Collagen Metabolism and Reversal of Aortic Medial Hypertrophy in Spontaneously Hypertensive Rats Treated With Methyldopa

L. ALLEN EHRRHART, PH. D. AND CARLOS M. FERRARIO, M.D.

SUMMARY Collagen synthesis, content, and concentration were determined in the hypertrophied intima media of thoracic aortas from 10-, 15-, and 20-week-old spontaneously hypertensive rats (SHR). Although the rates of aortic collagen synthesis declined with age, the dry weight of the intima media and the total collagen content increased proportionally. Collagen concentration thus remained unchanged. Methyldopa was administered orally to SHR when they were 12 to 15 weeks of age, when their body weights were identical to the untreated group. Blood pressure and the degree of aortic medial hypertrophy, judged by medial dry weight per kilogram body weight, were significantly lower compared with untreated SHR. Collagen synthesis was likewise decreased to a mean rate not significantly higher than age-matched normotensive Wistar-Kyoto controls. This reduction in collagen synthesis, however, was not sufficient to decrease measurably the total collagen content of the aortas compared with untreated SHR. Since medial dry weights were lower in the treated rats, collagen concentration in aortas from SHR given methyldopa for 3 weeks was actually increased. The increase in collagen concentration also suggests that medial hypertrophy was reversed.

KEY WORDS • collagen • methyldopa • aortic hypertrophy • spontaneous hypertension • reversal

A common structural response to hypertension of various etiologies is a thickening of the medial layer of both large and small arteries. Medial thickening is due to hyperplasia and hypertrophy of smooth muscle cells with concomitant increases in collagen, elastin, glycosaminoglycans, electrolytes, and water.1-3 Increases in the total arterial content of these components is not always accompanied by an actual increase in the concentration expressed per unit of wet or dry weight, deoxyribonucleic acid (DNA), or protein. Therefore, a distinction between alterations in arterial content and concentration is of considerable importance because it is directly relevant to our understanding of the mechanisms underlying the development of cardiovascular hypertrophy. Wolinsky,4 for example, showed that collagen content, but not concentration, was increased in the thickened media of thoracic aortas from renal hypertensive rats. Sen et al.5 likewise showed that total collagen content but not concentra-

tion was increased in hypertrophied ventricles of young adult spontaneously hypertensive rats (SHR) compared with normotensive controls. The latter also demonstrated that reversal of cardiac hypertrophy in SHR by treatment with methyldopa6,7 was not accompanied by a parallel decrease in myocardial collagen content. This finding may have some bearing upon the functional consequences of cardiac hypertrophy as well as its reversal. Skelton and Sonnenblick8 have suggested that alterations in cardiac collagen might influence ventricular compliance. Studies by Spech et al.9 and Pfeffer et al.10 provide additional, albeit indirect, evidence linking alterations in collagen metabolism with pump function.

The purpose of the present experiments was to determine whether the arterial wall in spontaneously hypertensive rats responds structurally and biochemically in a manner similar to that described for the myocardium of SHR and, furthermore, whether aortic collagen metabolism can be modified by antihypertensive therapy with methyldopa at a dose sufficient to normalize the elevated blood pressure (BP). With this in mind, we investigated the alterations in aortic hypertrophy and collagen metabolism in the thoracic aorta of rats with spontaneous hypertension both as a function of age and after administration of methyldopa for a 3-week period.
Methods

Animal Studies

Spontaneously hypertensive male rats from the Okamoto-Aoki strain were obtained from Charles River Breeding Laboratories (Wilmington, Massachusetts) and normotensive Wistar-Kyoto controls (WKY) from Taconic Farms (Germantown, New York) at 7 weeks of age. They were housed in plastic cages in groups of two to four and fed a pelleted rat diet (Teklad, Madison, Wisconsin). Systolic BPs were monitored once or twice weekly by a tail-cuff method similar to that described by Williams et al.5 Groups of SHR were terminated at 10, 15, and 20 weeks to examine the age-related changes in arterial collagen metabolism. Another group of SHR was given alphamethyldopa (Aldomet) daily when they were 12 to 15 weeks of age. Age-matched WKY animals served as controls. Methyldopa was administered by dissolving powdered Aldomet (Merck, Sharpe and Dohme, Rahway, New Jersey) in deionized drinking water at concentrations varying from 2.5 to 5.0 g/liter, to overcome drug tolerance and maintain the BP at or near normotensive levels.6

