Effects of Hypertension and Its Reversal on Aortic Metabolism in the Rat

PETER BRECHER, CATHERINE T. CHAN, CARL FRANZBLAU, BARBARA FARIS, AND ARAM V. CHOBANIAN

SUMMARY The relationship between aortic metabolism and hypertension was examined in spontaneously hypertensive rats (SHR) and in the normotensive Wistar-Kyoto (WKY) strain of rats. Comparative studies between age-matched animals of both strains showed in the SHR, enhanced activities of the aortic enzymes, N-acetyl β-glucosaminidase (NAGA) and acid phosphatase, which are associated with lysosomes. Similar increases were shown for aortic 5'-nucleotidase activity. Antihypertensive drug treatment of 30-week-old SHR for 17 weeks effectively lowered blood pressure and reduced the activity of aortic NAGA and acid phosphatase in the SHR to control levels. 5'-Nucleotidase activity remained significantly elevated, and aortic collagen and elastin concentrations were unaffected by antihypertensive drug treatment. Hypertension was produced in WKY rats by deoxycorticosterone treatment for either 4 or 7 weeks and then was reversed by discontinuing treatment and maintaining the pretreated animals on a low-salt diet for up to 11 weeks. At the end of the treatment period, aortic enzymatic activity was increased significantly, as were heart and aortic weights. Following the reversal of hypertension, the activity of the lysosomal enzymes was decreased significantly, but 5'-nucleotidase activity remained elevated. Collagen and elastin concentrations were not affected by mineralocorticoid treatment, but the total amount of connective tissue protein was increased and remained elevated following the reversal of hypertension, paralleling the changes in aortic weight. The studies indicate that etiologically different forms of hypertension result in characteristic changes in aortic metabolism which are not completely reversed by subsequent blood pressure reduction.

HYPERTENSION is known to be an important risk factor for atherosclerosis in man, but the biochemical basis for this relationship has not yet been established. Changes in the vascular endothelium of hypertensive animals may lead to increased vascular permeability and smooth muscle cell proliferation, thereby contributing to the development of atherosclerotic lesions.1,3 Wolinsky et al.4 have

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shown that, in rats, experimentally induced hypertension produced an increase in aortic lysosomal activity, and they proposed that changes in the activity of aortic lysosomal enzymes may be a causative factor in the development of atherosclerotic lesions.5

The spontaneously hypertensive rat (SHR) appears to be a useful animal model to study human essential hypertension as judged by several physiological and pathological criteria.6 Comparative studies on aortic tissue between the SHR and normotensive control rats have shown differences in several biochemical processes including connective tissue metabolism,7 cyclic nucleotide metabolism,8,9 glycolytic and lysosomal enzyme activity,10,11 noradrenaline and lysosomal enzyme activity,12 and calcium uptake.13,14

The present study was conducted to determine whether biochemical differences in aortic tissue between the SHR and the normotensive Wistar-Kyoto (WKY) rats were affected by the reversal of hypertension. We also examined the effect of mineralocorticoid-induced hypertension on aortic metabolism in the WKY strain, and the subsequent effect of returning the rats to a normotensive state.

Methods

Male SHR and WKY rats from the Okomoto-Aoki strain were used. Rats were supplied by Taconic Farms or Charles River Breeding Laboratories and, upon arrival, were housed individually, fed normal rat chow (Purina), and handled by the same person. Blood pressure was measured using a tail cuff method (Narco Bio Systems, Inc.) on unanesthetized rats maintained prior to measurement of blood pressure at 33–35°C for 5–10 minutes in a specially constructed chamber.

For the studies comparing biochemical characteristics of SHR and WKY rats between 6 and 21 weeks of age animals of both strains and of designated ages (5, 10, 15, and 20 weeks), were delivered weekly by the supplier over a 3-week period and were killed 7–10 days after arrival. On each of 3 successive weeks, aortic tissue was obtained from rats of each age group in both strains and of designated ages, and, upon arrival, were housed individually, fed normal rat chow (Purina), and handled by the same person. Blood pressure was measured using a tail cuff method (Narco Bio Systems, Inc.) on unanesthetized rats maintained prior to measurement of blood pressure at 33–35°C for 5–10 minutes in a specially constructed chamber.

