Collagen Phenotypes During Development and Regression of Myocardial Hypertrophy in Spontaneously Hypertensive Rats

Debabrata Mukherjee and Subha Sen

The myocardium contains collagen matrix that is a major determinant of its architecture, structural integrity, and mechanical properties. This fibrillar matrix consists primarily of type I and type III collagens having epimysial, perimysial, and endomysial components. The present study shows the alteration of collagen phenotypes during the evolution of hypertensive hypertrophy. Therapy with captopril, an angiotensin-converting enzyme inhibitor that regresses cardiac hypertrophy, not only reduces the total amount of collagen but reverses the altered distribution of type I and type III collagen. In normotensive rats, captopril did not significantly reduce collagen content or alter the ratio of type I to type III collagen. (Circulation Research 1990;67:1474-1480)

Collagen is responsible for the functional integrity of the myocardium, which allows interdigitation and transmission of force between contracting myocytes. The collagen network in the heart is largely influenced by the load. Collagen has been shown to be synthesized in the heart primarily by fibroblasts.1,2 It has been reported that pressure overload increases the collagen content within the left ventricular myocardium. Furthermore, evidence suggests that collagen probably plays a role in the stress–strain relation within the left ventricular myocardium during diastole and, perhaps, during systole.3,4 Bing et al5 noted that the velocity of shortening of hypertrophied left ventricular columnae carnea cordis muscles was reduced. In humans, it is recognized that the diastolic function of the hypertrophied pressure-overloaded myocardium and, in particular, early ventricular filling will be compromised.6 Various investigators have determined that, in pressure overload hypertrophy, there is a higher hydroxyproline content,7 and this may lead to the development of increased resting tension.7 But myocardial stiffness may be independent of collagen content. Thiedemann et al8 showed in Goldblatt hypertensive rats that at 4 and 8 weeks after surgery there is only a small increase in collagen content but a pronounced rise in the stress–strain relation of the left ventricle. Forty weeks after surgery, there was a 32% increase in collagen content, but a very small change in stress–strain relation. These findings suggest that ventricular compliance may be related to some other parameter, such as the type of collagen present.

Jalil et al9 used picrosirius red and polarization microscopy to monitor the transformation of the collagen matrix during cardiac hypertrophy. Perhaps this technique is inadequate for quantification of collagen phenotypes, because differential coloration is not due to specific interaction between sirius red and types I and III collagen but to the normal difference in diameter. Because type III frequently remains associated with type I, this method cannot quantify or distinguish type I from type III collagen.

To quantify collagen phenotypes, another important issue to consider is solubility of cardiac collagen. Most of the reports available to date10 have used pepsin to extract collagen and quantify various types of collagen. Cardiac collagen is extremely insoluble and highly cross-linked, especially during the chronic phase of myocardial hypertrophy.11 The present paper describes quantitation of collagen phenotypes using cyanogen bromide to solubilize collagen and the effect of captopril, a very commonly used antihypertensive agent, on collagen content and phenotype.

Materials and Methods

Study Groups

All spontaneously hypertensive rats (SHRs) used in this study were obtained from Taconic farm (Germantown, N.Y.). The normotensive control rats were age- and sex-matched Wistar-Kyoto (WKY) and

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American Wistar (Wistar) rats, which were obtained from Hilltop Farm, Scottsdale, Pa. Both normotensive and hypertensive rats were kept under the same conditions and were properly housed and fed with Purina rat chow. Arterial pressure was measured using a tail cuff in a method similar to that described by Sen and Bumpus.12 In all experiments, rats were killed after anesthetizing with ether, and ventricles were cut flush with the surface to remove aortic tissue and were cleaned thoroughly with phosphate buffered saline (PBS) to remove all traces of blood. The left ventricle then was separated from the right ventricle and atrial tissue. The left ventricular tissue was used for all experiments.

