Effects of Chronic Inhibition of Converting Enzyme on Mechanical and Structural Properties of Arteries in Rat Renovascular Hypertension

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The effect of hypertension and of therapy by converting enzyme inhibitor (S 9490-3, perindopril) on the function and structure of large arteries has been studied in two-kidney, one-clip Goldblatt hypertensive rats. After one month without treatment, clipped hypertensive rats (n = 24) and sham-operated rats (n = 24) were randomly allocated to treatment by S 9490, 1 mg/kg once a day (n = 24) or to placebo (n = 24) and pursued for 4 weeks. Hemodynamic parameters, including instantaneous pressure and aortic velocity measured by Doppler, were recorded under anesthesia at the end of the treatment period. Passive mechanical properties of carotid arteries were recorded in situ in the presence or the absence of smooth muscle cell activity (potassium cyanide poisoning). Morphological parameters of the aortic media, including medial thickness, nucleus density, and cross sectional area and relative density in proteins of interstitial matrix, were recorded by an automated morphometrical system. Hypertension was associated with an increase in characteristic impedance of the aorta and a decrease in compliance of the arterial system. Treatment with converting enzyme inhibitors completely reversed these in vivo markers of the rigidity of large arteries. Hypertension was associated with a shift of the passive pressure-volume relation in the carotid. Treatment with converting enzyme inhibitors normalized the carotid pressure-volume relation, whereas poisoning smooth muscle cells induced a disappearance of the curve differences between hypertensive and normotensive animals. Morphometric analysis of aortic walls permits us to report this functional change to structural modification of the arterial wall. Aortic media thickness was increased by hypertension; this phenomenon was reversed by treatment. Modification of aortic thickness was due to hypertrophy of smooth muscle cells with parallel modifications of absolute amount of collagen, whereas absolute amount of elastin did not change in this early phase of renovascular hypertension in young rats. Treatment with converting enzyme inhibitors reversed the thickness of aortic media without regression of the increase in absolute amount of collagen content whereas absolute amount of elastin content did not change.

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Chronic hypertension in both experimental animals1–3 and humans4–5 is characterized by an increase in systemic resistances due in part to an increase in arteriolar and arterial wall thickness and stiffness. The major contributors to the thickening of the arterial wall are the increase in smooth muscle cell mass6,7 and the increase in absolute amount in proteins of the interstitial matrix8 in the media of large arteries. These morphological modifications are associated with changes in arterial wall mechanical properties.1,9–11 There is general agreement that an increase in the stiffness of the arterial wall occurs in association with the development of sustained hypertension. From these data two questions arise: the first concerns the respective role of smooth muscle and proteins of the extracellular matrix in the modification of rigidity of arterial vessel walls during hypertension; the second concerns the reversal of these mechanical and structural alterations by appropriate therapy. Several authors reported that elastin and collagen play a predominant role in these modifications of stiffness and that the reversal of hypertension is not associated with a corresponding reduction of the increased arterial wall thickness12,13 and arterial rigidity.8
These results are difficult to interpret because of the differences in experimental models (e.g., Goldblatt, SHR, and DOCA-salt) and in antihypertensive therapy (e.g., β-blockers, ganethidine, and hydralazine). The recent development of converting enzyme inhibitors (CEI) allows us to use an antihypertensive drug that has specific actions that correct renal hypertension in two-kidney, one-clip Goldblatt rats. 

Thus, using hemodynamic and morphometric methodologies, an experimental design was performed to test the effect of converting enzyme inhibition on mechanical properties and structural parameters of the walls of large arteries in renovascular hypertensive rats.

Materials and Methods

Biological Model

Six-week-old male white Wistar rats weighing 130 ± 8 g were used in this study. Hypertension was produced by clipping the right renal artery (0.2 mm) under ether anesthesia. The left kidney was not disturbed. Thirty-five rats were clipped and 24 others were sham-operated. The animals were returned to their cages and fed a standard rat diet. Water was provided ad libitum. From the 1st to the 4th week after clipping the renal artery, systolic blood pressure (SBP) was measured every 2 weeks using a tail cuff method (BP recorder 8005, W + W electronic, Varese, Italy). The conscious rats were heated, with the cuff and the pulse wave transducer set around the tail about 10 minutes before SBP measurements. Body weight was measured weekly. Eleven rats were eliminated from the study because they either failed to develop hypertension (SBP<150 mm Hg) or they developed malignant hypertension (SBP>250 mm Hg, along with a major loss in body weight).