For the studies comparing biochemical characteristics of SHR and WKY rats between 6 and 21 weeks of age animals of both strains and of designated ages (5, 10, 15, and 20 weeks), were delivered weekly by the supplier over a 3-week period and were killed 7–10 days after arrival. On each of 3 successive weeks, aortic tissue was obtained from rats of each age group in both strains and aortic homogenates were prepared by pooling five aortas per homogenate for 6-week-old rats and three aortas per homogenate from the remaining age groups.

Antihypertensive Drug Treatment

Thirty-nine-week-old rats (WKY and SHR) were divided into three groups for each strain. Group I consisted of rats killed at the beginning of the experiment, (39 weeks old); group II consisted of rats fed a normal diet and left untreated for an additional 17 weeks; group III consisted of rats treated with antihypertensive drugs essentially as described by Freis.15 These animals were given tap water containing reserpine (1.4 mg/liter) hydralazine (100 mg/liter) and chlorothiazide (1 g/liter). Blood pressure and body weight were monitored weekly. At the end of the treatment period, eight rats from groups II and III were killed. Aortic tissue from all groups was weighed and homogenized using two aortas per homogenate.

Deoxycorticosterone (DOC) Treatment

Deoxycorticosterone pivalate (Percoten, Ciba) was administered subcutaneously (1.5 mg/100 g body weight) at the beginning of each treatment week to 11-week-old WKY rats, uninephrectomized 5–7 days prior to treatment. Rats treated with DOC had 1% saline added to the drinking water. In one series of experiments, animals were treated with DOC and saline for 4 weeks and then maintained on a sodium-deficient diet (Tecklad Test Diets) with no subsequent DOC-saline treatment for up to 8 additional weeks. Control rats were neither uninephrectomized nor given DOC, but were maintained on the identical food and water diet given to the treated animals. In addition, a small group of rats (6) was treated with DOC, but without saline. Blood pressure and body weight were monitored weekly throughout the entire experimental period. Rats from the control and treatment groups were killed 4 weeks after treatment was initiated, and 4 and 8 weeks following the cessation of DOC treatment. Three separate aortic homogenates (two aortas per homogenate) were prepared from each group at the time they were killed.

In a second series of experiments, uninephrectomized WKY rats were treated with DOC and saline for 7 weeks, then maintained on a low-salt diet for an additional 11 weeks. Control rats were uninephrectomized and maintained on the same diet as the treated animals, but were sham-injected with saline during the 7-week treatment period. Rats from both groups were killed after 7 weeks of treatment, and 3 and 11 weeks after treatment was discontinued, with three separate aortic homogenates prepared from each group after the rats were killed.

Tissue Preparation

Rats were killed by decapitation. Aortic tissue was removed from the heart to the iliac bifurcation, carefully stripped free of adventitia, and weighed. Routinely, aortas were pooled (2–5/group), minced, and homogenized in a 10-fold excess (wt/vol) of 0.15 M NaCl, 0.01 M Tris buffer, pH 7.4. Homogenization was performed at 0–2°C with a motor-driven, glass-glass apparatus and was continued until no visible particles of tissue remained. Samples of the homogenate were taken for enzymatic or chemical analysis.

Enzyme Determinations

All enzyme assays were performed in duplicate or triplicate under conditions that were optimized in preliminary experiments using aortic homogenates from WKY rats. Using the assay conditions described below, we found that reaction rates were linear with incubation time, directly proportional to
the amount of tissue protein added, and zero order with respect to substrate concentration. The pH values chosen for the assays were all within the pH optima range determined for each enzyme in preliminary experiments.

5'-Nucleotidase

Incubations were performed at pH 9.1 and contained 5 mM adenosine monophosphate, 10 mM MgCl₂, 0.1 mM glycine, and 20 µl of aortic homogenate in a total volume of 0.5 µl. Following incubation at 37°C for 30 minutes, the reaction was terminated by the addition of 2.5 ml of 8% trichloroacetic acid. The tubes were chilled on ice and centrifuged at 3000 g for 5 minutes at 5°C. Samples of the supernatant fluid were used for determination of inorganic phosphorus by the procedure of Fiske and Subbarow. Blanks were obtained in the absence of both tissue fraction and reagent mixture.

