>
<td>4.12±0.41 (A, B)</td>
<td>1.69±0.13 (A, B)</td>
<td>825±55 (A, B)</td>
</tr>
<tr>
<td>SHR</td>
<td>Pretreatment</td>
<td>4.7±1.5 (D) (n = 5)</td>
<td>0 (A) (n = 5)</td>
<td>4.38±0.27 (A, B)</td>
<td>1.94±0.08 (A, B)</td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>Pretreatment</td>
<td>5.0±1.1 (D) (n = 6)</td>
<td>0 (A) (n = 6)</td>
<td>3.92±0.27 (A, B)</td>
<td>1.79±0.10 (A, B)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Means with the same letter (in parentheses) are not significantly different (p > 0.05, analysis of variance, general linear models procedure, SAS Institute). SMC = smooth muscle cell.

*SMC volume was calculated as (smooth muscle content/aorta)/(SMC number/aorta).

†p = 0.07, ‡p = 0.06, compared with hydralazine-treated SHR.
§p = 0.12, compared with control SHR.

Discussion

Results of the present study provide several lines of evidence supporting a role for increased blood pressure or wall stress in the mediation of aortic SMC hypertrophy in the SHR. First, as observed in our previous studies, there was a close correlation between blood pressure levels and a variety of indices of hypertrophy, including aortic medial SMC content or mass and percentage of polyploid SMCs, even in animals treated with different antihypertensive drugs with different primary mechanisms of action. Second, the efficacy of drugs in preventing the development of medial hypertrophy and SMC polyploidism was the same as their efficacy in lowering blood pressure (i.e., captopril > hydralazine > propranolol). These results are consistent with our previous findings showing that normalization of blood pressure in SHR with a three-drug regimen consisting of reserpine, hydralazine, and chlorothiazide was extremely effective in preventing hypertrophic changes.

In addition, results of this study and previous studies show that increases in SMC polyploidism and medial hypertrophy are not present in prehypertensive SHR but occur predominantly after blood pressure has increased. In fact, most hypertrophic changes in the aorta occur after blood pressure has reached a plateau. Thus, there is considerable evidence, albeit indirect, that aortic medial hypertrophy represents an adaptive response to increased blood pressure or wall stress. Results are consistent with suggestions by Wolinsky that vascular wall mass is somehow regulated based on the level of vascular wall stress.

Several observations in the present study, however,
suggest that aortic medial hypertrophy is not simply a response to increased blood pressure or wall stress. First, at the doses used, captopril and hydralazine had similar blood pressure-lowering effects but captopril was much more effective in preventing increases in aortic SMC content, medial SMC weight, and percentage of polyplody SMCs. SMC volume was also reduced in captopril-treated SHR and WKY, while hydralazine had no significant effect. Second, propranolol lowered blood pressure by 26 mm Hg in SHR but had no effect on aortic medial hypertrophy or SMC volume. In fact, when propranolol results were normalized for the loss of body weight in this group, medial SMC content and SMC volume were significantly greater than untreated SHR. Third, analysis of covariance and two-stage regression analysis suggest that captopril was more effective in preventing the development of medial hypertrophy and SMC polyplodism than what is predicted by its blood pressure-lowering effect. Similar dissociation of blood pressure changes and aortic hypertension in SHR has been reported by Freslon and Giudicelli.30 These investigators treated SHR between 6 and 20 weeks of age with either captopril or hydralazine and examined changes in aortic weight or aortic wall/lumen ratios. They found that captopril was more effective than hydralazine in preventing aortic hypertrophy in SHR despite identical blood pressure-lowering effects of these two drugs. Similarly, Jespersen et al.31 found that hydralazine reduced blood pressure in SHR but was not effective in preventing structural alterations in mesenteric arteries.

Our results with the converting enzyme inhibitor captopril implicate a role for angiotensin II in the SMC hypertrophic response. Interestingly, captopril also significantly reduced SMC size and medial SMC content in WKY, suggesting that angiotensin II may play some role in the regulation of vascular wall mass in normotensive strains as well. Although the SHR are not considered to have a renin-dependent form of hypertension based on measurements of circulating renin,22 there is increased vascular renin activity in the SHR compared with the WKY,23,24 and the efficacy of captopril in reducing blood pressure in SHR is thought to be due to inhibition of local angiotensin II formation.4,25,26 Increased angiotensin II levels within the vascular wall could influence vascular growth, either by direct effects on vascular smooth muscle or indirectly by potentiation of sympathetic activity.14,22 Angiotensin II has been shown to induce increased protein synthesis in a variety of cell types, including adrenal cells19 and cardiac myocytes,20 and has been reported to increase growth rates and cell size in cultured SMCs grown in the presence of serum.29 We have recently reported30 that angiotensin II induces hypertrophy without cell proliferation in cultured rat aortic SMCs maintained in a defined serum-free medium. Increased catecholamine release secondary to angiotensin II-induced potentiation of sympathetic discharge may also play a role in SMC hypertrophy, although the rat aorta is poorly innervated by the sympathetic nervous system. Bevan and Tsuno31 have presented evidence supporting a role of the sympathetic nervous system in vascular SMC growth in hypertension, and Blaes and Boissel32 have demonstrated that epinephrine increased the growth rate of cultured vascular SMCs in the presence of serum, although it did not stimulate proliferation of quiescent cells maintained in platelet-poor plasma. Unfortunately, these investigators did not explore whether cellular hypertrophy occurred in epinephrine-treated cultures.

