Reduction of Transmural $^{125}$I-Albumin Concentration in Rat Aortic Media by Chronic Hypertension

Joël Belmin, Jean-Baptiste Michel, Patrick A. Curmi, Jean-Loup Salzmann, Lucienne Juan, and Alain Tedgui

Relative $^{125}$I-albumin concentration was measured in vivo in the aortic media of sham-operated ($n=10$) and hypertensive (two-kidney, one clip) rats, untreated ($n=8$) or treated ($n=10$) by an angiotensin converting enzyme inhibitor (CEI, Trandolapril). Blood pressure was acutely lowered to a normal level at the time of the experiment in hypertensive rats ($n=7$) to separate the direct effect of increased pressure from the effect of pressure-induced structural changes. Relative tissue concentration profiles of labeled albumin across the media were obtained using a serial frozen-sectioning technique. In hypertensive rats, the mean medial albumin concentration decreased by 35% in the ascending arch and 32% in the descending arch ($p<0.01$). When blood pressure was acutely lowered in hypertensive animals, this value decreased further by 56% in the ascending arch, 48% in the descending arch ($p<0.01$), and 22% in the thoracic aorta ($p<0.05$) as compared with controls. The medial thickness in hypertensive rats was significantly increased (more in the ascending arch than in the rest of the aorta). Four-week CEI treatment reversed hypertension and medial thickening, but the mean medial albumin concentration remained significantly lower in the arch (by 36% in the ascending part and 40% in the descending part, $p<0.01$). The collagen content in the thoracic aorta was significantly increased in hypertensive rats (by 40%, $p<0.01$) and remained increased (by 29%, $p<0.01$) after CEI treatment. These results suggested that the hypertension-induced structural changes might reduce the medial distribution volume for albumin, whereas elevated blood pressure per se tended to enhance albumin concentration within the media. However, the net result of chronic hypertension was a reduction of the mean medial albumin concentration. The aortic arch appeared to be more affected than the rest of the aorta. Fiber content, more than medial thickness, might be responsible for the observed differences in albumin concentration. Lowering of blood pressure seemed to be insufficient to restore normal albumin concentration profiles and perhaps those of other macromolecules. This finding may be relevant in evaluating some of the complications associated with hypertension. (Arteriosclerosis and Thrombosis 1991;11:334–343)

Mass transport is believed to play an important role in atherogenesis. Plasma proteins, like water and small-sized molecules, are transported across the wall of large arteries. At an early stage of the atheromatous process, accumulation of plasma materials in the intimal layer may result from increased influx through the endothelium or decreased efflux across the media. Previous studies have investigated the mechanisms by which protein transport occurs within the arterial wall and the factors that influence it. However, the effect of hypertension on this process has not been completely elucidated. Hitherto, most of the experiments have focused on the effect of acute experimental hypertension in vitro or in vivo and have reported an increase in macromolecular transport across the arterial wall. On the other hand, earlier studies dealing with chronic hypertension were only concerned with its effect on the permeability of the endothelium to medium-sized molecules in various animal models.

The present study was performed to examine the effect of chronic hypertension and its reversal by...
angiotensin converting enzyme inhibitor (CEI) treatment on the transmural distribution of radiolabeled albumin in the aortas of renovascular-hypertensive rats (two-kidney, one clip). In an attempt to separate the influence of elevated blood pressure per se from that due to the pressure-induced structural changes of the arterial wall, experiments were performed in hypertensive rats with acutely normalized blood pressures. The transmural distribution of $^{125}$I-albumin concentrations was determined using a serial frozen-sectioning technique.