Four weeks after clipping, hypertensive and sham-operated rats were randomly divided into two groups: the CEI-treated group or the untreated group. CEI (S 9490-3, perindopril; Institut de Recherches Internationales Servier) was administered daily by gavage at a dose of 1 mg/kg in 1 ml of distilled water over a 4-week period. This experimental drug is a specific angiotensin CEI with a half-life longer than 24 hours. The untreated rats received 1 ml of distilled water daily during the same period.

Four groups of 12 rats were thus studied: normotensive control (NC, n = 12), normotensive treated (NT, n = 12), hypertensive control (HC, n = 12), and hypertensive treated (HT, n = 12).

At the end of the treatment period, that is, 8 weeks after clipping, rats were used for hemodynamic study under pentobarbital anesthesia and were then killed for morphological study.

Hemodynamic Study

The basic surgical preparation and hemodynamic measurements have been described elsewhere and are presented here in brief. After induction of anesthesia with intraperitoneal pentobarbital (1 mg/kg), the trachea was cannulated and then connected to a rodent respirator (model 680, Harvard Apparatus, South Natick, Massachusetts). A Teflon catheter (i.d. 0.7 mm) connected to a Statham P23 ID pressure transducer (Gould, Cleveland, Ohio) was introduced into the right carotid artery and positioned into the ascending aorta. The catheter manometer system was checked with both time and frequency domain measurements and showed a flat response beyond 40 Hz. A midsternal thoracotomy was performed and the ascending aorta isolated, around which an adapted ultrasonic Doppler flow probe was placed for measurement of mean (cardiac output minus coronary blood flow) and phasic instantaneous aortic blood flow. Ascending aortic blood flow and pressure were simultaneously recorded and analyzed by a microcomputer system coupled with an analog/digital converter (Apple IIe, Cupertino, California, and A2D, San Francisco, California). At least 10 minutes after surgical preparation, when hemodynamics were stable, pressure and flow curves were recorded and digitized (500 Hz). Hemodynamic parameters were calculated during periods of 30 seconds and then averaged to provide hemodynamic values.

Basic study parameters were the systolic (SBP), diastolic (DBP), and mean (MBP) arterial blood pressure; cardiac output; and heart rate. Total peripheral resistance (TPR) was determined as the quotient of MBP and cardiac output. Fourier analysis was performed from aortic pressure and flow waves; the characteristic impedance was computed as the averaged value of the modulus of impedance for high frequencies (from the fourth to the tenth harmonic).

The systemic arterial compliance (SAC) was computed from a simple elastic model that discharges during diastole into a single resistance representing the TPR. Such a model has two fundamental characteristics: 1) it discharges monoexponentially as a function of time and 2) the time constant (t0) of the system, that is, the reciprocal of the exponential slope discharge, represents the product of the capacitance and the resistance. Thus, SAC is calculated as aortic compliance according to the formula SAC = t0/TPR. Validation of the model requires verification of the monoexponential form of the pressure decay during diastole and demonstration of a proportional through zero between the diastolic decay t0 and the TPR.

Measurements of Static Mechanical Properties of Carotid Artery

After recording of the hemodynamic parameters, the left carotid artery was catheterized with a 80-cm long nylon tube (i.d. 0.6 mm) filled with an Evans blue saline solution. The tube was connected to a manometer pressurized at adjustable pressure values. A three-way tap was connected between the
FIGURE 1. Schematic representation of the experimental hemodynamic system, permitting us to record instantaneous pressure and flow curves in the ascending aorta, measure in situ pressure-volume curve in the carotid artery, and remove the thoracic descending aorta for morphometric analysis.

manometer and the nylon tube, permitting a part of the tube to be filled in order to observe the position of the meniscus. The root of the left carotid artery was dissected, and a removable clamp was positioned at the junction of the aortic cross and the carotid artery. Finally, the lower part of the thoracic aorta was clamped. This preparation allowed us to exclude, in situ, about 18 mm of nonexposed carotid artery (Figure 1).

To start the measurement, the segment of isolated artery was submitted to atmospheric pressure for 5 minutes, and the position of the meniscus, representing changes in the contained volume within the artery, was noted. The artery was then submitted to a pressure increase of 50 mm Hg. The movement of the meniscus was followed and noted every 10 seconds during 5 minutes. During the first 30–45 seconds, the inflow was rapid and then became linear with time. The initial increase in volume with pressure would be expected to result from mechanical creep of the tissue and relaxation of vascular smooth muscle. The later linear inflow within the carotid artery after this initial increase in arterial volume could be attributed to the fluid filtration through the vascular wall. An estimate of the initial increase in volume was obtained by extrapolating the linear portion of the inflow curve to the time when the pressure was established (Figure 2).