**Determination of Hydroxyproline**

The amount of hydroxyproline in this tissue was determined by using a modified Stagemann procedure.13 Myocardial tissue was homogenized, and hydrolysis of the sample solution was done with 6N HCl at 110°C for 24 hours. The hydrolyzed samples were dried with a flash evaporator. Hydroxyproline standard solutions of 2, 4, 6, 8, and 10 μg/ml were made. A reagent blank was included in the procedure by substituting water for the hydroxyproline solution, and absorbance was corrected accordingly. One-half milliliter hydroxyproline standard solutions of different strengths and homogenates of heart samples were taken in glass tubes, and 1 ml isopropanol was added. The tubes then were vortexted. To this solution, 0.5 ml of oxidant (0.35 g chloramine T plus 5 ml water plus 20 ml citrate buffer) was added, vortexted, and allowed to stand for 4 minutes. Three and one-fourth milliliters Ehrlich’s reagent (3 ml Ehrlich’s plus 16 ml isopropanol) subsequently was added. The tubes were kept at 25°C for 18 hours, and the intensity of red coloration was measured using a Beckman DU50 spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.). The amount of hydroxyproline in unknown samples was calculated using the standard curve. Collagen content was estimated by multiplying the hydroxyproline content by a factor of 8.2.

**Quantitation and Typing of Collagen**

**Extraction of collagen.** Reference grade collagen standards were obtained from Calbiochem Corp., San Diego. The extraction and phenotyping of collagen was done following the procedure described by Laurent et al.14 In this method, the sodium dodecyl sulfate (SDS) was shown to remove the bulk of noncollagen proteins, leaving an insoluble residue that then could be reacted with cyanogen bromide without further purification. Second, cyanogen bromide was shown to solubilize essentially all the collagen in the residue, leaving an insoluble pellet whose amino acid analysis has been shown by Laurent et al14 to be similar to that of elastin.

The heart was homogenized in a 10-ml, all-glass Kontes tissue grind pestle (Kontes, Vineland, N.J.) with PBS at 4°C. The homogenate was centrifuged at 4,000g for 10 minutes. The supernatant, along with all subsequent supernatants, was retained so that proteins could be recovered by precipitation in 5% trichloroacetic acid. Next, the hydroxyproline content was determined. The residue was resuspended in 2% SDS and rehomogenized in the 10-ml glass homogenizer at room temperature. The homogenate again was centrifuged and rehomogenized in 2% SDS; the procedure was repeated four times. The remaining residue was further extracted three times with PBS to remove the excess SDS. The residue then was rehomogenized in acetone and centrifuged at 4,000g for 10 minutes; the supernatant was discarded. This step was repeated, and the pellet was dried under vacuum.

**Digestion of collagen with cyanogen bromide.** The acetone-dried powder was homogenized in an all-glass homogenizer with 0.6 ml 70% vol/vol formic acid per 100 mg original tissue. The homogenate was transferred to a 15-ml graduated polyethylene centrifuge tube, and any remaining residue was washed from the homogenizer with 0.6 ml formic acid. Homogenate was made up to a volume of 1.5 ml with 70% vol/vol formic acid per 100 mg original tissue. Cyanogen bromide crystals were added to this volume to produce a concentration of 20 mg/ml. Nitrogen gas then was bubbled through the mixture, tubes were sealed, and the reaction was allowed to proceed for 18 hours at 25°C. In our experiments, a digestion time of 18 hours was used because a 6-hour digestion, as recommended in the method of Laurent et al,14 could digest only 50% of the cardiac collagen. To facilitate mixing, the tubes were positioned at an angle of 30°. At the completion of the reaction, the digest was centrifuged at 5,000g for 20 minutes. The stopper was removed in a fume cupboard, and 0.3 ml of the supernatant was removed and dried under vacuum in a 1.5-ml microfuge tube. This material then could be dissolved in sample buffer in preparation for polyacrylamide gel electrophoresis. The amount to be loaded was determined by hydroxyproline estimation on the lyophilized powder obtained from 0.5 ml of supernatant.