Methods

Experimental Protocol

Hypertension was induced in 32 ether-anesthetized male Wistar rats (130-150 g) by placing a constricting clip with an internal diameter of 0.2 mm on the right renal artery. After 4 weeks, the hypertensive animals were randomly distributed into three groups. Seven hypertensive rats were used for albumin transport measurement at the end of these 4 weeks (4W-HR group). Fifteen hypertensive rats were kept untreated for another 4 weeks, and 10 were treated with a CEI (Trandolapril, Roussel Uclaf, France). Antihypertensive therapy was administered daily by gavage at a dose of 1 mg/kg in 1 ml distilled water ad libitum. They were weighed, and their systolic blood pressures (SBPs) were measured weekly using the tail-cuff method (blood pressure recorder model 8005, W& W Electronic, Varese, Italy). In all animals except those from the 4W-HR group, the tracer distribution in the aorta was measured 8 weeks after laparotomy.

Albumin Transport Measurement

Tracer. Rat serum albumin (fraction V, Sigma Chemical Co., St. Louis, Mo.) was iodinated with $^{125}$I (Amersham, Buckinghamshire, England) using the iodine monochloride method of McFarlane as modified by Bilheimer. Non-protein-bound radioactivity was removed by multiple dialysis against 0.9% NaCl for 24 hours at 4°C before use. Trichloroacetic acid (TCA) was used to precipitate the protein-bound label. The free/bound protein iodine ratio was approximately 0.2% before injection.

Animal procedures. Under ether anesthesia, each rat received a catheter inserted into the carotid artery and another into the jugular vein. The venous catheter served for $^{125}$I-albumin and drug injection. The arterial catheter was connected to a Statham Model P23ID pressure transducer (Gould, Cleveland, Ohio) for continuous monitoring of blood pressure and for blood sampling.

Rats were placed in a restraining box. After they completely recovered consciousness, the rats were injected with 500 µl $^{125}$I-albumin (=100 µCi). A blood sample was taken 5 and 90 minutes after injection for plasma radioactivity determination. Animals were usually killed with a lethal dose of sodium pentobarbital 90 minutes after injection of $^{125}$I-albumin. Two normotensive and two hypertensive rats were killed 3 hours after the injection of label to check that a steady state had indeed been achieved after 90 minutes. In these experiments, a blood sample was also taken at 3 hours.

After the rats were killed, the whole aorta from the heart to the iliac bifurcation was immediately excised and divided into several segments: one from the ascending part of the arch, one from the descending part of the arch, three from the descending thoracic aorta, and three from the abdominal aorta. Each segment was cleaned of adventitial debris. The segments from the ascending and descending parts of the arch were further divided into superior and inferior portions. The rest of the aorta was opened longitudinally. Each arterial segment was rinsed briefly in saline buffer to remove traces of blood, laid on a lightly greased microscope slide, and rapidly frozen in a cryostat at −20°C. The elapsed time between killing and freezing was about 10 minutes.

Estimation of radioactivity within the wall. The spatial distribution of tracer within the wall was obtained using a serial frozen-sectioning technique on a refrigerated microtome (SLEE TE, London, England). Frozen tissue-mounting medium was leveled with the microtome knife. A sample of frozen aorta was thawed slowly, and a small quantity of mounting medium was then spread on the adventitial surface. The microscope slide with the tissue sample (face down) was quickly lowered onto the frozen, leveled mounting medium using a spring device that made it possible to keep the microscope slide parallel to the cut face on the mounting medium. The edges of the segments were trimmed to remove overhanging material, and their surface areas (about 0.2 cm²) were measured. En face serial sections, 10 µm thick, were then cut, proceeding from the intimal surface. Consequently, the first slice was incomplete and was...
discarded when it was judged visually to be excessively thin (likely <5 μm thick). Therefore, the thickness of the first section kept for analysis ranged between 5 and 10 μm. The boundary between the media and the adventitia was defined by an alteration in the appearance of the sections. The sections were placed in tubes for radioactivity assay.

