Collagen Deposition and the Reversal of Coronary Reserve in Cardiac Hypertrophy

Shogen Isoyama, Nobuhiko Ito, Ken Satoh, and Tamotsu Takishima

The aim of this study was to clarify how collagen deposition or medial hypertrophy of the vascular wall affects the coronary dilator reserve in pressure-overloaded hearts and whether inhibition of collagen deposition reverses the abnormalities after relief of pressure overload. We used ascending aortic banding and debanding methods and superimposed β-aminopropionitrile in some of the banded rats (50 mg/kg i.p., twice a day). Ten weeks of banding increased in vivo peak systolic left ventricular pressure and produced medial hypertrophy, an increase in collagen deposition in the myocardial and perivascular tissues, and myocardial hypertrophy in the banded group without β-aminopropionitrile treatment. Superimposition of β-aminopropionitrile treatment on banding inhibited the increase in collagen deposition. In the groups debanded after the 10-week banding period, both with and without β-aminopropionitrile treatment, medial and myocardial hypertrophy regressed 4 weeks after debanding. We estimated coronary dilator reserve in Langendorff preparations perfused with modified Tyrode's solution containing oxygenated bovine red blood cells and serum albumin. The ratio of reactive peak flow after brief ischemia-to-resting flow decreased in both of the banded groups. After debanding, the ratio remained lower in the banded group without β-aminopropionitrile treatment than in the control group. However, debanding in the group with β-aminopropionitrile treatment increased the ratio to a level similar to that of the control group. Thus, in pressure-overloaded cardiac hypertrophy, coronary reserve seems to be determined by medial hypertrophy independently of collagen deposition, but collagen deposition plays an important role in the reversal of vasodilator reserve after relief of the overload. (Hypertension 1992;20:491–500)

KEY WORDS • collagen • coronary circulation • hypertrophy • rat studies

Pressure overload with coronary arterial hypertension causes myocardial hypertrophy and coronary vascular changes such as medial hypertrophy1,2 with an increase in collagen deposition in the interstitial,3-9 coronary vascular, and perivascular tissue. Myocardial hypertrophy10 and the vascular changes1 decrease coronary dilator reserve. The effects of collagen deposition in the myocardial tissue on ventricular or cardiac muscle functions have been vigorously investigated.6-8,11,12 However, it is not clear how the collagen deposition in hypertrophied hearts affects the coronary circulation.

In the present study, we attempted to clarify how the collagen deposition or medial hypertrophy of the vascular wall affects the coronary dilator reserve in pressure-overloaded hearts and whether the inhibition of collagen deposition reverses the abnormalities after relief of the overload. To inhibit collagen deposition in the vascular and extravascular myocardial tissue, we superimposed β-aminopropionitrile (β-APN) treatment on ascending aortic banding for 10 weeks in rats. In experimental models of systemic13 and pulmonary14-16 hypertension, inhibition of collagen deposition by β-APN lowered the blood pressure. Collagen deposition in the vascular wall plays a key role in sustaining the high blood pressure of the systemic or pulmonary circulation. In the present study, contrary to findings in studies of systemic or pulmonary circulation, β-APN did not affect the degree of pressure overload to the left ventricular muscle or coronary arterial hypertension, but did inhibit collagen deposition.

Methods

We used Wistar rats (6-8 weeks of age) (Funabashi Farms, Shizuoka, Japan) that were divided into six groups: a group with ascending aortic banding for a 10-week period (n=8), a control group treated with β-APN (n=8), a sham-operated group (n=3), a group debanded for 4 weeks after a 10-week period of banding (n=7), a group debanded for 4 weeks after a 10-week period of banding with β-APN treatment (n=7), and a sham-operated group (n=4). The two sham-operated groups were combined as a control group for the banded and debanded groups (n=7) since there was no difference in variables measured between those groups.

Surgical Procedures for Ascending Aortic Banding and Debanding

Details of the procedures have been described elsewhere.17-21 Briefly, the rats were anesthetized (metho-
were added to the Tyrode’s solution. The solution was pH of the perfusate were 33±0.4% (range 30-36), 3% CO₂, and 77% N₂. The hematocrit, Po₂, PcO₂, and Pco₂ were measured.