**Collagen Typing**

**Gel electrophoresis.** Polyacrylamide gel electrophoresis was performed on vertical gels (Protein II, Bio-Rad Laboratories, Richmond, Calif.) by stacking and separating gel concentrations of 4% and 12%, respectively. The gels were 1.5-mm thick, with 15 sample wells. Samples of a 10-μl volume were loaded into the wells using a Hamilton microsyringe (Hamilton Co., Reno, Nev.), and stacking was allowed to occur at a current of 25 mA. Once the samples entered the separating gel, the current was increased to 35 mA, and electrophoresis continued until the dye marker reached a level two thirds from the top. When electrophoresis was complete, the gel was removed and stained for 1 hour by gentle shaking in 250 ml of an aqueous solution containing 0.125% Coomassie blue R-250, 50% methanol, and 10% acetic acid. The gel was destained for 24–48 hours by
continuous shaking, with several changes of acetic acid. All staining and destaining reactions, as well as gel buffers, were filtered to minimize contamination for subsequent scanning.

Gel scanning. Gels were scanned using Helena Laboratories Quick Scan (Beaumont, Tex.). The myocardial collagen showed an electrophoretic pattern similar to those of collagen standards, with little background staining, making these gels suitable for densitometric scanning (Figure 1). The quantitation of relative amounts of type I:III collagen was accomplished by determining the relation between the amount of collagen applied to the gel and the relative peak, which was distinctive for the collagen types. The major bands (B, G, and I) of type I as well as the major single band (M) of type III gave good linear plots, with a correlation coefficient of about 0.98, as illustrated in Figure 2. Bands G and M were chosen because they contained very little interference from comigrating peptides of other collagen types.

Validation of quantification. To validate our quantification procedure, different amounts of cyanogen bromide digests of collagen (reference grade standards type I and type III) were loaded on an SDS-polyacrylamide gel, and a regression line was plotted. In three such experiments, the r value was about 0.98 for type I collagen and was 0.97 for type III collagen.

In another set of experiments, each of three lanes of the gel was loaded with 10 μg standard, 10 μg standard plus 10 μg myocardial collagen, and 10 μg myocardial collagen. In these experiments, the percent correlation of quantification was about 85–90%, as shown in Table 1.

Statistical Analysis

A two-way analysis of variance was done on data from the three rat strains—WKY and Wistar rats and SHRs—and each strain was used in two groups: one control group and one group receiving captopril. The Bonferroni (Dunn) t test analysis was used for individual group comparison.

Results

Blood Pressure and Left Ventricular Hypertrophy

Systolic blood pressure, left ventricular weight, and the left ventricular weight-to–body weight ratio are summarized in Tables 2, 3, and 4 for various experimental groups. Ten-week-old SHRs had significantly higher blood pressure (168±16 mm Hg) compared with that in WKY rats (120±16 mm Hg, p<0.001) and Wistar rats (120±18 mm Hg, p<0.001) (Table 2). The body weight at this age group did not differ significantly in either strain. SHRs had a higher mean left ventricular weight (948±16.6 mg) as compared with that in normotensive WKY rats (832±14.6 mg, p<0.05) and Wistar rats (876±18.8 mg, p<0.05). The left ventricular weight-to–body weight ratio also was significantly increased in SHRs (3.29±0.50 mg/g).

Table 1. Quantification of Collagen

<table>
<thead>
<tr>
<th>Collagen</th>
<th>10 μg CS</th>
<th>10 μg CS+</th>
<th>10 μg HC</th>
<th>10 μg HC</th>
<th>Predicted % correlation</th>
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<tbody>
<tr>
<td>Type I</td>
<td>27.00</td>
<td>44.00</td>
<td>19.00</td>
<td>90.00</td>
<td></td>
</tr>
<tr>
<td>Type III</td>
<td>19.00</td>
<td>35.00</td>
<td>19.00</td>
<td>88.00</td>
<td></td>
</tr>
</tbody>
</table>

HC, heart collagen; CS, collagen standard.
TABLE 2. Blood Pressure, Left Ventricular Weight, and Collagen in 10-Week-Old Rats

