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Effects of Aging and Antihypertensive Treatment on Aortic Internal Diameter in Spontaneously Hypertensive Rats

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Abstract—The effect of antihypertensive treatment on the development of large-artery remodeling in young animals has been widely studied, but reversal of established changes in older hypertensive animals has been largely ignored, although the latter represents a better paradigm for the human condition. We studied the effect of treatment with captopril plus hydrochlorothiazide, from 3 months onward, on geometry and wall stress of the thoracic aorta of adult (9 months, maturation) and old (15 months, senescence) spontaneously hypertensive rats; normotensive Wistar-Kyoto rats were used as controls. At 3 months of age, blood pressure, medial cross-sectional area, and internal diameter were higher in spontaneously hypertensive rats than in Wistar-Kyoto rats. In both strains, medial cross-sectional area and lumen diameter increased during maturation; there was little change with senescence. Changes in blood pressure were minor. Because medial hypertrophy failed to compensate for the wider lumen and higher intraluminal pressure in spontaneously hypertensive rats, medial stress was higher in these rats than in Wistar-Kyoto rats. Captopril plus hydrochlorothiazide rapidly lowered blood pressure and medial cross-sectional area. Despite a marked fall in blood pressure, the internal diameter of the thoracic aorta of treated animals was similar to that of untreated animals after 6 months of treatment and started to fall only after the animals had been treated for 1 year. Thus, under treatment with captopril plus hydrochlorothiazide, medial stress remained elevated, even after very-long-term treatment, because medial cross-sectional area was not adapted to internal diameter. We suggest that some changes in large-artery structure associated with hypertension and aging, such as the increase in diameter, take considerable time to regress after blood pressure is lowered, and this may explain why, despite treatment, wall stress remains elevated. (Hypertension. 1999;34:207-211.)

Key Words: medial stress • aorta • hypertension • rat • treatment • age

Hypertension is accompanied by increases in wall thickness and internal diameter of the large-caliber proximal arteries.1 The increase in wall thickness is thought to be an adaptive response allowing wall stress to remain constant despite the increase in intraluminal pressure and lumen diameter. Wall thickness is closely related to intraluminal pressure,2 it increases rapidly with increasing pressure in different hypertensive models, and it can be rapidly reversed by antihypertensive treatment.2–4 However, in various studies in normotensive and hypertensive animals, a drug-induced, long-term fall in blood pressure produced no significant change in aortic internal diameter.2–6

The structural increase in lumen diameter may be provoked by factors other than pressure, such as fracture of the elastin-retaining network under the long-term cumulative fatiguing effects of cyclic stress on the arterial wall.7 Thus, blood pressure alone may not be the major determinant in the severity of the effects of hypertension. Reversal of such changes may require a much longer period of treatment than those used in previous studies. If antihypertensive treatment does not lower internal diameter, then the wall thickness/internal diameter ratio may be too low for a given intraluminal pressure, and thus wall stress would remain elevated despite treatment.

The effect of short-term antihypertensive treatment on medial hypertrophy in young animals has been extensively studied, but the effect of long-term treatment on internal diameter in old hypertensive animals has received scarce attention. This should provide a better paradigm for the human situation, because hypertension is essentially a disease of the aged.

This study examined the effect of antihypertensive treatment with a combination of the angiotensin I–converting enzyme inhibitor captopril and the diuretic hydrochlorothiazide (CAP) on blood pressure, thoracic aorta lumen diameter, medial cross-sectional area, and stress in mature (9 months)
and old (15 months) spontaneously hypertensive rats (SHRs). The rationale for combined treatment with an angiotensin I–converting enzyme inhibitor and a thiazide diuretic is based on clinical observations showing that such a combination lowers blood pressure efficiently and may have a beneficial effect on aortic remodeling.

### Methods

The techniques used have been described in detail elsewhere. Animals and Long-term Drug Treatment

Three-month-old male SHRs (SHR/CON rats; body weight, 317±9 g; Iffa-Credo, L’Arbresle, France) and Wistar-Kyoto (WKY) rats (body weight, 308±2 g; Iffa-Credo) were given standard rat chow and water ad libitum. A third group of rats (SHR/CAP rats; body weight, 308±2 g; Iffa-Credo, L’Arbresle, France) and Wistar-Kyoto (WKY) rats (body weight, 301±6 g; Iffa-Credo) were given chow mixed with captopril (59 to 39 mg · kg⁻¹ · d⁻¹) and hydrochlorothiazide (30 to 19 mg · kg⁻¹ · d⁻¹) for 6 or 12 months. There were no deaths. In our laboratory, there is substantial mortality in untreated SHRs beyond 15 months such that there are no survivors at 18 months (unpublished results).