Glucose-6-phosphatase

Incubations were performed at pH 6.5 and contained 20 mM glucose-6-phosphate, 16.5 mM histidine, 1 mM EDTA, and 0.1 ml of aortic homogenate in a total volume of 0.5 ml. Following incubation at 37°C for 120 minutes, the reaction was terminated with 8% trichloroacetic acid and inorganic phosphorus determined as described above.16

Acid Phosphatase

Two methods for measuring acid phosphatase activity were used. Method 1: Incubation mixtures contained 1.0 ml of 0.05 M β-glycerophosphate in 0.1 M acetate buffer, pH 5.0, to which 0.1 ml of aortic homogenate was added. Incubations were conducted at 37°C for 2 hours, the reaction terminated with 1.0 ml of 10% trichloroacetic acid, and inorganic phosphorus determined.16 Method 2: Incubations were performed at pH 4.5 and contained 5.7 mM p-nitrophenyl phosphate, 56 mM sodium acetate, and 10 µl of aortic homogenate in a total volume of 400 µl. Following incubation at 37°C for 15 minutes, the reaction was stopped with 2.1 ml of 1.0 N NaOH, centrifuged at 3000 g for 10 minutes and the p-nitrophenol released as a result of hydrolysis measured at 400 nm. The activity determined by both methods was unaffected by 5 mM EDTA and was completely inhibited by 4 mM NaF.

N-Acetyl β-glucosaminidase

Activity was determined by measuring the release of p-nitrophenol resulting from the hydrolysis of p-nitrophenyl NAGA, using incubation conditions identical to those described previously by us in studies on rabbit aortic tissue.17

Analytical Methods

Protein was determined on 50-µl samples of tissue homogenates by a micro-Kjeldahl procedure.18 DNA was measured on samples of the total homogenate of 10,000 g pellets using the method of Burton.19 Collagen and elastin content was estimated by measuring the amount of hydroxyproline and desmosines in the aortic homogenates. Samples were hydrolyzed in 6.0 N HCl at 110°C for 20 hours. Hydrolysates were analyzed for hydroxyproline, desmosine, and isodesmosine on a Jenacl 6AH amino acid analyzer, and the results expressed as nmol of product per mg total aortic protein.

Results

Initially, experiments were performed to compare several characteristics of the SHR and WKY rat between the ages of 6 and 21 weeks. Comparative data for both strains are listed in Table 1. Blood pressure in the SHR increased progressively with age and was greater than that of WKY rats at all ages examined. WKY rats were heavier than SHR at all ages studied and grew at a more rapid rate. Aortic wet weights of the WKY rats also were slightly greater than those of the SHR for each age group, but if aortic weights were expressed as a function of body weight (e.g., mg aortic weight/g body weight), this ratio would be greater for the SHR than the WKY rats, particularly for the older animals.

Homogenates were prepared from pooled aortic tissue on the basis of tissue wet weight, and three

<table>
<thead>
<tr>
<th>Strain</th>
<th>Age (weeks)</th>
<th>Blood pressure (mm Hg)</th>
<th>Body weight (g)</th>
<th>Aortic wet weight (mg)</th>
<th>Homogenate protein (mg/ml)</th>
<th>Concentration (µg/ml protein)</th>
<th>Total content (µg/aorta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>6</td>
<td>121 ± 3.8</td>
<td>147 ± 7</td>
<td>46.7 ± 1.1</td>
<td>23.0 ± 0.6</td>
<td>8.17 ± 0.30</td>
<td>88.2 ± 3.5</td>
</tr>
<tr>
<td>WKY</td>
<td>11</td>
<td>134 ± 4.2</td>
<td>281 ± 5</td>
<td>71.1 ± 1.2</td>
<td>23.2 ± 0.2</td>
<td>6.35 ± 0.32</td>
<td>103.6 ± 4.1</td>
</tr>
<tr>
<td>WKY</td>
<td>16</td>
<td>137 ± 3.7</td>
<td>326 ± 3</td>
<td>78.0 ± 4.7</td>
<td>23.9 ± 1.0</td>
<td>7.59 ± 0.36</td>
<td>106.3 ± 3.6</td>
</tr>
<tr>
<td>WKY</td>
<td>21</td>
<td>130 ± 4.3</td>
<td>371 ± 11</td>
<td>84.3 ± 5.3</td>
<td>25.2 ± 0.9</td>
<td>9.21 ± 0.28</td>
<td>109.9 ± 3.3</td>
</tr>
<tr>
<td>SHR</td>
<td>6</td>
<td>146 ± 3.9***</td>
<td>131 ± 2*</td>
<td>44.2 ± 0.9</td>
<td>22.6 ± 1.0</td>
<td>9.15 ± 0.61</td>
<td>91.2 ± 3.6</td>
</tr>
<tr>
<td>SHR</td>
<td>11</td>
<td>169 ± 3.8***</td>
<td>248 ± 5***</td>
<td>66.6 ± 1.5*</td>
<td>23.0 ± 0.8</td>
<td>6.36 ± 0.69</td>
<td>97.1 ± 4.2</td>
</tr>
<tr>
<td>SHR</td>
<td>16</td>
<td>184 ± 4.4***</td>
<td>289 ± 11***</td>
<td>71.8 ± 1.9</td>
<td>24.4 ± 0.5</td>
<td>5.75 ± 0.36</td>
<td>100.3 ± 3.9</td>
</tr>
<tr>
<td>SHR</td>
<td>21</td>
<td>192 ± 5.1***</td>
<td>309 ± 4**</td>
<td>75.3 ± 3.7</td>
<td>25.0 ± 0.7</td>
<td>5.54 ± 0.25</td>
<td>104.8 ± 4.1</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE. Blood pressure, body weight, and aortic wet weight were obtained on groups containing 9 rats. Protein and DNA concentrations were obtained on three separate homogenates per group. Total content, µg DNA/aorta = DNA (µg/ml homogenate) x volume of original homogenate (ml) / number of aorta homogenized. Asterisks (*, **, *** ) indicate significant differences between SHR and age-matched WKY rats.