Biochemical Studies

Rates of collagen synthesis were determined in vitro on descending thoracic aortas consisting of the entire segment between the left subclavian and celiac artery branch points. Loosely adhering periadventitial tissue was quickly removed, and the aortas were incubated for 30 minutes at 37°C in 2 ml of Earle’s bicarbonate-buffered balanced salt solution containing 0.2 mM nonlabeled L-proline. After 5 µCi of [U-14C]-L-proline were added, the tubes were gassed with a mixture of 95% oxygen and 5% carbon dioxide, capped, and incubated for 4 hours. Protein synthesis and further proline hydroxylation were arrested by placing the incubated tissue in 15 ml of cold saline containing 100 µg/ml of cyclooxyamide and 1 mM α,α'-dipyridyl for 10 minutes, followed by two additional rinses in iced saline. Thoracic intima media (IM) was isolated completely from the adventitial layer by the dissection method of Wolinsky and Daley.7 Trichloroacetic acid was added to the incubation medium for subsequent determination of proline concentration8 and specific activity of labeled proline in the supernatant after centrifugation.

Wet weights of IM were recorded prior to obtaining dry defatted weights after repeated overnight extractions of lipid with acetone:ether (1:1) at 4°C. Collagen was then extracted by successive gelatinizations into 2.5 ml of water at 120°C for 18 hours and again for 3 hours. Exhaustive dialysis of the pooled extracts against water to remove any labeled free proline and polypeptides with molecular weights lower than 12,000 was followed by hydrolysis of collagen and other extracted proteins in 6 N HCl at 120°C for 18 hours. Hydrolysates were evaporated to dryness and redissolved in 5 ml of water, from which aliquots were removed for determination of hydroxyproline concentration.9 Separation of labeled hydroxyproline from proline by ion-exchange chromatography was performed as described by Ehrhart and Holderbaum10 and McCullagh and Ehrhart.11 Radioactivity in the hydroxyproline, isolated from the gelatinization extract, arises solely from incorporation of isotopically labeled proline into procollagen, which is subsequently hydroxylated by prolyl hydroxylase to form collagen-bound hydroxyproline. From the total 14C disintegrations per minute (dpm) in the hydroxyproline fraction and the specific activity of 14C-proline in the incubation medium, which was previously shown to be identical to that of free proline in the tissue itself,12 we calculated the moles of proline incorporated into the aortic collagen-bound hydroxyproline during the 4-hour incubation period. We then derived the amount of collagen synthesized by multiplying by 6.94 the quantity of hydroxyproline synthesized, assuming that hydroxyproline comprises 14.4% of the amino acid content of aortic collagen.13 We calculated in the same way the total collagen content in the aortic IM from the amount of hydroxyproline determined in the hot water extract of the aortic collagen. The significance of the differences in mean values between the groups was determined by the two-tailed Student’s t test.

Results

Effects of Aging on the Collagen Metabolism in the Thoracic Aortas of SHR

Changes in body weight, BP, and dry defatted weight of the thoracic aortic IM of SHR at 10, 15, and 20 weeks are shown in table 1. Systolic BP in these animals was elevated at all ages studied, reaching a plateau at 15 to 20 weeks of age. Compared to 10-week-old SHR, IM dry weight was markedly elevated at both 15 and 20 weeks of age. In the young adult SHR (20 weeks), the dry weight of the aortic media increased an average of 83% over values determined at 10 weeks of age. On the other hand, the ratio of medial dry weight to body weight did not reflect the changes in IM hypertrophy since the rats had a proportional weight gain of 78% between the 10th and 20th week of age (table 1). It should be noted, however, that the medial-dry-weight-to-body-weight ratios at the three ages studied were all significantly greater than that exhibited by the 15-week-old WKY controls (table 2). Medial dry weight per kilogram of body weight was slightly lower in 15-week-old SHR compared with the 10- and 20-week-old groups.