<table>
<thead>
<tr>
<th></th>
<th>WKY (n = 6)</th>
<th>Wistar (n = 6)</th>
<th>SHR (n = 6)</th>
<th>WKY vs. Wistar</th>
<th>WKY vs. SHR</th>
<th>Wistar vs. SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>120±16</td>
<td>120±18</td>
<td>168±16</td>
<td>NS</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>290±37</td>
<td>300±28</td>
<td>287±33</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LV weight (mg)</td>
<td>832±14.6</td>
<td>876±18.8</td>
<td>948±16.6</td>
<td>NS</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>LV wt/body wt (mg/g)</td>
<td>2.88±0.07</td>
<td>2.92±0.06</td>
<td>3.29±0.50</td>
<td>NS</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Collagen content (mg)</td>
<td>4.25±0.08</td>
<td>4.32±0.07</td>
<td>4.54±0.06</td>
<td>NS</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Collagen concentration (mg/g)</td>
<td>5.10±0.18</td>
<td>4.96±0.16</td>
<td>4.78±0.16</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rats; Wistar, American Wistar rats; SHR, spontaneously hypertensive rats; LV, left ventricular.

comparing with that in WKY rats (2.88±0.07 mg/g, p<0.05) and Wistar rats (2.92±0.06 mg/g, p<0.05).

At 34 weeks of age, SHRs had significantly higher blood pressure (169±18 mm Hg) compared with that in WKY rats (124±22 mm Hg, p<0.05) and Wistar rats (130±23 mm Hg, p<0.05) (Table 3). Captopril administered in a dosage of 50 mg/kg in drinking water for 6 weeks effectively controlled hypertension in SHRs, lowering blood pressure from 169±18 to 124±14 mm Hg (p<0.05), but it did not cause a significant drop in blood pressure in WKY and Wistar rats. Interestingly, there was a marked difference in the body weight of these animals at this age group. WKY rats had a body weight 1.5 times and Wistar rats about two times that of SHRs. This pronounced difference in body weights accounts for the lower left ventricular weight seen in SHRs at this age group (1,060±45 mg) compared with that in WKY rats (1,390±48 mg, p<0.01) and Wistar rats (1,710±90 mg, p<0.01). Captopril treatment significantly lowered the left ventricular weight in SHRs from 1,060±45 to 992±36 mg (p<0.05) but had no significant effect in WKY or Wistar rats. The left ventricular weight-to-body weight ratio was significantly higher in SHRs (3.19±0.04 mg/g) compared with WKY rats (2.60±0.22 mg/g, p<0.05) and Wistar rats (2.20±0.23, p<0.05). Captopril treatment significantly decreased this ratio in SHRs from 3.19±0.04 to 2.89±0.06 mg/g (p<0.05). There was no significant decrease in left ventricular weight-to-body weight ratio in captopril-treated WKY or Wistar rats (2.58±0.12 and 2.19±0.19 mg/g, respectively) (Table 3).

When 86-week-old SHRs were treated with captopril (50 mg/kg in drinking water) for 6 weeks, blood pressure decreased significantly from 196±28 to 119±23 mm Hg (p<0.001) (Table 4). There was no significant difference in the body weight in control and captopril-treated rats. There was a significant reduction in left ventricular weight from 1,234±78 mg in control rats to 970±53 mg (p<0.001). The left ventricular weight-to-body weight ratio also showed a significant decrease from 3.02±0.05 mg/g in control animals to 2.64±0.08 mg/g (p<0.001) in captopril-treated animals.

TABLE 3. Blood Pressure, Left Ventricular Weight, and Collagen in 34-Week-Old Rats and Effect of Captopril Treatment