* P < 0.05; ** P < 0.01; *** P < 0.001.
separate homogenates were made for each age group in both strains. The protein concentration of the homogenates was similar for all groups. DNA concentration expressed relative to aortic protein content decreased progressively with age in both species, but total DNA per aorta increased with age. No statistically significant differences in DNA concentration in the homogenates were observed between age-matched SHR and WKY rats.

Assays for several enzymes often considered as markers for specific subcellular fractions were conducted, using freshly prepared homogenates, and the data are shown in Table 2. In general, enzymatic activity was greater in the SHR than in the corresponding WKY age group. 5'-Nucleotidase activity tended to increase progressively with age in the SHR, almost doubling in specific activity between 6 and 21 weeks, but remained relatively constant in the normotensive controls. Glucose-6-phosphatase activity was greater in the SHR at all ages, although this difference was not statistically significant at 16 weeks. The enzymes, NAGA and acid phosphatase, which are associated at least in part with lysosomes, showed no pronounced age-related change in either the WKY or SHR, but NAGA activity did increase slightly in the SHR. This increase would be more pronounced if expressed relative to DNA rather than tissue protein. The studies summarized in Table 1 and 2 showed clearly that biochemical differences existed between the two strains and suggested that these differences could be due in part to the duration of hypertension.

To assess further the relationship between blood pressure and aortic metabolism, experiments were performed to determine whether the enhanced enzymatic activity observed in rats with established hypertension would be affected by a drug regimen designed to lower blood pressure. Thirty-nine-week-old SHR and WKY rats were treated with a combination of antihypertensive drugs added to their drinking water for a period of 17 weeks. They were killed at the beginning and end of the treatment period.

Table 3 shows the blood pressure, body weight, and heart and aortic wet weights for each of the six groups. At 39 weeks of age (group I), blood pressure in the WKY rats averaged 137 mm Hg, a value similar to that of WKY rats at 21 weeks of age (see Table 1). Blood pressure remained unchanged in the WKY rats during the subsequent 17 weeks of treatment with antihypertensive drugs. Blood pressure of the SHR at 39 weeks of age (SHR-group I) averaged 220 mm Hg and was markedly affected by drug treatment. By the 3rd week of treatment, average pressures were reduced to 150 mm Hg and fluctuated between 140 and 165 mm Hg for the remainder of the treatment period. Pressures in the untreated SHR remained in a range between 195 and 240 throughout the 17-week period.

Body weights of the WKY rats were consistently greater than those of the SHR. Weight gain during the 17-week experimental period was less in the drug-treated rats from both strains than in the corresponding untreated controls. Heart weights were greater in SHR than WKY rats, this difference being more pronounced if expressed as heart weight-body weight ratios. A reduction in heart weight was observed as a result of drug treatment in both groups. Aortic weights increased over the 17-week period in the untreated rats from both groups, but drug treatment appeared to suppress this increase, particularly in the SHR.