Whole bovine blood was centrifuged, and the red blood cells were washed twice with the Tyrode’s solution at 4°C. The washed red blood cells and serum albumin (15 g/l) were added to the Tyrode’s solution. The solution was oxygenated by bubbling with a gas mixture of 20% O₂, 3% CO₂, and 77% N₂. The hematocrit, Po₂, Pco₂, and pH of the perfusate were 35±0.4% (range 30–36), 126±4 mm Hg (99–140), 31±1 mm Hg (24–40), and 7.42±0.014 (7.34–7.49), respectively.

The coronary perfusion apparatus consisted of a coronary perfusion apparatus, a water-jacketed reservoir, and an air trap that was pressurized with a gas tank. Coronary perfusion pressure was controlled by a pressure regulator (Pressure Regulator Type 70, Bellofram, Burlington, Mass.). Perfusion pressure was measured from the side arm of the perfusion line using a strain gauge transducer (model TP-200T, Nihon Kohden Co., Ltd., Tokyo). Zero pressure reference was taken at the midlevel of the heart. Mean coronary flow rate was measured using an extracorporeal-type probe (1 mm i.d.) (model FF-010T, Nihon Kohden) and an electromagnetic flowmeter (model MFV-31, Nihon Kohden). The time constant of the electrical circuit used to obtain mean flow was 1.0 second. Calibration was performed by timed sampling of volume in each heart with the same solution used to perfuse the heart. The temperature of the perfusate was kept constant at 37°C. The heart rate was also maintained at 300 beats per minute by electrical pacing (Electrical Stimulator SEN 7103, Nihon Kohden).

After coronary flow rate had reached a steady state at 100 mm Hg of perfusion pressure, coronary flow was reduced to zero for 40 seconds and returned to 100 mm Hg of perfusion pressure. The same procedures were repeated at 50 and 150 mm Hg of perfusion pressure, and flow rates under resting conditions and at peak reactive hyperemia were obtained. To ascertain whether coronary vasculature was maximally dilated after a 40-second ischemia, we measured flow rate under vasodilation by administering a maximum dose of adenosine (5 μM/min), which was infused from the side arm of the perfusion line, in the sham-operated group (n=7). The flow rates at peak reactive hyperemia and under vasodilation by adenosine were as follows: 3.86±0.39 versus 2.44±0.51 ml/min ‘g”1 at 50 mm Hg of perfusion pressure, 7.95±0.78 versus 8.06±1.43 ml/min ‘g”1 at 100 mm Hg, and 12.13±1.07 versus 14.47±2.37 ml/min ‘g”1 at 150 mm Hg. The flow rates at peak reactive hyperemia tended to be higher at 50 mm Hg of perfusion pressure and to be lower at 150 mm Hg compared with the respective values under vasodilation by adenosine. However, the flow rates under the two conditions did not differ at any levels of perfusion pressure. In the present study, therefore, we determined coronary dilator reserve by peak flow rates during reactive hyperemia after ischemia. Coronary dilator reserve was estimated as the ratio of peak reactive flow/resting flow at the three levels of perfusion pressure.

Histological Examination

After measurements of coronary dilator reserve, the heart was isolated. A cannula was inserted into the ascending aorta. Through a left atrial incision, a drain was inserted into the left ventricular cavity to maintain the cavity in an empty state. The heart was perfused with oxygenated Tyrode’s solution containing bovine red blood cells and serum albumin (15 g/l). The composition of the modified Tyrode’s solution was as follows (mM): NaCl 106.0, KCl 6.0, CaCl₂ 2.5, NaH₂PO₄ 0.435, MgCl₂ 1.0, NaHCO₃ 36.0, and glucose 11.0. The preparation of the coronary perfusion apparatus consisted of a coronary perfusion apparatus, a water-jacketed reservoir, and an air trap that was pressurized with a gas tank. Coronary perfusion pressure was controlled by a pressure regulator (Pressure Regulator Type 70, Bellofram, Burlington, Mass.). Perfusion pressure was measured from the side arm of the perfusion line using a strain gauge transducer (model TP-200T, Nihon Kohden Co., Ltd., Tokyo). Zero pressure reference was taken at the midlevel of the heart. Mean coronary flow rate was measured using an extracorporeal-type probe (1 mm i.d.) (model FF-010T, Nihon Kohden) and an electromagnetic flowmeter (model MFV-31, Nihon Kohden). The time constant of the electrical circuit used to obtain mean flow was 1.0 second. Calibration was performed by timed sampling of volume in each heart with the same solution used to perfuse the heart. The temperature of the perfusate was kept constant at 37°C. The heart rate was also maintained at 300 beats per minute by electrical pacing (Electrical Stimulator SEN 7103, Nihon Kohden).