Carotid artery volume increases were recorded from 50 to 250 mm Hg by steps of 50 mm Hg. The pressure was maintained at each level for 5 minutes. The static compliance of the isolated segment of artery (carotid compliance) was calculated for each level of pressure as the quotient of the volume increase and the pressure step imposed (50 mm Hg). The clamp on the carotid excluding the root of the left carotid artery was then removed, and the artery was washed and filled with a saline solution of potassium cyanide (100 mg/liter). The extra fluid was prelevated from the right carotid artery, and the descending thoracic aorta was not submitted to potassium cyanide solution. The potassium cyanide solution was maintained in the carotid artery for 30 minutes, a period sufficient to poison the vascular smooth muscle. After isolating the same segment of carotid as used previously by clamping the root again, the measurement of the pressure-volume relation was performed in the potassium cyanide-treated vessel.

Morphological Study

At the end of the experiment, 2–3 cm of descending thoracic aorta were removed and fixed in Dubosq Brazil solution. After dehydration the aorta was longitudinally embedded in paraffin. Three successive sagittal sections of 5-μm thickness were treated by specific staining to obtain a monochromatic color associated with the various structures studied in the aortic media. Sirius red was used for collagen fiber staining, orcein for elastin, and hematoxylin after periodic acid oxidation for nucleus staining. Slides were submitted to an automatic image analysis processor (NS 1500, Nachet-Vision, Paris) based upon morphological mathematic principles. Different algorithms were developed to analyze each of the three structures shown by the staining in each of the three successive sections. The first algorithm analyzed the mean media thickness by measurements of the distance between the internal and external elastic lamina (70 measurements in each section). Medial elastin network was analyzed in terms of relative area and mean thickness of elastin lamella and lamina; the measurements and calcula-
tions were made in 10 fields in each section. The second algorithm analyzed the collagen matrix by measurement of relative area density and mean thickness of collagen fibers in 20 contiguous fields in each sirius red stained section. The third algorithm counted the number of nuclei within 20 fields of 7,442 μm² area of measurement in each section and measured the mean area of each nucleus. Repetitive measurements were performed, pooled, and averaged for the three algorithms in the corresponding stained sections of the aortic wall media of each animal.

**Statistical Analysis**

Results are expressed as mean±SD. The experimental design allowed us to use a two-way nested analysis of variance to provide evidence of differences relating to experimental models and/or treatment and interaction. The differences between groups were evaluated using the Newman-Keuls test. Analysis of covariance was performed to test the influence of the different experimental conditions on the passive pressure-volume relation in the carotid.

**Results**

**Hemodynamic**

Clipping of the renal artery induced a significant hypertension in rats. This hypertension did not significantly differ between HC and HT group before the beginning of treatment. Treatment by CEI decreased blood pressure and completely normalized blood pressure in the HT group during the 4 weeks of treatment (Table 1).

Although body weight was the same in each experimental group, there was a significant increase in the ratio of left ventricular weight to body weight in HC animals (p<0.001) and treatment by CEI decreased this ratio significantly (p<0.001). The left ventricular hypertrophy was particularly marked in HC group as compared with NC group (p<0.001).

**Table 1.** General Parameters Recorded In Four Experimental Groups Before (4 Weeks) and During Treatment With Converting Enzyme Inhibitor

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive (N)</th>
<th>Hypertensive (H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, 4 weeks (g)</td>
<td>Untreated (C)</td>
<td>286±18</td>
</tr>
<tr>
<td></td>
<td>Treated (T)</td>
<td>269±28</td>
</tr>
<tr>
<td>Before Treatment (T)</td>
<td>296±19</td>
<td>280±15</td>
</tr>
<tr>
<td>SBP, 4 weeks (mm Hg)</td>
<td>Untreated (C)</td>
<td>147±8</td>
</tr>
<tr>
<td></td>
<td>Treated (T)</td>
<td>192±32</td>
</tr>
<tr>
<td>Before Treatment (T)</td>
<td>149±6</td>
<td>210±40</td>
</tr>
<tr>
<td>BW, 8 weeks (g)</td>
<td>Untreated (C)</td>
<td>348±31</td>
</tr>
<tr>
<td></td>
<td>Treated (T)</td>
<td>355±33</td>
</tr>
<tr>
<td>SBP, 8 weeks (mm Hg)</td>
<td>Untreated (C)</td>
<td>149±11</td>
</tr>
<tr>
<td></td>
<td>Treated (T)</td>
<td>194±35</td>
</tr>
<tr>
<td>LVW/BW, 8 weeks</td>
<td>Untreated (C)</td>
<td>130±17</td>
</tr>
<tr>
<td></td>
<td>Treated (T)</td>
<td>136±18</td>
</tr>
<tr>
<td></td>
<td>190±0.08</td>
<td>2.35±0.35</td>
</tr>
<tr>
<td></td>
<td>1.76±0.13</td>
<td>1.93±0.21</td>
</tr>
</tbody>
</table>

NC, normotensive control; HC, hypertensive control; NT, normotensive treated; HT, hypertensive treated; BW, body weight; SBP, systolic blood pressure (tail cuff method); LVW, left ventricular weight.