Results of enzymatic studies on samples of aortic homogenates from the different groups are shown in Figure 1. NAGA activity was more than 2-fold greater in untreated SHR than in WKY rats at the beginning and end of the experimental period. Drug treatment of the SHR resulted in a marked reduction of NAGA activity to a value similar to that found in the WKY rats. In contrast, NAGA activity was unaffected by drug treatment of the WKY rats. Acid phosphatase activity was higher in the untreated SHR than the age-matched WKY rats at the beginning and end of the experiment; however, a statistically significant increase in activity (P < 0.01) did occur in the untreated groups of both strains over the 17-week period. Drug treatment lowered acid phosphatase activity in the SHR but

<table>
<thead>
<tr>
<th>Strain</th>
<th>Age (weeks)</th>
<th>NAGA (nmol/min per mg)</th>
<th>Acid phosphatase† (nmol/min per mg)</th>
<th>5'-Nucleotidase (nmol/min per mg)</th>
<th>Glucose-6-phosphatase (nmol/min per mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>6</td>
<td>2.07 ± 0.13</td>
<td>1.05 ± 0.03</td>
<td>15.0 ± 1.4</td>
<td>0.701 ± 0.030</td>
</tr>
<tr>
<td>WKY</td>
<td>11</td>
<td>1.64 ± 0.19</td>
<td>1.04 ± 0.02</td>
<td>18.7 ± 0.7</td>
<td>0.583 ± 0.013</td>
</tr>
<tr>
<td>WKY</td>
<td>16</td>
<td>1.86 ± 0.26</td>
<td>1.03 ± 0.04</td>
<td>19.1 ± 1.0</td>
<td>0.635 ± 0.057</td>
</tr>
<tr>
<td>WKY</td>
<td>21</td>
<td>1.72 ± 0.17</td>
<td>1.11 ± 0.01</td>
<td>18.9 ± 1.6</td>
<td>0.613 ± 0.036</td>
</tr>
<tr>
<td>SHR</td>
<td>6</td>
<td>3.63 ± 0.10***</td>
<td>1.36 ± 0.03**</td>
<td>16.8 ± 1.1</td>
<td>0.909 ± 0.037</td>
</tr>
<tr>
<td>SHR</td>
<td>11</td>
<td>4.10 ± 0.16***</td>
<td>1.15 ± 0.06**</td>
<td>22.6 ± 0.2**</td>
<td>0.839 ± 0.008***</td>
</tr>
<tr>
<td>SHR</td>
<td>16</td>
<td>4.08 ± 0.25**</td>
<td>1.20 ± 0.04*</td>
<td>27.9 ± 0.3**</td>
<td>0.830 ± 0.002</td>
</tr>
<tr>
<td>SHR</td>
<td>21</td>
<td>4.51 ± 0.33**</td>
<td>1.26 ± 0.03**</td>
<td>30.0 ± 0.2**</td>
<td>0.880 ± 0.040**</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE (n = 3). Asterisks indicate significant differences between SHR and age-matched WKY controls.

† Determined using 0-glycerophosphate as substrate (Method 1, see Methods section). See Table 1 for P values.
no change was observed in the treated WKY rats as a result of drug treatment. The values shown in Figure 1 for acid phosphatase activity were obtained using p-nitrophenyl phosphate as substrate (see Methods). In this series of experiments, acid phosphatase activity also was measured using β-glycerophosphate as substrate, and the relative differences between groups were similar with both methods, although activities were approximately 4-fold higher with the nitrophenyl ester.

5'-Nucleotidase activity was more than 3-fold greater in SHR than WKY rats at 39 weeks of age, and this difference was maintained throughout the 17-week period for the untreated animals. Drug treatment did not affect 5'-nucleotidase activity in the WKY rats. Although a partial reduction of 5'-nucleotidase activity did occur in the drug-treated SHR group, the activity remained significantly greater than that observed in the WKY rats.

Samples of the homogenates from groups II and III of both strains also were analyzed for collagen and elastin content. Table 4 shows these data expressed as the amount of hydroxyproline per milligram of homogenate protein, reflecting the collagen content, and the combined amount of desmosine and isodesmosine residues which are a measure of the elastin content. The data indicate that no appreciable differences in concentration of collagen or elastin were apparent between the treated or untreated groups from either strain.