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Table 1. In Vivo Hemodynamics in the Five Groups of Rats

<table>
<thead>
<tr>
<th>Hemodynamic measurements</th>
<th>Sham (n=7)</th>
<th>Banded (n=8)</th>
<th>Banded + β-APN (n=8)</th>
<th>Debanded (n=7)</th>
<th>Debanded + β-APN (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic LVP (mm Hg)</td>
<td>127±11</td>
<td>192±7*</td>
<td>178±9*</td>
<td>121±7</td>
<td>128±10</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>3±0.5</td>
<td>4±0.5</td>
<td>5±0.6</td>
<td>4±0.7</td>
<td>5±0.6</td>
</tr>
<tr>
<td>Systolic AoP (mm Hg)</td>
<td>118±7</td>
<td>122±6</td>
<td>121±10</td>
<td>113±7</td>
<td>117±8</td>
</tr>
<tr>
<td>Diastolic AoP (mm Hg)</td>
<td>98±7</td>
<td>99±5</td>
<td>105±6</td>
<td>88±7</td>
<td>91±12</td>
</tr>
<tr>
<td>Mean AoP (mm Hg)</td>
<td>105±7</td>
<td>108±5</td>
<td>109±7</td>
<td>96±6</td>
<td>100±10</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>332±24</td>
<td>392±16</td>
<td>376±17</td>
<td>347±10</td>
<td>383±31</td>
</tr>
</tbody>
</table>

Sham, sham-operated control group; banded, banded group; banded + β-APN, banded group with β-aminopropionitrile (β-APN) treatment; debanded, debanded group; debanded + β-APN, debanded group with β-APN treatment during aortic banding; systolic LVP, peak systolic left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; AoP, aortic pressure; bpm, beats per minute. Values are mean±SEM.

*p<0.01 compared between sham-operated and experimental groups.

were separated. The septum was considered to be a part of the left ventricle. After determination of their respective wet weights, the septum and the right ventricular free wall were dried to constant weight. The left ventricular dry weight was calculated from the dry/wet weight ratio of the septum and the total left ventricular wet weight.

The left ventricular free wall was placed in a fixative solution overnight and then washed in a 0.1 M phosphate buffer of pH 7.4 for 24 hours at room temperature. After fixation, the block of the left ventricular free wall was sliced into five strips in a transverse or parallel plane to the apex-to-base axis. The five strips were dehydrated for 2 hours in a series of increasing concentrations of ethanol (60-100%), cleaned in xylene (2 hours), and passed through three changes of paraffin (each change 2 hours before final embedding in paraffin). Subsequently, 4–5 μm cross-sections were cut on a microtome. The sections were mounted on glass slides and stained with Elastica-Goldner stain. The medial thickness of the coronary arterial trees and the degree of perivascular fibrosis were measured in each sample. Each of the coronary arteries and arterioles was photographed on color slide film (Fujichrome Rd 135, Fuji Photo Film Co., Ltd., Tokyo) using an objective with a magnification of x10 (Plan 10, n.a. 0.30, Nikon, Tokyo), x20 (Plan 20, n.a. 0.50), or x40 (Plan 40, n.a. 0.70). The slides were projected onto tracing paper through a screen (Neovision 102S, Sugiura Lab., Inc., Tokyo) at a final magnification of x490–1,960.

The long axis of the vessels was determined as the maximal internal diameter in the intravascular lumen. Vessels in which the long axes transversed the outlines of the lumen were excluded. Next, the short axis was determined as the longest internal diameter perpendicular to the long axis. Medial thickness was measured at the points where the internal diameter was determined. To avoid the effect of obliquely sectioned vessels, the medial thickness was calculated as the average value of the two minimum distances from the lumen to the outer edge of the media. The degree of vascular hypertrophy was expressed as the ratio of medial thickness/lumen radius. To estimate perivascular fibrosis, the minimum distance from the outer edge of the media to the outer edge of the adventitia was measured at the same points where the medial thickness was determined. The average value of the two minimum distances was construed as the thickness of perivascular fibrosis. Because the outer edge of the adventitia was irregular, particularly in the intramural arterial trees, we measured the thickness of circular collagen around the arterial trees. The medial thickness and the degree of perivascular fibrosis were divided into four categories according to the size of the lumen diameter (Φ): (μm) 10<Φ<31, 31<Φ<61, 61<Φ<101, and 101<Φ as was done in the previous study of Liu et al.22 Numbers of arteries and arterioles measured in each heart were as follows: 10–15 in the category of vessel size of 10<Φ<31 μm, 8–12 in the category of vessel size of 31<Φ<61 μm, 5–10 in the category of vessel size of 61<Φ<101 μm, and 3–5 in the category of vessel size of 101 μm<Φ. Mean values in each group of rats were obtained from average values in individual hearts. All histological measurements were made by a single observer.