*p<0.05
**p<0.01
***p<0.001
NS, not significant.

**Table 2.** Hemodynamic Parameters Obtained at 8 Weeks in Four Experimental Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive (N)</th>
<th>Hypertensive (H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>Untreated (C)</td>
<td>113±10</td>
</tr>
<tr>
<td></td>
<td>Treated (T)</td>
<td>103±18</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>Untreated (C)</td>
<td>81±12</td>
</tr>
<tr>
<td></td>
<td>Treated (T)</td>
<td>69±16</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>Untreated (C)</td>
<td>94±11</td>
</tr>
<tr>
<td></td>
<td>Treated (T)</td>
<td>81±16</td>
</tr>
<tr>
<td>Differential BP (mm Hg)</td>
<td>Untreated (C)</td>
<td>32±5</td>
</tr>
<tr>
<td></td>
<td>Treated (T)</td>
<td>34±8</td>
</tr>
<tr>
<td>CO (ml/min)</td>
<td>Untreated (C)</td>
<td>46±13</td>
</tr>
<tr>
<td></td>
<td>Treated (T)</td>
<td>419±43</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>Untreated (C)</td>
<td>54±13</td>
</tr>
<tr>
<td></td>
<td>Treated (T)</td>
<td>54±14</td>
</tr>
<tr>
<td>TPR (dyne · sec · cm⁻¹)</td>
<td>Untreated (C)</td>
<td>17,865±5,870</td>
</tr>
<tr>
<td></td>
<td>Treated (T)</td>
<td>13,290±5,718</td>
</tr>
<tr>
<td>Zc (dyne · sec · cm⁻¹)</td>
<td>Untreated (C)</td>
<td>9,022±4,071</td>
</tr>
<tr>
<td></td>
<td>Treated (T)</td>
<td>11,854±6,351</td>
</tr>
<tr>
<td>SAC (10⁻³ ml/mm Hg)</td>
<td>Untreated (C)</td>
<td>3.92±1.15</td>
</tr>
<tr>
<td></td>
<td>Treated (T)</td>
<td>4.76±1.33</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; BP, blood pressure; CO, cardiac output; HR, heart rate; TPR, total peripheral resistance; Zc, characteristic impedance; SAC, systemic arterial compliance.

*p<0.05
**p<0.01
***p<0.001
NS, not significant.
and treatment by CEI completely normalized left ventricular wt/body wt ratio in HT group as compared with NC group (HT versus NC, not significant; HT versus HC, \(p<0.001\)) (Table 1).

The basic hemodynamic values measured in the four experimental groups are reported in Table 2. Systolic and diastolic aortic pressure was significantly higher in the HC group than in the NC group (\(p<0.001\)). Treatment by CEI decreased both systolic and diastolic blood pressure; there was no significant difference in the pulse pressure.

Hypertension did not significantly change cardiac output as compared with the normotensive group. In contrast, treatment with CEI significantly increased cardiac output (\(p<0.05\)). There was a significant increase in cardiac output in HT and NT groups as compared with the HC group (\(p<0.01\)). Heart rate was unchanged by hypertension or by treatment. TPR was higher in the HC group (\(p<0.01\)), and treatment by CEI decreased TPR in a significant manner (\(p<0.001\)) in normotensive and hypertensive groups.

Characteristic impedance was increased by hypertension (\(p<0.05\)) and treatment by CEI decreased characteristic impedance in the HT as compared with the HC group (\(p<0.05\)). In the same way, SAC was decreased in the HC animals, and treatment by CEI increased SAC. This effect was particularly significant in the HT group as compared with the HC group (\(p<0.01\)).

**Mechanical Properties of Carotid Artery**

Figure 3 shows the volume curves of in situ isolated carotid, measured for different imposed pressures from 0 to 250 mm Hg, in the four experimental groups before and after poisoning of smooth muscle cells by potassium cyanide. The slopes of these curves represent the carotid compliance for different pressures. Analysis of covariance demonstrated a general effect of experimental conditions on the relation between volume and pressure in basal condition (\(F=12.5\), \(p<0.001\)). Poisoning of the smooth muscle cells by potassium cyanide induced an upper shift to higher volume and higher compliance at the same pressure levels than in basal conditions.