The enzymatic changes observed in the above experiment as a result of drug treatment indicated that the increased aortic enzymatic activity in the SHR was related to the hypertension. To determine whether normotensive rats would be affected by hypertension and its reversal in an analogous manner, and to compare the influence of two etiologically different forms of hypertension, we treated WKY rats with DOC and saline for a 4-week period to promote hypertension and they were then maintained on a low-salt diet with no subsequent DOC treatment to effect a lowering of blood pressure.

The data in Figure 2 indicate the characteristics of the DOC-saline-treated rats expressed relative to those of control animals. Systolic blood pressure rose to 170 mm Hg by the 2nd week of treatment and, by 4 weeks, averaged 192 mm Hg. After treatment was discontinued, blood pressure in the pretrained rats decreased steadily over a 3-week period and, by the end of the 4th week posttreatment, pressures were not significantly different from controls. Body weights were similar for control and DOC-treated groups throughout the experimental period (date not shown). Heart and aortic wet weights were increased significantly after 4 weeks of treatment, but decreased gradually toward control levels after treatment was discontinued.
TABLE 4  Effect of Antihypertensive Drug Treatment on Aortic Collagen and Elastin content

<table>
<thead>
<tr>
<th>Group</th>
<th>Homogenate protein (mg/ml)</th>
<th>Hydroxyproline (nmol/mg protein)</th>
<th>Desmosine plus isodesmosine (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY II (untreated)</td>
<td>23.6 ± 1.1</td>
<td>379 ± 19</td>
<td>6.90 ± 0.35</td>
</tr>
<tr>
<td>WKY III (treated)</td>
<td>23.8 ± 1.4</td>
<td>391 ± 21</td>
<td>7.82 ± 0.60</td>
</tr>
<tr>
<td>SHR II (untreated)</td>
<td>22.9 ± 0.5</td>
<td>352 ± 9</td>
<td>7.40 ± 0.30</td>
</tr>
<tr>
<td>SHR III (treated)</td>
<td>23.2 ± 0.4</td>
<td>414 ± 15</td>
<td>8.38 ± 0.05</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE (n = 3).

DOC-induced hypertension in the WKY rats clearly enhanced the activity of the enzymes studied. NAGA activity was increased almost 2.5-fold over controls, and both acid phosphatase and 5'-nucleotidase levels approximately doubled. No significant changes in enzymatic activity occurred in either the non-DOC-treated control rats over the 12-week experimental period or in a separate group of DOC-treated rats lacking saline in their drinking water over a 4-week treatment period. Of particular interest were the effects observed after treatment was discontinued and blood pressure was reduced. The activities of the lysosomal enzymes NAGA and acid phosphatase decreased nearly to control levels by the 4th week posttreatment and remained essentially unchanged after 4 additional weeks. In contrast, 5'-nucleotidase activity remained significantly greater than control levels even after 8 weeks on the low-salt diet. These relative changes in enzymatic activity in the WKY strain as a result of induction and reversal of hypertension were similar to those already noted in the SHR following antihypertensive drug treatment, (Fig. 1).

An additional series of experiments was performed to determine whether the reversibility of the biochemical changes induced by DOC-salt treatment for 4 weeks also would occur if the duration of hypertension was prolonged. WKY rats were treated with DOC and saline for 7 weeks, at which time drug treatment was discontinued, and the rats were maintained for up to 11 additional weeks without treatment. Figure 3 summarizes the data obtained in these experiments. Blood pressure rose gradually during DOC treatment to levels of systolic pressure ranging from 220 to 263 mm Hg. Pressures decreased slightly 2 weeks after treatment was discontinued (215 ± 8 mm Hg) and then decreased progressively, reaching normotensive levels (137 ± 4 mm Hg) before the 6th week posttreatment.

Heart weights were greater in the hypertensive rats (average wet weight = 1.08 g) than in the controls (0.81 g) at the end of the treatment period, and this relative difference persisted 3 weeks after treatment was discontinued. Eleven weeks after treatment, the differences in heart weight between the groups was reduced, but average weights were still greater in the pretreated rats (1.23 g vs. 1.09 g). Average wet weight of aortic tissue increased almost 150% over controls as a result of DOC treatment for 7 weeks, (117 mg vs. 81 mg), and this relative difference was maintained throughout the posttreatment period.