Systolic arterial blood pressure was recorded by the tail-cuff method before and 6 and 12 months after the onset of treatment. Experiments were performed in conformity with the recommendations of the European Union and Nancy University on animal welfare.

### Aortic Structure

Rats were infused for 30 minutes under pressure with 10% formal containing PBS (mmol/L: NaCl, 120; KCl, 2.7; and PBS, 10; pH 7.4 at 25°C) via a cannula implanted in the right common carotid artery under ether anesthesia. Pressure was fixed at a level corresponding to the central aortic mean pressure calculated from tail artery systolic pressure. The thoracic aorta was excised and immersed in 10% formol. A 1-cm specimen was dehydrated in graded ethanol solutions and embedded in paraffin. Three 20-μm-thick sections were cut and stained with Weigert’s reagent (for elastin) for determination of lumen diameter and medial thickness.

Morphometric analysis was performed with the Optilab algorithm. Each section was examined 3 times in a blinded manner. Medial cross-sectional area (mm²) was defined as the area between the internal and external elastic lamina. Medial stress was calculated as follows: (systolic arterial blood pressure · lumen radius)/medial thickness (dynes · cm⁻² · mm⁻¹).

The outward hypertrophic remodeling ratio was defined as the slope of the linear positive relationship between medial cross-sectional area (mm²) and lumen diameter (mm).

### Left Ventricular Mass

The heart was removed, and the left ventricle (plus septum) was dissected free and weighed. Because body weights were significantly different in some cases, left ventricular mass was expressed as left ventricular weight (g) divided by body weight (kg).

### Statistical Analysis

Results are mean±SE. ANOVA followed by the Bonferroni test was used for the comparison of means; P<0.05 was considered statistically significant. Interactions between different variables were also evaluated by multiple regression analysis.

### Results

#### Body Weight, Systolic Arterial Blood Pressure, Left Ventricular Mass, and Drug Intake

<table>
<thead>
<tr>
<th>Parameter and Months</th>
<th>WKY</th>
<th>SHR/CON</th>
<th>SHR/CAP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>3</td>
<td>307±4 (16)</td>
<td>317±13 (9)</td>
<td>323±18 (9)</td>
</tr>
<tr>
<td>Systolic arterial blood pressure, mm Hg</td>
<td>3</td>
<td>146±4</td>
<td>194±5</td>
<td>198±5</td>
</tr>
<tr>
<td>Left ventricular mass, g · kg⁻¹</td>
<td>3</td>
<td>1.14±0.07</td>
<td>1.21±0.06</td>
<td>1.20±0.06</td>
</tr>
</tbody>
</table>
| Drug consumption (CAP), mg · kg⁻¹ | 3     | 59±1/30±1 | 41±1/20±0.4 | 39±1/19±0.4 | *Numbers in parentheses are the number of animals in each group.*
Thoracic aorta lumen diameter (mm), medial cross-sectional area (mm²), and stress (dynes · cm⁻² · 10⁶) in WKY (●), SHR/CON (■), and SHR/CAP (▲) rats. For lumen diameter, $P_{\text{group}}<0.0001$, $P_{\text{age}}<0.0001$, and $P_{\text{group} \times \text{age}}=0.0937$ (ANOVA). For medial cross-sectional area, $P_{\text{group}}<0.0001$, $P_{\text{age}}<0.0001$, and $P_{\text{group} \times \text{age}}=0.0001$ (ANOVA). For calculated medial stress, $P_{\text{group}}<0.0001$, $P_{\text{age}}=0.0014$, and $P_{\text{group} \times \text{age}}=0.8000$ (ANOVA). For the number of rats in each group, see Table 1.

Medial cross-sectional area increased with maturation in all 3 groups (WKY, +41%; SHR/CON, +78%; SHR/CAP, +27%; all, $P<0.05$). With aging, there was an additional slight but significant increase in WKY rats (+13%, $P<0.05$) but not in SHR/CON rats (+11%, $P>0.05$) or SHR/CAP rats (−5%, $P>0.05$). CAP normalized medial cross-sectional area (−26% at 9 months; −37% at 15 months; both, $P<0.05$ versus SHR/CON rats).

Medial stress in SHRs was higher than in WKY rats by an average of 0.60 dynes · cm⁻² · 10⁶ in SHR/CON rats and 0.50 dynes · cm⁻² · 10⁶ in SHR/CAP rats (both, $P<0.05$). Maturation had no effect on medial stress, but aging produced a slight significant decrease (average, −0.24±0.20 dynes · cm⁻² · 10⁶; $P<0.05$). CAP had no effect on medial stress.