In basal conditions, carotid artery pressure volume relations were significantly different in hypertensive and normotensive control groups (\(p<0.01\)). The largest carotid compliance was observed at 100 mm Hg where hypertension significantly reduced carotid compliance as compared to normotensive untreated group (Table 3) (\(p<0.001\)). Poisoning of the smooth muscle cells by potassium cyanide similarly increased the compliance in both NC and HC, which remained significantly different (\(p<0.05\)).

Treatment with CEI induced a significant increase in carotid compliance in both hypertensive and normotensive rats (\(p<0.05\)). Particularly, treatment with CEI normalized carotid compliance in the HT group as compared with the NC group. Potassium cyanide treatment did not induce a supplementary effect in the HT group, whereas it induced an increase in carotid distensibility in all other groups, including the NT group.

**Morphometry**

Figure 4 shows a typical aspect of morphometric analysis of the aortic media in the different experimental conditions after staining of elastin lamellas and cell nucleus.

Aortic media thickness was significantly increased in hypertensive group(s) (\(F=5.80\), \(p<0.02\)) (Table 4). The thickness of aortic media was increased by 25% in the HC versus the NC group (\(p<0.001\)). Treatment with CEI decreased aortic media thickness (\(F=4.25\), \(p<0.05\)). This effect was particularly significant in the HT group where treatment completely reversed hypertrophy of the media as compared with the NC group. Analysis of covariance demonstrated no difference in the slope of the
relation between medial thickness and SBP measured during the treatment period in hypertensive and normotensive animals. Thus, these results show a general intergroup positive correlation between aortic media thickness and SBP ($y = 0.42x + 41$; $r = 0.64, F = 36, p < 0.001$) (Figure 5).

In parallel, the relative density of elastin was significantly reduced in the aortic wall by hypertension ($p < 0.01$). Treatment with CEI had no significant effect on elastin density, but there was a general negative correlation between the relative amount of elastin and the media thickness ($r = -0.38$, $p < 0.05$). Neither hypertension nor treatment modified the mean thickness of elastin fiber or the number of elastin lamellae.

Conversely, hypertension induced a significant increase in the relative density of collagen (Figure 6) ($F = 6.84, p < 0.02$); treatment by CEI did not
reverse the increase in collagen density ($F = 1.09$, not significant). The mean thickness of the collagen fibers was significantly increased in hypertensive groups ($F = 6.77$, $p < 0.02$) and was not reversed by treatment ($F = 0.45$, not significant).

Morphometric measurements of stained nuclei (Figure 7) by periodic acid oxidation did not show any effect neither of hypertension nor treatment on the number of nuclei per unit area ($F = 0.66$, and $F = 2.31$, not significant, respectively). The hypertensive group had significantly larger nucleus cross sectional area than the normotensive one ($F = 6.45$, $p < 0.02$). Treatment did not induce any significant effect on the size of the nucleus ($F = 3.19$, not significant). Moreover, the mean nucleus area was positively correlated with the thickness of media ($r = 0.58$, $F = 24$, $p < 0.001$) and with SBP ($r = 0.53$, $F = 18.3$, $p < 0.01$).

These data showed that in the present experimental design, the increase in medial thickness was predominantly due to the hypertrophy of smooth muscle cells in hypertensive rats.

**Discussion**

Functional and morphological consequences of hypertension and its treatment are heterogeneous depending on both the experimental model and the class of drugs used. In the renovascular model, it has been shown that hypertension in association with the activation of the renin-angiotensin system is associated with significant modification of function and morphology of the heart and of the vessels. Converting enzyme inhibition could completely normalize blood pressure and block the angiotensin II production without activation of the sympathetic nervous system. Our results confirm that converting enzyme inhibition completely normalizes blood pressure in experimental two-kidney-one-clip renovascular hypertension and reverses the cardiac hypertrophy.

The effects of renovascular hypertension and its treatment with CEI on the function and the structure of the arterial walls have not been extensively studied in relation to their actions on the heart. In this model, hypertension is associated with a significant increase in the thickness of arterioles, which could change their resistance. Renovascular hypertension also induces significant changes in function and structure of large vessel walls as shown by Wolinsky and demonstrated in the present study.