Enzymatic activity was essentially unchanged in the control rats over the 18-week experimental period. The activities of all three enzymes increased after 7 weeks of DOC-saline treatment, the relative increase for NAGA averaged 257%, acid phosphatase, 196%, and 5'-nucleotidase, 161% of control values. During the 11-week posttreatment period, NAGA and acid phosphatase activity both decreased but NAGA still remained significantly elevated. In contrast, 5'-nucleotidase activity remained constant during the posttreatment period despite the reversal of hypertension.

Total collagen and elastin content of aortas of

FIGURE 2  Changes produced by DOC treatment of WKY rats for a 4-week period are expressed as the percent increase over untreated age-matched WKY controls. Values for blood pressure, heart weight, and aortic weight were obtained from six rats per group. Enzymatic data were obtained on three separate homogenates for each group. Asterisks indicate significant differences between treated rats and age-matched controls killed at the same time: *P < 0.05; **P < 0.01; ***P < 0.001.
the DOC-treated rats increased significantly, this increase being proportional to the increased wet weight of aortic tissue. However, when expressed on the basis of tissue protein, no changes in the concentrations of either collagen or elastin were apparent as a result of hypertension or its reversal. Hydroxyproline concentration was 435 ± 21 nmol/mg total protein (mean ± se for three homogenates) after 7 weeks of DOC treatment, and was 406 ± 15 nmol/mg in the corresponding controls. Eleven weeks after treatment, the values for treated and control tissues averaged 441 and 437 nmol/mg, respectively. The average concentrations for desmosine and isodesmosine residues, reflecting elastin content, ranged between 4.8 and 5.6 nmol/mg in all groups.

Discussion

These studies have examined the effects of reversal of hypertension on aortic metabolism. Differences in enzymatic activity between SHR and WKY rats were apparent even in 6-week-old animals despite only mild elevations in blood pressure for a relatively brief period. Age-related increases in NAGA and 5'-nucleotidase activity were observed in the SHR but not WKY rats, suggesting that these progressive changes were related to the duration of hypertension. Although strain-specific differences between the WKY and SHR could have accounted for the different enzymatic activities, this is unlikely, since hypertension in the WKY rats induced by DOC-saline treatment produced increases in enzymatic activity analogous to those measured in the SHR.

Enzymatic activity was determined by assaying samples of the aortic homogenate, thereby providing a quantitative estimate of total tissue activity. The vigorous homogenization procedure required to disrupt aortic tissue precluded the use of conventional subcellular fractionation procedures to isolate intact organelles. However, other studies employing sophisticated fractionation techniques to isolated aortic smooth muscle cells from the rabbit indicated the localization of acid phosphatase and NAGA activity in a lysosomal fraction.11,22 Histochemical studies also localized both enzymatic activities within rat aortic lysosomes.4 Neither of those studies showed an exclusive localization of either NAGA or acid phosphatase within lysosomes, and it is possible that enzymatic activity also is associated with other cellular fractions. 5'-Nucleotidase activity was shown to be localized in plasma membrane-enriched fractions from rat aorta14 and would appear to be a reliable marker for that membrane. Glucose-6-phosphatase activity has been used as a marker for endoplasmic reticulum of liver cells,23 but there is no evidence that this activity reflects the endoplasmic reticulum of vascular smooth muscle.

The most definitive results establishing the relationship between hypertension and aortic enzyme activity were obtained from experiments in which hypertension was reversed. This was accomplished by drug therapy in the 39-week-old SHR with established hypertension and by withdrawal of DOC and salt in WKY rats. NAGA and acid phosphatase were sensitive to the effect of hypertension, and when hypertension was reversed in the SHR with prolonged drug therapy, these lysosomal enzymes decreased to control levels. In the WKY group pretreated with DOC and saline for 4 weeks, a similar normalization of lysosomal activity was observed. However, in rats treated with DOC for 7 weeks, NAGA activity did not return completely to control levels even after treatment was discontinued for 11 weeks.