<table>
<thead>
<tr>
<th></th>
<th>WKY (n = 3)</th>
<th>Treated (n = 3)</th>
<th>Control (n = 3)</th>
<th>Treated (n = 3)</th>
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<tr>
<td>Blood pressure (mm Hg)</td>
<td>124±22</td>
<td>100±14</td>
<td>130±23</td>
<td>129±21</td>
<td>169±18</td>
<td>124±14</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td>534±87</td>
<td>540±91</td>
<td>777±96</td>
<td>732±88</td>
<td>340±67</td>
<td>345±61</td>
</tr>
<tr>
<td>LV wt (mg)</td>
<td>1,390±48</td>
<td>1,388±22</td>
<td>1,710±90</td>
<td>1,604±98</td>
<td>1,060±45</td>
<td>992±36</td>
</tr>
<tr>
<td>LV wt/body wt (mg/g)</td>
<td>2.60±0.22</td>
<td>2.58±0.12</td>
<td>2.20±0.23</td>
<td>2.19±0.19</td>
<td>3.19±0.04</td>
<td>2.89±0.06</td>
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<tr>
<td>Collagen content (mg)</td>
<td>6.87±0.17</td>
<td>6.70±0.38</td>
<td>8.64±0.66</td>
<td>7.89±0.61</td>
<td>4.87±0.34</td>
<td>4.33±0.98</td>
</tr>
<tr>
<td>Collagen concentration (mg/g)</td>
<td>4.90±0.69</td>
<td>4.83±0.33</td>
<td>5.02±0.22</td>
<td>4.84±0.08</td>
<td>4.49±0.40</td>
<td>4.37±0.69</td>
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Statistical significance

<table>
<thead>
<tr>
<th></th>
<th>WKY control vs. WKY treated</th>
<th>WKY control vs. SHR control</th>
<th>SHR treated vs. SHR control</th>
<th>Wistar control vs. Wistar treated</th>
<th>Wistar control vs. SHR control</th>
<th>WKY control vs. Wistar control</th>
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</thead>
<tbody>
<tr>
<td>Blood pressure</td>
<td>NS</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td>NS</td>
<td>p&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Body wt</td>
<td>NS</td>
<td>p&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LV wt</td>
<td>NS</td>
<td>p&lt;0.01</td>
<td>p&lt;0.05</td>
<td>NS</td>
<td>p&lt;0.01</td>
<td>NS</td>
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<tr>
<td>LV wt/body wt</td>
<td>NS</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td>NS</td>
<td>p&lt;0.05</td>
<td>NS</td>
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<tr>
<td>Collagen content</td>
<td>NS</td>
<td>p&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>p&lt;0.05</td>
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<tr>
<td>Collagen concentration</td>
<td>NS</td>
<td>NS</td>
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</table>

WKY, Wistar-Kyoto rats; Wistar, American Wistar rats; SHR, spontaneously hypertensive rats; LV, left ventricular.
Collagen Content and Concentration of Hypertrophy

The collagen content and concentration are summarized in Tables 2, 3, and 4. In 10-week-old rats, SHRs had a significantly higher collagen content (4.54±0.06 mg) compared with WKY rats (4.25±0.08 mg, p<0.05) and Wistar rats (4.32±0.07 mg, p<0.05) (Table 2). There was, however, no significant difference in the collagen concentration between SHRs (4.78±0.16 mg/g), WKY rats (5.10±0.18 mg/g) and Wistar rats (4.96±0.16).

The collagen content was low in 34-week-old SHRs (4.87±0.34 mg) compared with that in WKY rats (6.87±0.17 mg, p<0.05) and Wistar rats (8.64±0.66 mg, p<0.05) (Table 3). This difference is due to a significant difference in their left ventricular weight, as hypertensive animals had much lower body weight. Captopril treatment at 50 mg/kg doses for 6 weeks did not lower the collagen content significantly in either group. The values of collagen content for treated animals are SHRs, 4.33±0.98 mg; WKY rats, 6.70±0.38 mg; and Wistar rats, 7.89±0.61 mg. There was no significant difference in the collagen concentration between SHRs (4.49±0.40 mg/g), WKY rats (4.90±0.69 mg/g), and Wistar rats (5.02±0.22 mg/g).

Captopril treatment had no significant effect on collagen concentration either in SHRs (4.37±0.69 mg/g) or in WKY rats (4.83±0.33 mg/g) and Wistar rats (4.84±0.08 mg/g).

In 86-week-old SHRs, the collagen content was significantly higher in control animals (5.42±0.18 mg) as compared with captopril-treated animals (4.70±0.22 mg) (Table 4). There was no significant difference in the collagen concentration between control rats (4.40±0.15 mg/g) and treated rats (4.80±0.32 mg/g) (Table 4).