Hydroxyproline Measurements

In the three additional groups of rats, i.e., the sham-operated group (n=5) and the banded groups with (n=5) and without (n=6) β-APN treatment, hydroxyproline concentration was measured as in a previous study.23 Samples from the free wall of the left ventricle were chopped into pieces and immersed by reflux in ethanol and in ether, where soluble elements were extracted. Then, the dregs were dried and weighed. Approximately 25 mg of the dregs were hydrolyzed in 6 ml of 6N HCl for 16 hours at 118°C. The hydrolysate, after being evaporated at 65°C under vacuum, was dissolved in distilled water. After the oxidation and reduction steps were performed, tolune was added, and the hydroxyproline in organic phase was extracted. A part of the extract was colored by adding Ehrlich’s reagent, and the concentration of hydroxyproline was evaluated by measuring the intensity of absorbance with a spectrophotometer.

Statistical Analysis

Variables measured are expressed as mean±SEM. The statistical significance of differences in mean values was assessed by variance analysis and Scheffé multiple comparison test. When the value of p<0.05, the difference in mean values was considered significant.

Results

Table 1 and Figure 1 show in vivo hemodynamics in the five groups of rats. Peak systolic left ventricular pressure significantly increased in the banded groups,
Figure 1. Bar graphs show peak systolic left ventricular pressure and peak-to-peak pressure difference between the left ventricle and the aorta in the five groups of rats. Open bars indicate the value in the sham-operated group, striped bars the values in the banded or debanded groups without &-aminopropionitrile treatment during aortic banding, and filled bars the values in the banded and debanded groups with &-aminopropionitrile treatment during aortic banding. Values are mean±SEM. LVP, left ventricular pressure; &P, change in pressure; SO, sham-operated group; B, banded group; DB, debanded group. **p<0.01 compared with the value in the sham-operated group.

Table 2 summarizes the changes in body weight and heart weight in the five groups of rats. Treatment with &-APN superimposed on aortic banding significantly decreased body weight. The left ventricular wet and dry weights in the two banded groups were greater than those in the sham-operated group. The left ventricular weights in both of the debanded groups did not differ from that in the sham-operated group. Aortic banding did not change the right ventricular wet or dry weights.

Figure 2 shows the degree of myocardial hypertrophy estimated by the ratio of left ventricular dry weight/body weight in the five groups. The ratio increased by 33% above sham-operated controls in the banded group without &-APN treatment (p<0.01) and by 31% in the banded group with &-APN treatment (p<0.01). &-APN treatment tended to decrease the extent of myocardial hypertrophy. After debanding, the ratios decreased to 11% and 12% above the value in the sham-operated group, respectively.

Table 2. Body Weight and Heart Weight in the Five Groups of Rats

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Sham (n=7)</th>
<th>Banded (n=8)</th>
<th>Banded +&amp;-APN (n=8)</th>
<th>Debanded (n=7)</th>
<th>Debanded +&amp;-APN (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>327±7</td>
<td>347±11</td>
<td>281±*</td>
<td>342±3</td>
<td>286±18*</td>
</tr>
<tr>
<td>LV wet weight (mg)</td>
<td>749±17</td>
<td>1,045±92t</td>
<td>869±21*</td>
<td>871±27</td>
<td>757±36</td>
</tr>
<tr>
<td>LV dry weight (mg)</td>
<td>149±4</td>
<td>217±19t</td>
<td>166±7</td>
<td>176±6*</td>
<td>147±9</td>
</tr>
<tr>
<td>RV wet weight (mg)</td>
<td>181±9</td>
<td>215±21</td>
<td>189±13</td>
<td>203±10</td>
<td>167±9</td>
</tr>
<tr>
<td>RV dry weight (mg)</td>
<td>34±2</td>
<td>41±3</td>
<td>34±2</td>
<td>43±3†</td>
<td>31±2</td>
</tr>
</tbody>
</table>

Sham, sham-operated control group; banded, banded group; banded +&-APN, banded group with &-aminopropionitrile (&-APN) treatment; debanded, debanded group; debanded +&-APN, debanded group with &-APN treatment during aortic banding; LV and RV, left and right ventricles. Values are mean±SEM.