**Mechanical Properties of Arteries**

In the present study, hypertension seems to be secondary to the increase in peripheral resistance, but the hemodynamic parameters corresponding to the function of the arterial wall in large arteries are also modified: characteristic impedance of the aorta was increased and systemic arterial compliance was decreased leading to a proportional increase in both systolic and diastolic blood pressure in this model. This associated effect of clipping one renal artery on the function of large arteries could be due to dif-

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**TABLE 4. Morphometric Parameters Measured in Media of Aortic Wall Obtained by Automated Image Analysis After Staining by Orceine, Sirius Red and Periodic Acid Oxidation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive (N)</th>
<th>Hypertensive (H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media thickness (µm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (C)</td>
<td>$97.5 \pm 4.4$</td>
<td>$122.3 \pm 3.7$</td>
</tr>
<tr>
<td>Treated (T)</td>
<td>$90.7 \pm 2.7$</td>
<td>$103 \pm 4.8$</td>
</tr>
<tr>
<td>Elastin density (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (C)</td>
<td>$38.6 \pm 12.8$</td>
<td>$31.9 \pm 8.3$</td>
</tr>
<tr>
<td>Treated (T)</td>
<td>$42.5 \pm 9.2$</td>
<td>$34.9 \pm 5.7$</td>
</tr>
<tr>
<td>Thickness of elastin fiber (µm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (C)</td>
<td>$3.21 \pm 0.40$</td>
<td>$3.25 \pm 0.26$</td>
</tr>
<tr>
<td>Treated (T)</td>
<td>$3.38 \pm 0.42$</td>
<td>$3.15 \pm 0.16$</td>
</tr>
<tr>
<td>Collagen density (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (C)</td>
<td>$7.3 \pm 2.4$</td>
<td>$8.6 \pm 3.6$</td>
</tr>
<tr>
<td>Treated (T)</td>
<td>$7.0 \pm 2.14$</td>
<td>$10.6 \pm 2.8$</td>
</tr>
<tr>
<td>Thickness of collagen fiber (µm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (C)</td>
<td>$1.29 \pm 0.04$</td>
<td>$1.38 \pm 0.12$</td>
</tr>
<tr>
<td>Treated (T)</td>
<td>$1.30 \pm 0.04$</td>
<td>$1.37 \pm 0.07$</td>
</tr>
<tr>
<td>Nucleus density (numbers/field)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (C)</td>
<td>$38.4 \pm 4.2$</td>
<td>$35.4 \pm 5.5$</td>
</tr>
<tr>
<td>Treated (T)</td>
<td>$40.7 \pm 5.2$</td>
<td>$39.3 \pm 5.3$</td>
</tr>
<tr>
<td>Nucleus cross-sectional area (µm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (C)</td>
<td>$7.38 \pm 0.78$</td>
<td>$8.58 \pm 1.54$</td>
</tr>
<tr>
<td>Treated (T)</td>
<td>$7.55 \pm 0.74$</td>
<td>$7.47 \pm 0.94$</td>
</tr>
</tbody>
</table>

*p < 0.05  **p < 0.01  ***p < 0.001  NS, not significant.

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**FIGURE 5. Positive intergroup correlation between medial thickness and blood pressure.**

These data showed that in the present experimental design, the increase in medial thickness was predominantly due to the hypertrophy of smooth muscle cells in hypertensive rats.
ferent phenomena: 1) Angiotensin II could act directly on the function of smooth muscle cells leading to an increase in active tension. Stone and Dujardin\textsuperscript{33} have shown that in an acute situation an increase in arterial stiffness could be related to the activation of smooth muscle cell contraction in aortic wall independent of the arterial blood pressure. Moreover, this effect was probably due to the activation of the vascular sympathetic nervous system and was reversed by a blocking agent.\textsuperscript{34} In renovascular hypertension, the high level of circulating angiotensin II could act on large arteries possibly by an action on endothelium\textsuperscript{35} and by direct action on smooth muscle cells in the media. Thus, angiotensin II could increase the stiffness of the arterial wall by a direct or indirect effect on the activation of smooth muscle cell contraction. 2) High blood pressure directly reduced the arterial
compliance in relation to the higher strain of the arterial wall. However, the passive distensibility of the isolated carotid artery measured at the same immediate level of pressure in absence of angiotensin evidenced an increase in stiffness of the arterial wall. Furthermore, our result concerning the passive mechanical distensibility of carotid artery demonstrated that the effect of renovascular hypertension on compliance was unrelated to the immediate level of pressure. At least, the structural changes of the wall of large arteries induced by hypertension could have acted on the function of the media. The structural increase in wall stiffness could be due to an increase in the protein content of the extracellular matrix of the arterial wall and/or to an increase in smooth muscle mass.