As the presence of free labeled iodide in plasma could have led to an error in the estimate of tissue albumin concentration, 500 μl 1% unlabeled albumin solution and 500 μl 20% TCA were added to the samples of the descending thoracic aorta of each animal. The mixture was centrifuged at 2,500g for 15 minutes, and half of the supernatant (500 μl) was removed carefully. TCA-precipitable radioactivity was obtained by subtraction of the radioactivity of the 500 μl of supernatant from that of the rest of the mixture. The same procedure was used to measure TCA-precipitable labeled albumin in duplicate 20-μl aliquots of plasma. In a preliminary study, the TCA-soluble radioactivity fraction in the aorta was found to be similar in the different parts of the aorta. Therefore, the TCA-soluble fraction obtained in the thoracic aorta was used to calculate protein-bound radioactivity in other parts of the aorta.

The 125I radioactivity was assayed on a gamma counter (Kontron Gammamatic, Basel, Switzerland). The tissue counts ranged from 100 to 1,000 cpm (after subtraction of the background, which was about 30 cpm). The samples were counted for 3 minutes.

The relative 125I-albumin tissue concentration, C_t/C_p, was calculated for each section as counts per minute per unit volume of wet tissue (C_t) divided by the counts per minute per milliliter of plasma sampled 5 minutes after injection (C_p). The C_t/C_p value of the first section was underestimated because its actual thickness was less than the 10-μm value used for calculation of the volume of the wet tissue. However, as the unknown actual thickness ranged between 5 and 10 μm (averaging 7.5 μm), the error might be estimated to average 25%. The C_t/C_p values for the various sections (including the first) cut from a single segment were plotted as a function of their distance from the luminal surface to the external surface. Distances for each segment were normalized by dividing by the medial thickness, calculated as the number of tissue sections multiplied by 10 μm. Mean concentration profiles were constructed by averaging the C_t/C_p values at equal intervals across the media. As the number of tissue sections varied between animals within the same group, the number of intervals was chosen from the mean number of tissue sections obtained in each group.

For each aortic site, a mean relative albumin concentration in the media (MRAC) was calculated as the average of the medial C_t/C_p values, excluding the first, which was underestimated. The albumin concentration in the adventitia was obtained using the C_t/C_p values of the first two complete sections of the adventitia.

### Morphological Study

Medial thickness was calculated in all arterial segments using the number of sections cut from the sample and the thickness of each section (10 μm). A ring of the descending thoracic aorta was fixed with Bouin’s solution. After dehydration with alcohol, the aortic ring was embedded in paraffin. Five-micron-thick transverse sections were specifically stained with monochromatic color. Red Sirius was used to stain collagen fiber, and orcein was used to stain elastin. The slides were subjected to an automatic image analysis processor (NS model 1500, Nachet-Vision, Evry, France). The mean medial thickness was measured as the distance between the internal and external elastic lamina and compared with the corresponding calculated value.

The collagen density was determined by measuring the Red Sirius-stained area in the media and was expressed as a percentage of the medial surface area. The amount of medial collagen was calculated as the product of medial thickness multiplied by collagen density and expressed in arbitrary units.

### Statistical Methods

Results are expressed as mean±SD. Blood pressures, body weights, measured medial thicknesses, collagen densities, and collagen amounts were compared using one-way analysis of variance (ANOVA) between experimental groups. In the thoracic aorta, calculated medial thickness was compared with measured values using a linear regression model. A two-factor repeated-measures ANOVA was constructed with the MRAC and calculated media thickness data to test the effect of experimental group and aortic site (level of significance at p<0.05). The differences between experimental groups were evaluated using Bonferroni's test. The differences between aortic sites were evaluated using Student's paired t test.

### Results

#### Body Weight and Blood Pressure

Body weight at the time the rats were killed, SBPs measured 4 and 8 weeks after laparotomy, and carotid mean blood pressure (MBP) at the time of experiment are given in Table 1. Body weights in 4W-HR and CEI groups were significantly lower than in the C group (p<0.01). Those in the HR and LPHR groups did not differ significantly from control.