Collagen Phenotypes

Even though the collagen concentration did not show a significant difference between normotensive and hypertensive animals, there were marked changes in collagen phenotypes during evolution of hypertensive hypertrophy, as shown in Figure 3. Ten-week-old SHRs had a significantly decreased type I:III ratio (1.83±0.13) as compared with that in WKY rats (3.54±0.47, p<0.05) and Wistar rats (3.52±0.66, p<0.05).

At 34 weeks of age, there was a significant increase in type I:III ratio in normotensive WKY (6.03±0.28,

Table 4. Blood Pressure, Left Ventricular Weight, and Collagen in 86-Week-Old Spontaneously Hypertensive Rats and Effect of Captopril Treatment

<table>
<thead>
<tr>
<th></th>
<th>Control SHR (n=6)</th>
<th>Captopril-treated SHR (n=4)</th>
<th>Control vs. captopril-treated SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>196±28</td>
<td>119±23</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td>408±73</td>
<td>367±82</td>
<td>NS</td>
</tr>
<tr>
<td>LV weight (mg)</td>
<td>1,234±78</td>
<td>970±53</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>LV wt/body wt (mg/g)</td>
<td>3.02±0.05</td>
<td>2.64±0.08</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Collagen content (mg)</td>
<td>5.42±0.18</td>
<td>4.70±0.22</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Collagen concentration (mg/g)</td>
<td>4.40±0.15</td>
<td>4.80±0.32</td>
<td>NS</td>
</tr>
</tbody>
</table>

SHR, spontaneously hypertensive rats; LV, left ventricular.
p<0.05) and Wistar rats (6.86±0.86, p<0.05) as compared with 10-week-old WKY and Wistar rats, suggesting an increase in type I collagen with aging. SHRs at 34 weeks showed a further increase in type I:III ratio (10.31±0.69, p<0.05) as compared with normotensive WKY and Wistar rats at that age. Captopril treatment caused a significant reduction in type I:III ratio in SHRs (7.06±0.17, p<0.05) but had no significant effect on WKY (6.26±0.49) or Wistar rats (6.41±0.52) (Figure 3).

At 86 weeks of age, there was a marked decrease in the type I:III ratio in SHRs (1.94±0.32, p<0.001) as compared with 34-week-old SHRs (10.31±0.69). Captopril treatment of this age group significantly increased the type I:III ratio to 3.12±0.69 (p<0.05) (Figure 3).

Discussion

It is now appreciated that the myocardium contains a collagen matrix that is a major determinant of its architecture, structural integrity, and mechanical properties.15 The collagen matrix of the heart consists primarily of type I and type III collagens. The interrelations among the structural features of these components, their biochemical characteristics and biophysical properties, and the function they serve in the myocardium have not been completely defined. Since Thiedemann et al18 showed that myocardial stiffness does not correlate with total collagen content, the possibility that the alteration of collagen phenotypes may be responsible for compromised function in hypertensive heart disease has been suggested.

Our study showed that myocardial collagen phenotype is perhaps more important than the total amount of collagen present in the myocardium. The dramatic decrease in the type I:III ratio in the established phase of hypertensive hypertrophy emphasizes that the type of collagen may play an important role in myocardial dysfunction. Many investigators10 have used pepsin to solubilize cardiac collagen to determine its phenotype. We have shown that pepsin could solubilize only about 12% of collagen. Any quantification based only on pepsin digestion is likely to be misleading. In our studies, we used cyanogen bromide, which solubilized about 82–88% of collagen. This is in agreement with the studies of Laurent et al,14 who used cyanogen bromide to extract collagen from lung tissue.

To elucidate how hypertensive hypertrophy affected the collagen in the heart, the total collagen content, collagen concentration, and the ratio of type I:III collagen were determined. In agreement with other investigators,11 we found that the collagen concentration did not significantly differ from that of normal rats in hypertensive hypertrophy. It has been proposed that the elevation in resting tension is related to an abnormality in the collagen component in the heart. Because no significant alteration was evident in the collagen concentration, we hypothesized that the type of collagen, rather than the total amount, may play a more important role in determining myocardial function.