*p<0.05, **p<0.01 compared between sham-operated and experimental groups.
Table 3 summarizes coronary flow rates during resting conditions in the five groups of rats. There were no significant changes among the different operation categories or between groups with and without B-APN treatment on aortic banding.

Figure 3 shows coronary dilator reserve estimated by the ratio of peak reactive hyperemic flow/resting flow at the three levels of perfusion pressure in the five groups of rats. The ratio in the banded groups without B-APN treatment decreased at all levels of perfusion pressure compared with the value in the sham-operated group (p<0.01 at 50 and 100 mm Hg of coronary perfusion pressure; p<0.05 at 150 mm Hg). In the banded group with B-APN treatment, the ratio decreased to a value similar to that of the banded group without B-APN treatment (not significant at any level of coronary perfusion pressure). In the debanded group without B-APN treatment on aortic banding, the ratio remained unchanged at all levels of coronary perfusion pressure. However, in the debanded group with B-APN treatment on aortic banding, the ratio increased to the level of the sham-operated group at all levels of perfusion pressure. The ratio was significantly greater than that in the debanded group without B-APN treatment during aortic banding.

Figure 4 shows micrographs of arteries obtained from hearts of the five groups of rats. In an artery of the sham-operated group the media was thin, and the ratio of the thickness-to-lumen radius was small. Collagen deposition around the artery was also thin. In an artery of the banded group without B-APN treatment, the media and perivascular collagen were thicker compared with those of the artery in the sham-operated group. In the banded group with B-APN treatment, the media was thicker, but perivascular collagen was not so thick. In both of the debanded groups, the medial thickness was decreased to the same level as in the sham-operated group. The perivascular collagen also regressed but was thicker in the debanded group without B-APN treatment during aortic banding.

Figure 5 shows changes in the ratio of medial thickness/lumen radius in the five groups of rats. The ratio increased in the banded groups with and without B-APN treatment in categories of smaller vessel size. After debanding, the ratio decreased to the same level as in the sham-operated group in each category of vessel size; there was no significant difference between the debanded groups with and without B-APN treatment during aortic banding. As summarized in Table 4, there were no significant differences in lumen diameter among the five groups of rats in each category of vessel size.

Figure 6 shows the degree of collagen deposition estimated by the thickness of perivascular fibrosis in the five groups of rats. In the banded group without B-APN treatment, the thickness of perivascular fibrosis was significantly greater than that in the sham-operated group in each category of vessel size. B-APN treatment during aortic banding significantly inhibited the deposition of collagen around the arteries and arterioles in each category of vessel size (p<0.01 versus banded group). After debanding, the thickness of perivascular fibrosis decreased, but was significantly greater compared with the value in the sham-operated group (p<0.05 in the categories of vessel size, 31 μm and 101 μm≤φ and with the value in the debanded group with B-APN treatment during aortic banding (p<0.05 in the categories of vessel size larger than 31 μm). The thickness in the debanded group with B-APN
FIGURE 4. Micrographs of arteries from hearts of the five groups of rats. Left lower panel: Long axis of the vessels was determined as the maximal internal diameter in the intravascular lumen. Next, the short axis was determined as the longest internal diameter (φ) perpendicular to the long axis. Medial thickness was defined as the average value of the two minimum distances (M1 and M2) from the lumen to the outer edge of the media. Minimum distance from the outer edge of the media to the outer edge of the perivascular collagen was measured at the same points where the medial thickness was determined. Average value of the two minimum distances (F1 and F2) was construed as the thickness of perivascular fibrosis. β-APN, β-aminopropionitrile.

treatment during aortic banding did not differ from that in the sham-operated group.

Figure 7 shows changes in hydroxyproline concentration in the three groups of rats. The concentration was higher in the banded group without β-APN treatment than in the sham-operated group (p<0.01). β-APN treatment significantly decreased the concentration compared with that in the banded group without β-APN treatment (p<0.01).