Hypertension was achieved 2 weeks after clipping the renal artery in all hypertensive rats. Four weeks after clipping, SBP measured by the tail-cuff procedure was similar in the different groups of hypertensive animals (Table 1). In untreated hypertensive rats (LPHR and HR groups), SBP did not vary significantly until the time of the experiment. In the CEI group, SBP fell to normal values as early as the first week after starting treatment and did not change significantly during the 4-week treatment. The SBP of control rats remained in the physiological range during all 8 weeks.
Table 1. Body Weight and Blood Pressure

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=10)</th>
<th>4W-HR (n=7)</th>
<th>HR (n=8)</th>
<th>LPHR (n=7)</th>
<th>CEI (n=10)</th>
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<tr>
<td>Body weight (g)</td>
<td>398±23</td>
<td>250±9</td>
<td>384±18</td>
<td>376±20</td>
<td>315±27</td>
</tr>
<tr>
<td>SBP at 4 weeks (mm Hg)</td>
<td>126±8</td>
<td>191±10</td>
<td>186±12</td>
<td>188±11</td>
<td>189±12</td>
</tr>
<tr>
<td>SBP at 8 weeks (mm Hg)</td>
<td>132±17</td>
<td>...</td>
<td>191±18</td>
<td>193±15</td>
<td>112±7</td>
</tr>
<tr>
<td>Carotid MBP (mm Hg)</td>
<td>108±7</td>
<td>162±18</td>
<td>150±21</td>
<td>162±12</td>
<td>91±11</td>
</tr>
</tbody>
</table>

*Values of body weight, systolic blood pressure (SBP) measured using the tail-cuff method at 4 weeks and 8 weeks after laparotomy, and mean blood pressure (MBP) measured via carotid catheter at the time of the experiment for each experimental group: 4W-HR, 4-week hypertensive; HR, untreated hypertensive; LPHR, low-pressure hypertensive; and CEI, angiotensin converting enzyme inhibitor-treated hypertensive rats. Values are mean±SD. Statistical comparisons are given in text.

At the time of the experiment, the MBP obtained by direct measurement via a carotid catheter was found to be similar in all untreated hypertensive animals. In the LPHR group, MBP after nicardipine injection fell to a value similar to that of controls. MBP of CEI-group animals was found to be significantly lower than that of the C group (p<0.05). The MBP of each animal, including that of the LPHR group, was stable during the duration of the experiment.

Studies of Albumin Distribution

TCA-precipitable plasma radioactivity decreased by 24±9.0% (N=42) between 5 and 90 minutes after tracer injection. This decrease was similar in all groups. The TCA-soluble/TCA-precipitable plasma radioactivity ratio was 0.0012±0.0007 (N=42) at 5 minutes and increased to 0.0030±0.0013 (N=42) at 90 minutes. The mean medial TCA-soluble/TCA-precipitable tissue radioactivity in the thoracic aorta was 0.12±0.054 (N=42).

In experiments conducted for 3 hours in two animals from the C group and two from the HR group, the Cr/Cpo values were not significantly different from those obtained in 90-minute experiments, indicating that a steady state was achieved after 90 minutes. In the following analyses, values obtained in 3-hour experiments were pooled with those from 90-minute experiments.

The Cr/Cpo values found in the adventitia did not vary significantly between sites nor between groups (ranging from 0.045 to 0.087). The repeated-measures ANOVA showed that experimental group and aortic site both affected MRAC and that significant interaction occurred between these two factors. No significant difference was found between the upper and lower parts of the ascending arch, and of the descending arch. Therefore, these data were pooled, and only four aortic sites (ascending arch, descending arch, descending thoracic, and abdominal aorta) will be considered subsequently. These data are presented in Table 2.

Figure 1 shows the average profile of the relative 125I-albumin concentration across the media in normotensive animals. The average profiles of the relative 125I-albumin concentration across the media of the ascending arch, the descending arch, the thoracic aorta, and the abdominal aorta in each experimental group are shown in Figures 2-5, respectively.