Rates of collagen synthesis expressed both as ng/100 mg of IM/4 hrs and as a fractional rate of synthesis (ng/mg of collagen/4 hrs) decreased from 10 to 20 weeks (table 3). Although the collagen content in the total thoracic IM increased significantly between 10 and 20 weeks of age, the change was proportional to increases in IM weight. This accounts for the lack of significant increases in collagen concentrations as the animals matured.
Effect of Methyldopa on Blood Pressure, Aortic-Medial Hypertrophy, and Vascular Collagen Metabolism

A significant reduction of BP was achieved by administering methyldopa to SHR for 3 weeks (table 2). Comparisons between untreated and treated SHR and corresponding normotensive WKY controls at 15 weeks of age showed that the systolic BP of treated SHR was significantly less than in the untreated group (200 mm Hg vs 147 mm Hg) and, moreover, the residual hypertension amounted to only 17 mm Hg above the normotensive values of WKY controls (table 2).

Hypertrophy of the thoracic aorta IM, defined as IM dry weight per kg body weight, was significantly less in SHR receiving methyldopa from the 12th to 15th week compared with untreated SHR both at 15 weeks (table 2) and 10 weeks of age (table 1). Although these data indicate that aortic hypertrophy has been reversed in the treated group, these changes also could be interpreted to reflect a retarded rate of aortic growth relative to the whole body in the drug-treated group. Additional, and perhaps stronger, evidence for an actual reversal of aortic hypertrophy stems from the observed increase in collagen concentration in the thoracic aorta from rats given methyl-

### Table 1. Age Related Changes in SHR from 10 to 20 Weeks

<table>
<thead>
<tr>
<th>Age (wks)</th>
<th>Blood pressure (mm Hg)</th>
<th>Body wt (g)</th>
<th>Aortic media (mg dry weight)</th>
<th>Medial dry weight Body wt (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>172 ± 2</td>
<td>181 ± 7</td>
<td>4.91 ± 0.20</td>
<td>27.10 ± 0.49</td>
</tr>
<tr>
<td>15</td>
<td>200 ± 5</td>
<td>277 ± 6</td>
<td>7.14 ± 0.17</td>
<td>25.78 ± 0.37</td>
</tr>
<tr>
<td>20</td>
<td>205 ± 4</td>
<td>323 ± 12*</td>
<td>9.00 ± 0.15†</td>
<td>28.06 ± 0.88†</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE. Groups consisted of 10 rats at 10 weeks and nine rats each at 15 and 20 weeks of age. The p values represent comparisons with the 10-week-old group.

†p < 0.001 with respect to 15-week-old group.

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Blood pressure (mm Hg)</th>
<th>Body wt (g)</th>
<th>Aortic media (mg dry weight)</th>
<th>Medial dry wt (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR (untreated)</td>
<td>200 ± 5</td>
<td>277 ± 6</td>
<td>7.14 ± 0.17</td>
<td>25.78 ± 0.37</td>
</tr>
<tr>
<td>SHR (treated)</td>
<td>147 ± 1*</td>
<td>276 ± 7</td>
<td>6.58 ± 0.23</td>
<td>23.76 ± 0.44</td>
</tr>
<tr>
<td>WKY</td>
<td>130 ± 3*</td>
<td>274 ± 4</td>
<td>5.61 ± 0.14†</td>
<td>20.51 ± 0.61*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE. Groups contained nine rats each. Differences between groups where no p value is given were not significant (p > 0.1); p values represent comparisons with untreated SHR.

†p < 0.005 with respect to treated SHR.