The idea that contractile agonists might play some direct role in mediating hypertrophic responses is intriguing, since it could explain how vascular smooth muscle alters its mass in response to changes in work load. It is of interest that vasoactive agonists and a number of polypeptide growth factors elicit several common signals in smooth muscle cells including increased phosphatidylinositol turnover, mobilization of Ca2+, and activation of protein kinase C.33-36 Furthermore, one potent mitogen for vascular SMCs, platelet-derived growth factor, has recently been shown to induce SMC contraction,37 while a number of contractile agonists have growth-promoting activity in smooth muscle38,39 as well as nonmuscle cells.35,36 Significantly, however, these contractile agonists do not initiate replication in quiescent cells that are growth-arrested using either serum starvation or plasma-derived serum,29,30,31 the two common methods for inducing quiescence in vitro.37,38 Results of this study and our previous studies5-8 demonstrate that aortic medial hypertrophy in SHR is due primarily to cellular hypertrophy rather than to hyperplasia and that reductions in medial SMCs with antihypertensive treatment were due to decreased cell size without detectable changes in cell number. It is thus interesting to speculate that contractile agonists may serve as partial growth factors for SMCs, initiating the increased cell mass associated with cell cycle progression but not cellular proliferation. In contrast, hyperplasia may occur upon exposure of SMCs to multiple growth factors, as occurs following deliberate vessel injury29 or following sudden onset of hypertension where there is evidence of endothelial injury or dysfunction.8 This hypothesis obviously remains to be tested, and at present, the cellular and molecular controls of hypertrophic versus hyperplastic SMC growth represent poorly understood areas of SMC biology.

Whereas our data and those of others showing that blood pressure changes can be dissociated from SMC hypertrophy clearly suggest that factors other than blood pressure per se are involved in the hypertrophic response of aortic SMCs, some caution must be exercised in deducing the specific mechanisms involved based on the differential effects of various pharmacological agents in vivo. This need for caution derives from the fact that blood pressure changes reflect the net effect of a large number of variables, including cardiac output, heart rate, peripheral resistance, plasma volume, and plasma ion concentrations, that could be influenced either directly or indirectly by the drugs used. Alternatively, specific drugs may have direct unknown effects on cellular growth. Thus, the relative
effectiveness of one drug versus another on aortic hypertrophy may be complicated by complex interactions of drug effects. For example, the reduced effectiveness of hydralazine as compared with captopril in preventing hypertrophic changes may be due to the increased sympathetic discharge characteristic of hydralazine-treated animals, which could counteract overall blood pressure-lowering effects.40 The failure of propranolol treatment to prevent SMC hypertrophy may be due to a reflex increase in vasoactive tone (peripheral resistance) evoked following drug-induced decreases in cardiac output.13, 40 The reduced weight gains that occurred in the propranolol-treated group may also be due to a reduction of myocardial hypertrophy. If anything, one might expect to see less medial hypertrophy in this group. However, aortic medial smooth muscle content (see Table 3) of propranolol-treated SHR was the same as that of untreated SHR, despite a lower body weight. Given the complexity of interpreting pharmacological intervention studies, identification of the specific cellular controls of SMC hypertrophy clearly will be difficult to elucidate in vivo, and development of an in vitro model for studying SMC hypertrophy would be extremely beneficial.

Finally, results of these studies suggest that reductions in blood pressure alone may not accurately reflect the efficacy of drugs in preventing pathological cardiovascular alterations. Although hydralazine and propranolol both significantly reduced blood pressure, they were not particularly effective in preventing aortic hypertrophy. As previously reported by Sen et al.16 and others41 hydralazine lowered blood pressure in SHR in the present study but did not prevent myocardial hypertrophy. Limas et al.41 found that short-term diuretic treatment of SHR between 21 and 28 weeks of age was effective in reversing aortic intimal changes despite having little effect on blood pressure. These observations suggest that factors in addition to blood pressure reduction should be considered in evaluating the beneficial effects of antihypertensive drug therapies.

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