Effect of Aortic Sites

MRAC varied significantly with aortic site (p<0.001). Analysis of the difference between sites regardless of the experimental group showed that MRAC increased from the ascending part of the arch to the descending thoracic aorta. MRAC increased by 12.7% in the descending arch (p<0.01) and by

Table 2. Mean Medial Relative Concentration (x10^-2)

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<tbody>
<tr>
<td>C (n=10)</td>
<td>1.99±0.68</td>
<td>2.24±0.71</td>
<td>1.93±0.38</td>
<td>1.86±0.37</td>
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<td>4W-HR (n=7)</td>
<td>1.48±0.34</td>
<td>1.56±0.39</td>
<td>2.05±0.48</td>
<td>1.19±0.43</td>
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<td>HR (n=8)</td>
<td>1.30±0.21</td>
<td>1.52±0.36</td>
<td>1.75±0.34</td>
<td>1.73±0.41</td>
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<td>LPHR (n=7)</td>
<td>0.87±0.15</td>
<td>1.16±0.29</td>
<td>1.50±0.37</td>
<td>1.45±0.35</td>
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<tr>
<td>CEI (n=10)</td>
<td>1.27±0.40</td>
<td>1.33±0.28</td>
<td>2.04±0.35</td>
<td>2.09±0.43</td>
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</table>

Values are mean medial values of relative concentrations of 125I-albumin±SD for the different aortic sites: Asc. arch, ascending arch; Desc. arch, descending arch; Thor. Ao., descending thoracic aorta; Abd. Ao., abdominal aorta of each experimental group: C, control; 4W-HR, 4-week hypertensive; HR, untreated hypertensive; LPHR, low-pressure hypertensive; CEI, angiotensin converting enzyme inhibitor-treated group. Statistical comparisons are given in text.
31.7% in the descending thoracic aorta \((p<0.001)\) as compared with that in the ascending arch. MRAC values from the descending arch and the descending thoracic aorta were significantly different \((p<0.001)\), whereas those from the ascending arch were not. In contrast, in normotensive animals, MRAC did not vary significantly with the aortic sites.

The transmural profiles of \(C_{\text{p}}/C_{\text{m}}\) also showed differences with the aortic sites. In the arch, these profiles were U-shaped (Figures 2 and 3), whereas in the abdominal aorta, the \(C_{\text{p}}/C_{\text{m}}\) values increased quasilinearly from the luminal side to the outer media (Figure 5). In the descending thoracic aorta, the profiles were practically flat on the inner two thirds of the media and showed a slight gradient in the outer media (Figure 4).

**Effect of Experimental Group**

Irrespective of the aortic site, MRAC decreased by 21.7% in the HR group as compared with C-group animals \((p<0.001)\). MRAC values were not significantly different in 4W-HR and HR groups. In the LPHR group, MRAC was significantly decreased by 20.7% as compared with HR-group values \((p<0.05)\) and by 37.9% as compared with controls \((p<0.001)\). In the CEI group, MRAC values were decreased by 16.2% as compared with controls \((p<0.001)\) and did not differ significantly from those in the HR group.

**Interactions**

The effect of the experimental group on MRAC varied with the aortic site, resulting in a significant interaction between groups and sites as revealed by ANOVA \((p<0.001)\). Analysis of interaction showed that the ascending and descending parts of the aortic arch were markedly affected by the experimental group \((p<0.001)\), whereas the descending thoracic aorta was less affected \((p<0.05)\). In the abdominal aorta, the effect of the experimental group was absent except in the 4W-HR group, in which MRAC...
was significantly less than that in the other groups. At each aortic site, the typical shape of the average transmural profile of \( C_v/C_p \) was similar regardless of the experimental group (Figures 2–5).