Chronic blockade of the renin-angiotensin system by converting enzyme inhibition decreases periph-

**Figure 7.** Examples of morphometric analysis of smooth muscle cell nucleus density and cross sectional area of nucleus in the aortic wall of the four experimental groups (periodic acid oxidation staining) (x40). NC, normotensive control; HC, hypertensive control; NT, normotensive treated; HT, hypertensive treated.
eral resistance but also normalizes characteristic impedance of the aorta and SAC. These data demonstrate that chronic treatment by CEI acts also on the mechanical properties of the wall of large arteries and particularly improves the "Windkessel" function of large arteries. Similar results have already been reported in man with CEI in essential hypertension. Renovascular hypertension is a model where this effect of CEI is particularly powerful in relation to the decrease in the level of angiotensin II production and to the decrease in blood pressure in the absence of activation of the sympathetic nervous system. The improvement in the compliant function of large arteries could have some beneficial effect on the afterload for the left ventricle and on the peripheral blood flow during diastole. Moreover, in the present work the effects of treatment on systemic arterial compliance are predominant in the hypertensive group but probably also exist in normotensive animals.

Notably, in our study the effect of CEI on peripheral resistances and on function of large arteries led to an improvement of cardiac output without significant changes in heart rate. The mechanical properties of the large arteries have been evaluated by three independent methods. The characteristic impedance was calculated from frequency analysis of aortic pressure and flow; characteristic impedance is mainly related to the elastic modulus of the wall of the ascending aorta where the pressure and flow were recorded. SAC was calculated from the late diastolic decay of the aortic pressure and from the calculated systemic resistances; SAC represents an equivalent value of the whole arterial compliance. Both characteristic impedance and SAC need a simplified hypothesis to be calculated. In contrast, the direct measurement of the carotid distensibility provides information about the mechanical behavior of the carotid wall in control conditions and after poisoning of the vascular smooth muscle for well-defined pressure steps. The carotid arteries were studied in situ, nonisolated, nonexposed and maintained to their physiological longitudinal stress. These experimental conditions allowed us to measure more physiological values than those obtained on strips or in vitro arteries.

The mechanical properties of the arterial wall, as studied by these three different ways, did not show discrepancies. The same groups had stiffer arterial walls regardless of the studied parameter: characteristic impedance, SAC, or carotid distensibility.

Mechanical passive properties of the carotid arterial wall were modified by hypertension and by treatment by CEI. In an earlier study, Cox had shown that renal hypertension increased both the passive stiffness of carotid artery and the active response of smooth muscle cell activation. Although we did not expose or introduce a catheter into the studied carotid artery, we have no evidence to exclude that the manipulation of the vessel had not influenced the integrity of the endothelium and the subsequent responses of the vasculature. Our present data clearly confirm this study. Renovascular hypertension decreased the compliance of the carotid wall for a definite pressure. Chronic treatment by CEI normalized the compliance of the arterial wall. These effects are probably mediated by smooth muscle cell activity as shown by the effect of potassium cyanide on these parameters. In control condition CEI normalized the compliance of carotid artery in hypertensive group, but did not change carotid compliance after the potassium cyanide treatment. CEI seems to normalize the vascular muscle tone thus, potassium cyanide poisoning could not still further decrease the stiffness of the carotid wall. Moreover, the effect of hypertension after potassium cyanide poisoning of smooth muscle cells was less marked than in basal conditions. Thus the action of hypertension and of its treatment on the function of large arteries in this model seems to be predominantly mediated by the smooth cell activity or mass.

**Morphological Analysis**

The morphological study was performed on unloaded samples of aorta. As shown by Wolinsky there are differences in morphometric parameters, especially in the thickness of the media, the density of proteins of the extracellular matrix and of nuclei, between their in situ values and those measured in unloaded conditions. Nevertheless, we may compare the values obtained in different groups in similar unloaded conditions. Two months of renovascular hypertension was associated with a significant 25% increase in the aortic wall thickness. These results are concordant with preceding studies demonstrating an increase in the thickness of the aortic wall in different models of hypertension.