Discussion

Important findings in the present study are that inhibition of collagen deposition with β-APN treatment did not improve coronary dilator reserve in pressure-overloaded hearts and that after relief of pressure overload, inhibition of collagen deposition improved the decreased reserve, which was not improved by relief of the overload alone in spite of the regression of coronary vascular medial and myocardial hypertrophy.

In hypertrophied hearts by pressure3,6,9-24 or volume25 overload, effects of increased collagen deposition and its inhibition26 on left ventricular and muscle functions have been investigated. However, the effects of collagen deposition and its inhibition on coronary circulation have not been reported. In the systemic and pulmonary circulation, inhibition of excess collagen deposition by β-APN13,14 or the proline analogue cis-4-hydroxy-L-proline15 decreased blood pressure in experimental models of deoxycorticosterone-salt treatment13 or hypoxic pulmonary hypertension.14,15 However, in our experimental model the situation concerning coronary arterial hypertension and the effect of β-APN treatment is quite different, because β-APN inhibited collagen deposition but never decreased coronary arterial hypertension and pressure overload to the left ventricle. Our data show that changes in coronary circulation caused by β-APN treatment were the result of inhibition of collagen deposition in the presence of coronary arterial hypertension and myocardial hypertrophy. To date, it remains unclear how collagen deposition affects coronary circulation in pressure-overloaded hearts before and after relief of the overload.

Methodological Considerations

In the study of Nissen et al,27 hypertension produced by deoxycorticosterone-salt treatment accelerated the turnover of collagen in the arterial wall such as the aorta and mesenteric artery from a half-life of 60–70 days to 17 days. In the pulmonary artery of rats, increased collagen and elastin by short-term (14 days) hypoxia completely regressed within 3 and 7 days, respectively.16 In our previous study,18-20 the abnormalities of the coronary circulation in cardiac hypertrophy were not fixed but were fully reversible in short-term pressure

[Image 7x25 to 588x818]
overload (4 weeks). However, decreased vasodilator reserve in long-term pressure-overloaded hearts (10 weeks) did not reverse after relief of the overload. Thus, the duration of pressure overload and coronary hypertension, and the time after its relief may be important factors that modulate collagen metabolism in the heart. The ascending aortic banding and debanding methods enabled us to choose those periods precisely. In the present study, we examined the effects of collagen deposition on coronary circulation abnormalities 10 weeks after aortic banding and 4 weeks after debanding.

We estimated coronary dilator reserve in an experimental model of isolated, empty hearts perfused with crystalloid solution containing bovine red blood cells and serum albumin. The differing levels of perfusion pressure, i.e., ascending aortic pressure, and extravascular compression forces between pressure-overloaded and control hearts may affect coronary circulation in vivo. Flow measurements at the three same levels of perfusion pressure in the five groups of rats could cancel the effects of different perfusion pressure levels on the coronary circulation in situ measurements by the microsphere method. The coronary dilator reserve was estimated as the ratio of peak flow/resting flow at the three levels of coronary perfusion pressure since the flow rates under resting conditions in each level of perfusion pressure did not differ among the sham-operated, banded, and debanded groups, as reported in situ studies by other investigators.

We estimated the degree of collagen deposition by two methods: measurement of hydroxyproline concent-

### Table 4. Lumen Diameter in the Four Categories of Vessel Size That Were Histologically Examined

<table>
<thead>
<tr>
<th>Lumen diameter (µm)</th>
<th>Sham (n=7)</th>
<th>Banded (n=8)</th>
<th>Banded + β-APN (n=8)</th>
<th>Debanded (n=7)</th>
<th>Debanded + β-APN (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10≤φ&lt;31</td>
<td>20.1±1.7</td>
<td>22.5±1.1</td>
<td>22.8±1.0</td>
<td>23.8±0.9</td>
<td>23.3±0.8</td>
</tr>
<tr>
<td>31≤φ&lt;61</td>
<td>43.2±1.1</td>
<td>42.9±1.0</td>
<td>44.4±1.2</td>
<td>46.0±1.5</td>
<td>42.5±1.8</td>
</tr>
<tr>
<td>61≤φ&lt;101</td>
<td>76.6±2.0</td>
<td>76.8±1.4</td>
<td>79.6±1.9</td>
<td>79.3±2.8</td>
<td>78.8±1.4</td>
</tr>
<tr>
<td>101≤φ</td>
<td>149.3±5.9</td>
<td>161.5±4.3</td>
<td>149.4±14.2</td>
<td>160.0±9.2</td>
<td>163.1±7.6</td>
</tr>
</tbody>
</table>