### Table 3. Collagen Synthesis, Content, and Concentration in Aortic Media of SHR

<table>
<thead>
<tr>
<th>Rat age (wks)</th>
<th>Collagen synthesis (ng/100 mg media/4 hrs)</th>
<th>Collagen synthesis (ng/mg collagen/4 hrs)</th>
<th>Collagen content (µg/total thoracic media)</th>
<th>Collagen concentration (mg/100 mg media)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2579 ± 331</td>
<td>355 ± 57</td>
<td>412 ± 19</td>
<td>8.2 ± 0.2</td>
</tr>
<tr>
<td>15</td>
<td>1729 ± 287</td>
<td>255 ± 51</td>
<td>600 ± 24</td>
<td>8.4 ± 0.2</td>
</tr>
<tr>
<td>20</td>
<td>1321 ± 171</td>
<td>160 ± 29</td>
<td>794 ± 27†</td>
<td>8.9 ± 0.3</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE. Groups are the same as in table 1. Differences between groups where no p value is given were not significant (p > 0.1); p values represent comparisons with 10-week-old group.

*p < 0.001 with respect to 15-week-old group.
dopa (table 4). Increases in collagen concentration also have been consistently demonstrated in the hearts of hypertensive rats after reversal of cardiac hypertrophy by administration of antihypertensive drugs.1-4

A striking decrease in the rate of aortic collagen synthesis was noted in methyldopa-treated SHR. Collagen synthesis fell from 1729 in 15-week-old untreated SHR to 790 ng/100 mg IM/4 hrs in the treated group, a rate that was not significantly higher than the values observed in age- and weight-matched WKY animals. Differences between these groups in fractional rates of collagen synthesis, expressed as ng synthesized/mg collagen/4 hrs, were comparable to those in which the absolute rates were compared (table 4).

Discussion

These experiments have shown that 15-week-old SHR exhibit aortic medial hypertrophy compared with normotensive WKY rats of the same age and weight. Three weeks of antihypertensive therapy by oral administration of methyldopa caused a reduction of aortic medial dry weight per kg body weight in the treated SHR to a level significantly lower than that of untreated SHR, but still higher than that seen in WKY animals. Since dry-weight-to-body-weight ratios were used as an index of hypertrophy, the reduction in aortic medial hypertrophy seen in SHR treated with methyldopa cannot be explained by loss of water from the aortic media of treated rats. Reduction of hypertrophy also would have been evident had wet weights been compared, since data not presented showed no differences in aortic IM wet-weight-to-dry-weight ratios between treated and untreated SHR.

Decreased medial dry weight per kg body weight may well be regarded by itself as insufficient evidence to establish a true reduction or reversal of aortic hypertrophy in the absence of direct measurements of wall thickness or morphometric analyses. Although it could be argued that the changes in aortic weight per kg body weight could have arisen merely due to a slowing of aortic growth relative to the whole body, this is probably not the case. The observed increase in medial collagen concentration in treated rats supports the conclusion that methyldopa reversed aortic hypertrophy. Since the quantity of collagen is calculated from hydroxyproline determinations, this conclusion rests on the assumption or proof that the degree of proline hydroxylation in collagen from the aortas of treated SHR is not higher than in untreated rats. Hydroxyproline-to-proline ratios in aortic collagen-containing extracts from methyldopa-treated rats were, in fact, found to be no greater than those from untreated SHR. Thus, both the reduction of medial dry weight per kg body weight and the increase in medial collagen concentration suggest strongly that reversal of hypertension was accompanied by amelioration of aortic hypertrophy.

We are currently investigating the effects of antihypertensive agents other than methyldopa in experiments similar to the present design (Ehrhart and Sen, unpublished data). Initial studies have thus far shown that normalization of BP in SHR treated with a combination of reserpine, hydralazine, and hydrochlorothiazide from 12 to 16 weeks of age produces changes in aortic weight and collagen concentration similar to those just described for methyldopa. Medial dry weight per kg body weight was decreased 11% (p < 0.05), and collagen concentration was increased from 8.4% of dry weight in untreated SHR aortas to 9.8% in treated animals. Reduction or reversal of aortic medial hypertrophy by lowering the BP with methyldopa therefore does not appear to be a response unique to methyldopa. However, it cannot yet be stated with certainty that the reduction or normalization of BP with antihypertensive regimens other than the two just discussed will produce similar patterns of change in aortic weight and composition, indicative of a consistent response to the lowering of BP regardless of the mode of antihypertensive therapy.