In our study, the increase in medial thickness of the aortic wall associated with renovascular hypertension in its early phase (2 months) was predominantly due to a hypertrophy of smooth muscle cells. The hypertrophy is demonstrated by the unchanged relative nuclear density despite the reverse increase in the stiffness of the media. The data of the literature concerning hypertrophy versus hyperplasia of smooth muscle cells is conflicting. Some authors using morphometric methods reported a hypertrophy of smooth muscle cells associated with different types of hypertension. Using chemical methods, Owens and Schwartz reported a hypertrophy associated with polyploidy. A proliferation of smooth muscle cells (hyperplasia) in response to aortic coarctation in rats and in spontaneously hypertensive rats was recently reported. These differences could be due to the different models used, the different methods of investigation, and to the different stage of hypertension. Our results demonstrate that in early renovascular hypertension in rats the significant increase in smooth muscle mass was predominantly due to a hypertrophy of smooth muscle cells without significant prolifer-
ation as demonstrated by nucleus density. On the other hand, the increase in cross-sectional area of each nucleus with hypertension is concordant with results reporting an increase in DNA content and polyploidy in hypertension. This increase in mass of nuclei is probably associated with an increase in proteins synthesis by smooth muscle cells: contractile proteins, elastin, and collagen.

The published data concerning proteins of the interstitial matrix in the aortic walls during hypertension are also conflicting, depending predominantly on the method of expression of the results as a relative or absolute amount of collagen and elastin and on the early or late phase of hypertension. In the present study, the relative amount of elastin decreased with hypertension in relation to the increase in smooth muscle mass. The absolute amount of elastin did not change significantly with hypertension in this experimental model. Moreover, the number of elastin lamellae did not change with hypertension, and the thickness of each lamella remained constant. These results suggest that hypertrophy of smooth muscle cells is not associated with a significant increase of elastin biosynthesis in our experimental model. Conversely, the modification of relative collagen density is a different matter than that of elastin. The absolute and relative amount of collagen and the thickness of each fiber increased with smooth muscle cell hypertrophy. These data show that hypertrophy of smooth muscle cells is associated with a significant increase in the absolute amount of collagen corresponding probably to an increase in biosynthesis. Our experimental design concerned young rats in the early period of severe hypertension, and an increase in the collagen content into the arterial wall could be a more predominant phenomenon with the late phase of hypertension.

Treatment decreases wall thickness and reverses smooth muscle cell hypertrophy as demonstrated by the increase of relative nuclear density per unit area and the decrease of the cross-sectional area of the nuclei. Furthermore, treatment significantly increases the relative amount of elastin without significantly modifying the increase in collagen density. There are few data concerning the effect of treatment on the vascular wall than the effect of hypertension. In an early study, Wolinsky showed that surgical treatment could reverse morphological changes due to two-kidney/one-clip renovascular hypertension in female hypertensive rats but not in male rats. The discrepancy between our results and the data reported by Wolinsky may be related to three differences in the respective experimental protocols: 1) the renal artery was clipped at the same age in both experiments, but hypertension was maintained during 10 weeks in Wolinsky's study instead of 4 weeks as in the present experiment. 2) The morphological measurements were performed in older animals by Wolinsky (28 weeks vs. 10 weeks). The animals studied in the present work were relatively young and were still growing. It is uncertain whether our results are applicable to adult mature animals or are only relevant to growing animals.

Of particular interest was the observation that renal hypertension markedly modified functional and structural properties of large arteries. Three mechanisms may be involved to explain the increase in arterial resistance and stiffness during renal hypertension:

1) **Altered artery wall thickness.** We have found a hypertrophy of the medial cells associated with an increase in collagen content.

2) **Altered passive stiffness of vessel walls.** Aortic input impedance, systemic arterial compliance and distensibility of the isolated carotid artery showed a concordant increase in the stiffness of large arteries in hypertensive untreated rats. These changes may be partly attributed to the increase in collagen and smooth muscle mass of the arterial wall as shown by the altered compliance of the carotid artery after poisoning the myocytes in the untreated and treated hypertensive and normotensive groups.

3) **Altered active vascular muscle tone.** There is evidence of elevated active contractile force in hypertensive arteries which may be partly related to the increased medial smooth muscle mass.

In the present study, CEI treatment increased the in vivo hemodynamic properties of distensibility of the arterial wall. This effect of CEI could be explained by interference of treatment with the three mechanisms of arterial wall distensibility. In both normotensive and hypertensive rats, treatment decreased the thickness of the media and the passive stiffness of the vessel wall without change in collagen content and the active contractile tone of smooth muscle cells. Thus, the decrease in arterial distensibility associated with hypertension and its reversal by specific treatment seems to be mainly due to the change in smooth muscle cell mass activity. The functional modifications of the wall of large arteries could play a major role in the value of the left ventricular afterload and in the peripheral diastolic perfusion by the arterial wind kessel. They are associated with correspond-
ing changes in the structure and the composition of the arterial media. The hypertrophy of smooth muscle cells induced by renovascular hypertension and its reversion by CEI treatment could be of importance for the understanding of the relationship between hypertension and the development of atheroma.

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