**Morphological Study**

**Medial thickness.** Figure 6 shows the linear regression obtained between the values of medial thickness of the descending thoracic aorta measured by histological analysis and those values calculated from the number of frozen serial sections (\( r=0.79, \ p<0.0001, \ N=42 \)). The highly significant linear relation between the two methods indicates that the serial frozen-sectioning technique could be used to estimate and compare the aortic medial thickness in different sites and groups. A repeated-measures ANOVA was constructed with the calculated medial thickness data. As in the MRAC study, no difference was found between the upper and lower parts of the ascending and descending arch. Therefore, their data were pooled. Results are presented in Table 3. Both aortic site and experimental group affected the medial thickness (\( p<0.001 \)) with a significant interaction between these two factors (\( p<0.001 \)), indicating that hypertension or its treatment with CEI affected the medial thickness in a different manner in the different aortic sites. Irrespective of experimental group, the medial thickness significantly decreased from the ascending arch to the abdominal segment. As compared with the ascending arch, the medial thickness was decreased by 10.9% in the descending arch (\( p<0.001 \)), by 23.7% in the descending thoracic aorta (\( p<0.001 \)), and by 36.7% in the abdominal aorta (\( p<0.001 \)). Irrespective of aortic site, the medial thickness was significantly increased in untreated hypertensive rats (by 35.3% in the HR group and 50.0% in the LPHR group) as compared with controls (\( p<0.001 \)). The thicknesses in the LPHR and HR groups were not significantly different. In the 4W-HR group, medial thickness was significantly
Abdominal Aorta

FIGURE 5. Line plot of transmural distribution of mean relative $^{125}$I-albumin tissue concentration ($\times 10^{-2}$) across the media of the abdominal aorta in each experimental group. Normalized distance equals 1 at medial-adventitial boundary. Number of animals are given in parentheses. Bars represent SEM. -■-, Control; -○-, 4W-HR, 4-week hypertensive group; -▲-, HR, untreated hypertensive group; -△-, LPHR, low-pressure hypertensive group; -◆-, CEI, angiotensin converting enzyme inhibitor–treated group.

increased by 27.8% as compared with controls ($p<0.01$). After CEI treatment, the medial thickness was brought back to normal and was not significantly different from controls. Analysis of interaction showed that in all untreated hypertensive animals, the relative increase in medial thickness was higher

FIGURE 6. Scatterplot showing linear regression between values of medial thickness (µm) for the descending thoracic aorta calculated from the number of serial frozen sections (y axis) and those measured using morphometric method (x axis).

TABLE 3. Medial Thickness

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<tbody>
<tr>
<td>C (n=10)</td>
<td>77.5±11.8</td>
<td>75.5±9.8</td>
<td>62.2±8.7</td>
<td>54.3±4.7</td>
</tr>
<tr>
<td>4W-HR (n=7)</td>
<td>97.9±14.4</td>
<td>90.7±15.4</td>
<td>84.6±8.8</td>
<td>71.1±9.1</td>
</tr>
<tr>
<td>HR (n=8)</td>
<td>120.1±25.3</td>
<td>96.2±11.9</td>
<td>81.9±8.4</td>
<td>66.4±9.6</td>
</tr>
<tr>
<td>LPHR (n=7)</td>
<td>128.6±33.2</td>
<td>110.2±20.8</td>
<td>91.1±13.4</td>
<td>74.3±10.6</td>
</tr>
<tr>
<td>CEI (n=10)</td>
<td>76.5±15.9</td>
<td>70.5±10.9</td>
<td>60.8±8.9</td>
<td>49.7±8.3</td>
</tr>
</tbody>
</table>

Values of medial thickness (µm) calculated using numbers of serial frozen sections for the different aortic sites: Asc. arch, ascending arch; Desc. arch, descending arch; Thor. Ao., descending thoracic aorta; Abd. Ao., abdominal aorta of each experimental group: C, control; 4W-HR, 4-week hypertensive; HR, untreated hypertensive; LPHR, low-pressure hypertensive; CEI, angiotensin converting enzyme inhibitor–treated group. Values in parentheses represent medial thickness measured in the thoracic aorta using morphometric technique. Values are mean±SD. Statistical comparisons are given in text.
in the aortic arch than in the rest of the aorta. Moreover, medial thickness values from thoracic and abdominal aortas did not differ significantly between the 4W-HR group and the HR group, while those from the aortic arch were lower in the 4W-HR group ($p<0.05$).