Based on our hypothesis, we determined the type I:III ratio in 10-, 34-, and 86-week-old animals. In 10-week-old hypertensive rats, there was a significant increase in type III collagen, with a decrease in the type I:III ratio, in comparison with normotensive rats. This is in support of what Medugorac and Jacob10 reported in Goldblatt hypertensive and aortstenotic rats. Our studies in 34-week-old normotensive animals showed a significant increase in type I collagen in comparison with 10-week-old normotensive rats, suggesting there is an increase in type I collagen with aging.

In 34-week-old hypertensive animals, there was a further significant increase in type I collagen in comparison with normotensive 34-week-old animals. In 86-week-old SHRs, however, there was a marked increase in type III collagen, with a sharp fall in the type I:III ratio. Bing et al16 have shown unequivocal myocardial dysfunction at 18 months, with normal cardiac function before that age group in spite of myocardial hypertrophy. We therefore decided to use 86-week-old hypertensive animals in our studies. Myocardial dysfunction during 18 months of age in SHRs has been documented in a number of studies.17–20 The degree of myocardial dysfunction documented is variable, but almost all studies show some alteration in myocardial function, namely, prolonged electromechanical delay and time to peak tension. Pfeffer et al17 demonstrated a marked decline in the peak pumping ability from 52 to 90 weeks of age in the SHR. Mirsky et al18 showed that in the normotensive rats, the myocardial stiffness constant remained within normal limits until 18 months. At this time, a significant increase in this index of myocardial stiffness occurred. They also showed that baseline and maximal cardiac indexes and ejection fraction index of SHRs were normal from 6 to 18 months but were markedly reduced at 24 months. They stated that a depression in the contractile state of the SHR occurred at 18 months of age. We were unable to procure 86-week-old normotensive controls for this study; however, if we were to extrapolate the information that Medugorac21 reported with pepsin-digestible collagen in 104-week-old Wistar rats (type I:III ratio, 8.8:1), this would mean a pronounced increased in type III collagen in hypertensive animals in this age group. Because impaired myocardial function is present in this age group, it is logical to assume that increased deposition of collagen type III is associated with a transition from compensated hypertrophy to decompensation or myocardial dysfunction.

In humans,22 during the stages of compensated hypertensive heart disease, the hypertrophy principally thickens the left ventricular wall at the expense of left ventricular chamber volume. This is referred to as concentric hypertrophy. This stage may be associated with increased deposition of thick bundles of type I collagen, as seen in 34-week-old hypertensive rats.
With the onset of decompensation, dilatation of the left ventricle occurs, and this stage may be associated with a marked increase in the deposition of type III collagen fibers, as seen in 86-week-old hypertensive rats.

Sen and Bumpus\textsuperscript{12} were the first to show the different effects that antihypertensive medications may have on collagen metabolism. Their studies showed that captopril reduced the collagen content and that hydralazine had no significant effect, whereas \alpha-methyldopa actually increased the collagen content of the myocardium. In our studies, captopril consistently decreased myocardial hypertrophy and collagen content. More importantly, this inhibitor was successful in correcting the increase in type I collagen at 34 weeks of age and brought the ratio toward normal at 86 weeks of age. Pfeffer et al\textsuperscript{23} reported that with captopril, cardiac function improved in SHR's. This improvement could be due, in part, to amelioration of the type I:III ratio.

Several laboratories\textsuperscript{24} have reported different effects on total collagen content with the use of different antihypertensive drugs. The exact mechanism of action of various drugs on cardiac collagen metabolism is still to be elucidated, but it may be due to an interplay of hemodynamic, neural, and humoral factors. The different effects of various drugs on collagen metabolism suggest that the factors governing myocardial connective tissue proliferation and regression may be independent of those governing muscle fiber hypertrophy. It is now becoming clear that the pathophysiology of myocardial dysfunction in hypertensive heart disease is multifactorial, with altered collagen matrix playing an important role. The present study should help define the importance of collagen phenotypes in chronic hypertrophy and its transition to heart failure, and it may have relevance in antihypertensive drug therapy for the future.

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