Because a large pool of medial collagen was already present at the time that methyldopa therapy was begun, and because collagen turnover is known to be extremely slow compared with other mammalian proteins, a reduced rate of aortic collagen synthesis was not paralleled by a decrease in collagen content. The decrease in medial hypertrophy was therefore due to a reduction in other aortic components, possibly

<table>
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<td>255 ± 51</td>
<td>600 ± 24</td>
<td>8.4 ± 0.2</td>
</tr>
<tr>
<td>SHR (treated)</td>
<td>[p &lt; 0.025]</td>
<td>[p &lt; 0.01]</td>
<td>610 ± 30</td>
<td>[p &lt; 0.05]</td>
</tr>
<tr>
<td>WKY</td>
<td>619 ± 129</td>
<td>69 ± 16</td>
<td>498 ± 12*</td>
<td>8.9 ± 0.2</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE. Groups are the same as in table 2. Differences between groups where no p value is given were not significant; p values represent comparisons with untreated SHR.

* p < 0.005 with respect to treated SHR.
noncollagen proteins, glycosaminoglycans, or electrolytes, which have been shown to increase in hypertensive vessels. Interpretations of the effects of experimentally induced hypertension on aortic structure, mass, and connective-tissue protein synthesis depend on several factors, including the specific animal model, age of the animals, and availability of suitable age- and weight-matched controls. The age-related decrease in the rates of collagen synthesis observed in the thoracic aortas of SHR in our study can also be inferred from the data of Ooshima et al. They found that aortic prolyl hydroxylase activities were lower in both SHR and WKY rats at 17 weeks of age compared with those at 7 weeks. Furthermore, Yamori also reported that the in vivo incorporation of labeled proline into aortic collagen, expressed as radioactivity per micromole of collagen-bound hydroxyproline, was decreased in both SHR and WKY between 4 and 10 weeks of age.

Although hypertension in SHR was present at 10 weeks of age, the mean systolic BP (172 ± 7 mm Hg) in this group had not yet reached the plateau level seen in 15- and 20-week-old rats. During the same time, their body weights and IM dry weights increased in approximately the same proportion. It is known that increases in medial thickness are associated in SHR with hyperplasia of the arterial smooth muscle cells but the proportionally greater increase in ribonucleic acid (RNA) than in DNA is also indicative of the hypertrophy of the medial smooth muscle cells themselves. In our experiments, thickening of the arterial wall in SHR at each of the ages studied was chiefly due to medial thickening, since there was no evidence of thickening of the intima as judged by light microscopy. Since the aortic intima in both SHR and WKY rats consisted primarily of a single layer of endothelial cells, IM weights can be considered to represent weights of the thoracic media. In addition, there was no increase in IM weight per kg body weight in rats between 10 and 20 weeks of age. Thus, medial tissue hypertrophy is apparently already well established at 10 weeks, an age at which the BP has not yet reached a hypertensive plateau. As with the dry weights of IM, collagen content (μg/total IM) was significantly increased from 10 to 15 weeks and again from 15 to 20 weeks (table 3). Although a trend toward higher concentrations was seen with increasing time, there was no significant increase in mg collagen/100 mg IM when the oldest group was compared with the youngest. These age-related increases in the medial collagen content but not concentrations have also been noted by Hollander et al. in SHR and by Wolinsky in rats with experimental renal hypertension.