**Collagen density and amount.** Collagen density of the thoracic aorta, measured by histomorphometry (Table 4), was significantly increased in the 4W-HR and LPHR groups as compared with the C group ($p<0.05$). The increase in collagen density observed in the HR group was not significant when compared with controls. In the CEI group, collagen density was significantly higher than that in the C group or the HR group ($p<0.05$) and did not significantly differ from that obtained in the 4W-HR or LPHR group. The amount of medial collagen in the thoracic aorta (Table 4), calculated as the product of medial thickness and collagen density, was increased in all untreated hypertensive rats (4W-HR, HR, and LPHR groups) compared with controls ($p<0.05$). In the CEI group, despite normalization of medial thickness, the amount of collagen was markedly larger than that of controls ($p<0.01$) and did not differ significantly from that of untreated hypertensive rats.

**Discussion**

The present findings indicate that chronic hypertension decreases albumin concentration in the aortic media, with the extent of the reduction dependent on the region of the aorta. A delay in the albumin penetration in the wall due to differences in wall thicknesses in hypertensive animals is unlikely to explain this, since a steady-state level of labeled albumin accumulation was reached. In chronic hypertension, the albumin accumulation is expected to be influenced by both elevated blood pressure and hypertension-induced structural changes of the arterial wall. In our experiments performed on hypertensive rats with acutely lowered blood pressure, the medial albumin concentrations were reduced when compared with those in hypertensive animals with elevated blood pressures. This cannot be accounted for by a direct effect of the drug used to lower blood pressure, since it has been shown that this drug enhances albumin uptake by rabbit aortas in vitro under controlled transmural pressure.\(^{20}\) Therefore, it seems that high blood pressure per se contributes to enhancement of albumin influx, in agreement with previous studies showing that albumin uptake increases with high transmural pressure in vitro\(^{7,9,11}\) or with acute hypertension in vivo.\(^{12,13}\) Nevertheless, we observed a decrease in albumin accumulation in the aortas of hypertensive rats. We suggest that the mechanisms responsible for this might be related to some structural changes caused by hypertension.

Albumin transport across the arterial wall is a complex process involving the entrance and exit of protein across the endothelium, entrance and exit from the adventitia, and diffusion/convection across the media. Thus, the observed reduction of the albumin tissue concentration in hypertensive animals might result from alterations in any of these processes. Several authors\(^{15,21}\) have shown that chronic hypertension either induced an increase in the endothelial permeability to macromolecules or did not change it, depending on the model of experimental hypertension and on the duration of the hypertensive state of the rats. This suggests that a reduced entrance from the lumen is unlikely. It is also unlikely that the observed changes in medial albumin concentrations were due to an alteration of the adventitial source of albumin because the distribution of adventitial vasa vasorum seems to be unaltered by hypertension.\(^{22}\) On the other hand, the albumin concentration in the adventitial layer was similar in all groups. Therefore, it is most likely that the differences observed in hypertensive animals resulted from changes affecting the albumin transport within the media. First, an increased rate of drainage from the media by pressure-driven convection might decrease the medial concentration of the macromolecule. Yet, this is not in agreement with our finding, which showed that albumin uptake decreased further when hypertensive animals were made acutely normotensive. Second, a pressure-induced structural alteration of the internal elastic lamina could reduce the influx of albumin into the media and, to a lesser extent, in those animals with elevated pressure in which the internal elastic lamina is stretched, than in hypertensive animals with acutely lowered pressure. Even though this cannot be ruled out, we believe that the most likely possibility is a reduction in the space available to albumin in the entire media. This conclusion is supported by our finding that the medial fibrous content was increased in hypertensive animals as well as in CEI-treated rats.