Remodeling and Medial Stress
All groups showed outward hypertrophic remodeling because medial cross-sectional area increased in parallel with the increase in lumen diameter. This occurred in a similar way in WKY and SHR/CON rats; in SHRs, CAP lowered the slope relating medial cross-sectional area to lumen diameter at 15 months (Table 2).

In all groups, calculated wall stress decreased as outward hypertrophic remodeling ratio increased. This occurred in a similar way in WKY and SHR/CON rats; in SHRs, CAP increased the slope relating medial stress to remodeling ratio at 15 months (Table 3).

Discussion
In Situ Fixation Under Pressure and Hypertrophic Remodeling
If the wall is stiffer in SHRs, then the internal diameter actually measured may not depend only on remodeling, and this may be more important at higher pressures. Mulvany et al.¹¹ state that “... the term ‘remodeling’ should be confined to situations in which there is a change in the lumen of a relaxed vessel, measured under a standard intravascular pressure, and where changes in the characteristics of the wall material (ie, wall stiffness) do not account for the change in...
vascular lumen." Thus, if the wall is stiffer in SHRs, then this may affect internal diameter. This effect may be more important at higher than at lower, or "relaxed," intravascular pressures if the slope relating wall stiffness to wall stress is steeper in SHRs. We hypothesize that as wall stress, defined as (pressure×radius)/thickness, is linearly related to pressure\(^2\) (if wall elasticity is related to wall stress in a similar manner in SHRs and WKY rats), then measuring internal diameter at different pressures in different groups should not induce an effect on diameter (via an effect of pressure on wall stiffness) beyond that to be expected from an effect of pressure on remodeling. Were there to be changes in the relative composition of the wall (amounts of collagen, elastin and smooth muscle) or in the way in which the wall elements are linked together, this might not be true.

In this article, remodeling is defined on the basis of changes in the ratio of medial cross-sectional area to lumen diameter. With structural dilation of the thoracic aorta, there was an increase in medial cross-sectional area. This implies that there was not simply a rearrangement of existing medial cells and extracellular matrix around a larger diameter but that as diameter increased, more cells and matrix were formed. Thus, to use the term suggested by Mulvany et al,\(^1\) outward hypertrophic remodeling occurred.

### Effect of Aging and Hypertension on Cardiovascular Remodeling

Aging in the absence of any marked increase in intraluminal pressure may induce an increase in internal diameter through the long-term cumulative fatiguing effects of cyclic stress on the arterial wall.\(^2\) In this study, such a phenomenon may have been occurring in normotensive WKY rats, in which internal diameter increased significantly with age (3 to 15 months, +16%; \(P<0.05\)) but blood pressure did not (+8%; \(P>0.05\)). Medial hypertrophy may then follow to keep wall stress constant. Multivariate regression analysis with the use of medial cross-sectional area as the dependent variable and blood pressure and lumen diameter as the independent variables revealed a significant effect of diameter (\(P<0.0001\)) but not pressure (\(P=0.1386\)). The increase in diameter cannot be the only factor involved, however, because medial cross-sectional area increased to values beyond those necessary to compensate for dilatation (slopes in Table 2 are positive and greater than unity). Thus, the ratio of medial cross-sectional area to internal diameter increased by 38% from 3 to 15 months (0.22±0.03 to 0.30±0.03 mm; \(P<0.05\)). This suggests that age has a supplementary effect on medial hypertrophy beyond that to be expected by age-linked dilatation.

Pressure seems to be less important than aging in relation to another aspect of cardiovascular remodeling in WKY rats, the increase in left ventricular mass. There was a 40% (\(P<0.05\)) increase in left ventricular mass from 3 to 15 months, once again in the absence of any significant increase in blood pressure (see above). Blood pressure was not a significant determinant (\(P=0.1832\)) of left ventricular mass in multivariate analysis (independent variables were pressure and age).

Hypertension in the absence of aging can be studied in young (3-month-old) SHRs. In young SHRs, blood pressure was substantially increased (+33% versus age-matched WKY rats; \(P<0.05\)), yet internal diameter was only moderately increased (+10%; \(P<0.05\)), and the ratio of medial cross-sectional area to internal diameter was not significantly higher (+6%; \(P>0.05\)). This suggests that pressure is not an important determinant of large-artery remodeling or that the effects of pressure take time to develop. Thus, there may be a significant interaction between hypertension and aging. In our data, there is evidence for and against this. In multivariate analysis, blood pressure is a significant determinant of lumen diameter at 9 months (\(P=0.0520\) but not at 3 (\(P=0.281\)) or 15 months (\(P=0.9141\)). Blood pressure did increase significantly from 3 to 15 months. The increase was small (+11%), whereas a substantial increase occurred in the medial cross-sectional area/internal diameter ratio (+60% from 0.23±0.03 to 0.37±0.03 mm; \(P<0.05\)). Aging appears to have a proportionally greater effect than blood pressure.