Body weights of 15-week-old SHR were nearly identical to WKY normotensive controls and to 15-week-old SHR treated with methyldopa for 3 weeks, affording the opportunity to compare groups of rats that were both age- and weight-matched. Differences in medial dry weights were therefore also significant when normalized for body weights, as commonly done to show organ hypertrophy. Relative to normotensive WKY animals, untreated SHR exhibited a 26% increase in aortic medial hypertrophy. Less hypertrophy was seen in rats receiving methyldopa, but the 16% increase over WKY was still significant (p < 0.005, table 2). It is interesting to note that the difference in hypertrophy shown here between SHR treated with methyldopa and untreated SHR is of the same magnitude as the difference in myocardial hypertrophy observed by Spech et al. in 17- to 29-week-old SHR treated for 3 to 6 weeks with methyldopa. Thus, it appears that the heart and aorta of SHR may respond in a similar fashion to hypertension and its reversal with methyldopa. Increased aortic wet-weight-to-body-weight ratios have been observed in SHR compared with American Wistar and WKY rats by Brecher et al., Newman and Langner, and Fischer. Rates of collagen synthesis, expressed either as nanograms of collagen synthesized/100 mg IM/4 hrs or nanograms synthesized/1 mg aortic collagen/4 hrs, were three- to fourfold greater in 15-week-old SHR thoracic aortas than in 15-week-old normotensive WKY controls (table 4). Aortic synthesis of collagen from labeled lysine in vivo was also shown to be slightly higher in 8-week-old SHR compared with age-matched WKY. Prolyl hydroxylase activities, often regarded as an indicator of collagen synthesis, were shown by Ooshima et al. to be increased in SHR at 7, 17, and 34 weeks of age compared with corresponding WKY controls. Collagen synthesis as a percentage of total aortic protein synthesis was found by Newman and Langner to decline with age in SHR and American Wistar rats. The distribution of labeled proline incorporated into collagen and noncollagen protein was similar in both groups of rats up to 23 weeks of age, at which time the percentage incorporated in collagen was greater in SHR than in Wistar controls. They also observed comparable increases in prolyl hydroxylase activities in the same SHR after 23 weeks. Yamori reported that in vivo incorporation of labeled proline, expressed as dpm/μmole hydroxyproline, into collagenous protein of aortas was higher in 10-week-old SHR than in WKY. Thus, it appears that both BP and aging have an important influence on the rates of collagen synthesis in SHR and normotensive controls, with collagen synthesis generally being higher in the aortas of SHR than in age-matched normotensive controls. The increased rates of synthesis in SHR in our experiments were accompanied by increases in the total collagen content of the thickened medial layers in hypertensive rats, but the resulting collagen concentrations in terms of mg collagen/unit dry weight were not significantly elevated above control levels. Increases in aortic total collagen content but not in the concentration in SHR compared with age-matched WKY have also been observed by Hollander et al. and by Brecher et al. Increased collagen content without increased concentration has also been shown by Wolinsky and Ehre hart et al. in the aortas of renal hypertensive rats.
Methyldopa, even in a relatively short time, was shown to decrease collagen synthesis in SHR to rates not significantly higher than those of WKY of the same age and weight. This decrease was not sufficient to affect a measurable decrease in the aortic collagen content, however, and, in fact, since medial dry weights were lower in the treated rats, collagen concentration in the aortas of rats given methyldopa for 3 weeks was actually increased. These observations in aortas where medial hypertrophy has been reduced are thus analogous to the pattern seen with reversal of cardiac hypertrophy in SHR by treatment with methyldopa. Further studies will be necessary to determine whether administration of methyldopa and other anti-hypertensive drugs for a prolonged period will be effective in reversing or even preventing the appearance of hypertension, aortic thickening, and associated accumulation of collagen and other aortic proteins.

It is apparent from our studies together with those of other investigators that the onset of hypertension and the initial stages of its reversal represent dynamic changes in arterial wall structure, composition, and metabolism of connective tissue proteins. Elucidation of these changes and their resulting effects on cardiovascular structural integrity and performance are necessary both for our understanding of the pathogenesis of hypertension and for the selection of proper therapy to arrest and control the adverse effects of high blood pressure.

Acknowledgments

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References

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