In contrast to the lysosomal enzymes, the elevated levels of 5'-nucleotidase activity diminished only slightly after either antihypertensive drug therapy or cessation of DOC. Possibly, longer periods of normotension would result eventually in a return of 5'-nucleotidase activity to control levels,
but the data clearly indicate that the changes in this enzyme are less reversible that those of the lysosomal enzymes. 5'-Nucleotidase has been used as a marker enzyme for aortic plasma membrane and has been shown to be higher in aortic plasma membrane fractions from SHR than in Wistar rats. Adenosine, a product of 5'-nucleotidase activity, is known to be an effective vasodilator and has been implicated as a potential agent for regulating blood pressure however additional studies are required to determine whether the changes in aortic 5'-nucleotidase activity reflect a physiological adaption to hypertension or result from other causes, such as nonspecific changes in membrane structure.

No change in the concentration of either hydroxyproline or desmosines relative to total protein or aortic wet weight was observed as a result of hypertension in the genetic or mineralocorticoid-treated rat models. The data were expressed directly as the amounts of these amino acids, as measured on the amino acid analyzer, rather than assuming an undetermined molar ratio of these amino acids in collagen or elastin and calculating total amounts of those proteins. Our findings are consistent with those of Wolinsky who found that hypertension in young rats produced by renal clipping resulted in absolute increases in aortic collagen and elastin, although the relative proportions of these proteins were not altered. Hollander et al. also found absolute but not relative increases in connective tissue protein both in DOC-saline treated rats and in the SHR model.

An increase both in aortic lysosomal enzyme activity and connective tissue content was observed in rats following the production of hypertension by renal artery clipping, and these effects were suppressed by treatment with estrogen or methylprednisolone during the hypertensive phase. In our studies, WKY rats treated with DOC for 7 weeks had increased total amounts of connective tissue proteins, and following an 11-week period when the hypertension was reversed, aortic weights remained greater than controls, as did the total amount of connective tissue proteins. However, activity of the lysosomal enzymes did decrease substantially during the period of reversal. In a separate study, the increased amount of collagen and elastin found in rat aortic tissue following experimental renal hypertension for a 10-week period also was not decreased after the reversal of hypertension. Several markers of collagen biosynthesis were shown to be increased in the aorta of hypertensive rats and subsequent reduction of blood pressure by reserpine or chlorothiazide were observed to diminish collagen biosynthesis. β-Aminopropionitrile, an inhibitor of lysyl oxidase, an enzyme necessary for cross-linking in collagen and elastin, also reduced aortic collagen biosynthesis in hypertensive rats and lowered the blood pressure as well. Our studies on the SHR with well-established hypertension indicated that changes in the vascular connective tissue component did not occur following antihypertensive drug therapy.

As reported here, the relationship of the enzymatic and chemical changes to the pathological effects of hypertension is uncertain and remains to be determined. Alteration in the continuity of the endothelial cell layer could lead to increased infiltration of plasma constituents, an effect which has been suggested to be a fundamental pathogenetic process in the development of vascular damage. Prior investigations have demonstrated increased endothelial permeability of the vasculature with hypertension, and recently it was shown that endothelial cell replication in the rat aorta was increased 10-fold after renal hypertension of 2-3 weeks' duration. In collateral studies we have observed evidence of subendothelial thickening and infiltration of the subendothelial space with an amorphous material, even with brief periods of hypertension (unpublished observation).

It is possible that enhanced lysosomal activity and changes in connective tissue metabolism are part of the response of medial smooth muscle cells to alterations occurring in the intima. It is interesting to note that endothelial cell damage has been proposed as an initiating factor in the development of atherosclerosis, and vascular changes associated with atherosclerotic disease include enhanced lysosomal activity and an increase in connective tissue. Although both humoral and hemodynamic factors have been implicated in the production of intimal injury, it is also possible that such factors could directly affect medial smooth muscle cell metabolism independently of intimal damage.

The accelerating effect of hypertension on atherogenesis appears to be facilitated by the presence of hyperlipoproteinemia in a variety of species. Although those complications of hypertension which are directly attributable to a blood pressure elevation such as congestive heart failure and strokes can be prevented or reduced in incidence by adequate antihypertensive therapy, the complications related to atherosclerosis such as myocardial infarction may be uninfluenced or affected only minimally by lowering of blood pressure. The major clinical studies relating to the influence of antihypertensive therapy on the incidence of complications generally have involved middle-aged individuals with long-standing hypertension. Most such patients could be expected to have significant atherosclerotic disease, which might not be influenced by blood pressure lowering once it had become established. The current studies are potentially relevant in this regard in that they have demonstrated that certain biochemical alterations induced by hypertension in the arterial wall may persist despite correction of the hypertension.

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