In SHRs, remodeling did not compensate for increased pressure because wall stress was higher than in WKY rats at all ages. This suggests that active force could be important in the "protection" of the aorta against high blood pressure. Another possibility is that wall composition changes. Results on the change in the elastin/collagen ratio with hypertension are conflicting, with some studies showing an increase\(^1\) and others a decrease.\(^2\) Studies in old hypertensive models are lacking. Changes in other wall components or in cell-matrix attachments may also be important.

CAP treatment reduced medial cross-sectional area. This is not an original observation. It is interesting to note, however, that thoracic aorta remodeling (Table 2) and the relationship between wall stress and remodeling (Table 3) were different in 15-month-old SHR/CAP rats after 1 year of CAP treatment and SHR/CON rats. The medial cross-sectional area/internal diameter ratio was lower in SHR/CAP rats (0.26±0.03 mm) than in SHR/CON rats (0.37±0.03 mm; \(P<0.05\)). Thus, CAP lowered medial cross-sectional area but not wall stress. This suggests that some change in active force, wall composition, or cell-matrix attachments occurred because this combination of drugs appears to lower arterial stiffness.\(^9\) Here again,

### TABLE 3. Medial Stress (dynes cm\(^{-2}\) 10\(^{6}\)) Versus Remodeling (mm) in the Thoracic Aorta of WKY, SHR/CON, and SHR/CAP Groups

<table>
<thead>
<tr>
<th>Group and Month</th>
<th>Origin, dynes cm(^{-2}) 10(^{6})</th>
<th>Slope, dynes cm(^{-2}) 10(^{6}) \cdot mm(^{-1})</th>
<th>(P)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY 3</td>
<td>4.14±0.37</td>
<td>−10.36±1.70</td>
<td>&lt;0.0001</td>
<td>16</td>
</tr>
<tr>
<td>9</td>
<td>3.89±1.23</td>
<td>−7.83±1.80</td>
<td>0.0141</td>
<td>10</td>
</tr>
<tr>
<td>15</td>
<td>2.40±0.06</td>
<td>−3.74±1.02</td>
<td>0.0274</td>
<td>16</td>
</tr>
<tr>
<td>SHR/CON 3</td>
<td>3.38±0.65</td>
<td>−3.66±0.79</td>
<td>0.0226</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>4.80±0.70</td>
<td>−6.73±2.12</td>
<td>0.0130</td>
<td>10</td>
</tr>
<tr>
<td>15</td>
<td>3.26±1.34</td>
<td>−6.34±1.62</td>
<td>0.0451</td>
<td>14</td>
</tr>
<tr>
<td>SHR/CAP 3</td>
<td>2.84±2.10</td>
<td>−6.94±1.70</td>
<td>0.0507</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>3.56±0.46</td>
<td>−4.89±1.89</td>
<td>0.0290</td>
<td>11</td>
</tr>
<tr>
<td>15</td>
<td>6.39±0.88</td>
<td>−16.44±3.42</td>
<td>&lt;0.0001</td>
<td>18</td>
</tr>
</tbody>
</table>
previous results are similar, with some showing that antihypertensive treatment reduces wall collagen content\(^3\) and others showing no effect.\(^2,5\) Some authors\(^14\) describe a “protective” effect of angiotensin I–converting enzyme inhibitors on elastin, whereas others\(^2\) do not find this effect.

**Animal Model of Human Aortic Remodeling**

In WKY rats and SHRs, the main changes in aortic inner diameter and medial cross-sectional area occurred during maturation, with less change occurring with aging. This is not the case in humans, in whom aortic diameter increases linearly with age up to the eighth decade.\(^15\) The increase in diameter is associated with extensive calcification,\(^15\) which, although it occurs in WKY rats and SHRs, is generally far less marked in animal models than in humans.\(^16\) Thus, in humans, the process of elastocalcinosis (accumulation of calcium on medial elastin fibers followed by breakdown of the elastin fiber–retaining network) may be more important than in the WKY or SHR model, and degradation of the aortic media may play a more important role in structural dilatation with age.

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**References**