### Table 4. Collagen Density and Amount

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=10)</th>
<th>4W-HR (n=7)</th>
<th>HR (n=8)</th>
<th>LPHR (n=7)</th>
<th>CEI (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen density (%)</td>
<td>9.82±0.68</td>
<td>11.6±0.39</td>
<td>10.4±0.53</td>
<td>12.7±1.16</td>
<td>11.9±0.96</td>
</tr>
<tr>
<td>Collagen amount (AU)</td>
<td>900±88</td>
<td>1,401±126</td>
<td>1,255±200</td>
<td>1,634±227</td>
<td>1,161±158</td>
</tr>
</tbody>
</table>

Values of collagen density (%) and collagen amount in arbitrary units (AUs) measured in the thoracic aorta of each experimental group using histomorphometric technique. Values are mean±SD. Statistical comparisons are given in text. 4W-HR, 4-week hypertensive; HR, untreated hypertensive; LPHR, low-pressure hypertensive; CEI, angiotensin converting enzyme inhibitor–treated group.
In hypertensive animals, the albumin concentration in the media was decreased much more in the aortic arch than in other parts of the aorta. The observed regional variations in medial albumin concentrations might be accounted for by regional differences in the structure of the media, including extracellular matrix components, possibly associated with regional differences in local hemodynamics and mechanical stresses applied to the aortic wall. In particular, the aortic arch with its high elasticity is subjected to larger cyclic distensions than are other parts of the aorta, and there is evidence that cyclic stretching of smooth muscle cells may be a stimulus for collagen and mucopolysaccharide synthesis. Indeed, we found that the aortic arch showed the most marked changes in medial thickness and albumin concentration with hypertension.

In the abdominal aorta, the transmural distribution of albumin tissue concentrations strongly differed from that in the thoracic aorta, showing a quasilinear increase from the inner to the outer media. This might be accounted for by a structural heterogeneity of the media, resulting in a distribution volume for albumin that increases from the inner to the outer media. However, this is unlikely since the distribution volume for albumin has been found to be practically constant across the rabbit aortic media. Most likely, the low albumin concentration values on the intimal side of the media suggest that the albumin influx occurred mainly from the adventitial vasa vasorum.

The 4-week treatment with CEI was started while the media was already thickened, and at the end of the treatment, medial hypertrophy was reversed in the entire aorta. Conversely, the collagen content, which increased in hypertensive animals, did not return to control values with hypertension reversal. These results are in agreement with previous observations. The persistence of increased collagen content, associated with reversal of the medial thickening, offered us the opportunity to examine the respective contribution of these two structural factors to the changes in albumin accumulation in the aortic media. The CEI treatment did not completely reverse albumin concentration changes after 4-week treatment. This might be accounted for by the persistence of an increased collagen content in treated rats although our data cannot prove a causal relation. However, if this is the case, it suggests that the accumulation properties of the media to albumin were mainly determined by the extracellular connective tissue components, such as collagen, which might decrease solution space available in the media for macromolecules.

Even though the accelerating effect of hypertension on atherosclerosis is well documented, the actual mechanism by which it occurs has not been established. Wolinsky and Hadjiiski et al, from studies showing that experimental hypertension caused structural changes in the media of hypertensive rats, suggested that interstitial fibrosis and hypertrophy of the media might decrease the permeability of this layer to plasma substances that diffuse through the wall. The present study supports this hypothesis. With chronic hypertension, decreased available space to macromolecules might slow the transmural diffusion and efflux of macromolecules through the arterial wall, which might accelerate the atherogenic process by increasing the tendency of the vessel wall to trap materials in the subendothelial space. Indeed, Bretherton et al found increased intimal entry of low density lipoprotein in the aorta of hypertensive rabbits. The lack of reversal of vascular functional changes with correction of hypertension might be related to the reported lack of effect of certain antihypertensive therapies to reduce the extent of atherosclerosis.

### References

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KEY WORDS  • aorta • albumin concentration • hypertension • rats
Reduction of transmural 125I-albumin concentration in rat aortic media by chronic hypertension.

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