Sham, sham-operated control group; banded, banded group; banded + β-APN, banded group with β-aminopropionitrile (β-APN) treatment; debanded, debanded group; debanded + β-APN, debanded group with β-APN treatment during aortic banding; φ, lumen diameter. Values are mean±SEM.
vascular and perivascular structural changes. In the present study, we performed physiological and morphological studies in the same individual hearts of three groups of rats. Values are mean±SEM. Open bar indicates the value in the sham-operated group, striped bar the value in the banded group without β-aminopropionitrile treatment, and filled bar the value in the banded group with β-aminopropionitrile during aortic banding. LV, left ventricle; SO, sham-operated group; B, banded group; DB, debanded group. **p<0.01 compared with the value in the sham-operated group.

In the present study, we performed physiological and morphological studies in the same individual hearts of each group and contrasted coronary dilator reserve and vascular and perivascular structural changes. In the morphological study, we measured the ratio of medial thickness/lumen radius and thickness of perivascular collagen. From the changes in hydroxyproline concentration in the myocardial tissue and collagen in the perivascular tissue. These measurements revealed that collagen deposition was increased after aortic banding and that β-APN treatment during aortic banding inhibited the deposition. The outer edge of perivascular collagen was often irregular, particularly in the intramural arterial trees, and merged with adjacent connective tissue septa. Therefore, the morphological measurement of collagen was limited to arteries or arterioles around which collagen was relatively well defined and circular. In the two methods, we did not directly measure the degree of collagen deposition within the media, which may well be more limited to vasodilation than perivascular collagen. From the changes in hydroxyproline measured in the myocardial tissue and collagen in the perivascular tissue, however, we suppose that those changes correlate with the degree of collagen deposition within the media and that β-APN inhibits the collagen deposition in the media as well as in the other tissues.

In the present study, we performed physiological and morphological studies in the same individual hearts of each group and contrasted coronary dilator reserve and vascular and perivascular structural changes. In the morphological study, we measured the ratio of medial thickness/lumen radius and thickness of perivascular fibrosis in the hearts fixed at a perfusion pressure of 100 mm Hg and classified coronary arterial trees into four categories by lumen diameter. However, we did not determine the number of all arterial trees in each category of vessel size. In addition, some shifts in lumen diameter might have occurred after aortic banding or debanding, or both. From the present study, it is not clear how the changes in the ratio quantitatively altered the entire coronary vascular resistance through the changes in lumen diameter after aortic banding with and without β-APN treatment or debanding. Also, it is not clear how functional changes in the vascular wall or smooth muscles, or both, resulted in differences in coronary dilator reserve between the two debanded groups with and without β-APN treatment. Our morphological data provide one possible mechanism responsible for the regression of coronary dilator reserve after relief of pressure overload.

Determinant Factor or Factors of Coronary Reserve in Hypertrophied Hearts

Aortic banding decreased coronary dilator reserve at all levels of perfusion pressure compared with sham-operated controls. The ratio of medial thickness/lumen radius was increased in both of the banded groups with and without β-APN treatment. The coronary vascular hypertrophy in banded groups is consistent with the observations concerning spontaneously hypertensive rats by Anderson et al. The increase in the ratio of medial thickness/lumen radius in the present study would be the result of intimal thickening, hypertrophy, or hyperplasia of the vascular smooth muscle or increased connective tissues. Therefore, it was possible that β-APN treatment decreased the ratio through inhibition of collagen deposition in the media. However, the ratio of medial thickness/lumen radius was only slightly decreased by β-APN treatment. The β-APN treatment inhibited the increase in collagen deposition that was observed in the banded group without β-APN treatment. Thus, the coronary dilator reserve in hypertrophied hearts decreased independently of the degree of collagen deposition in the myocardial tissue. These results indicate that an increase in the ratio of medial thickness/lumen radius or myocardial hypertrophy, or both, rather than collagen deposition is more important for coronary dilator reserve in pressure-overloaded hearts, probably through reduced lumen diameter of arterioles.

Determinant Factor or Factors of Reversibility of Decreased Coronary Reserve After Relief of Pressure Overload

Decreased coronary dilator reserve did not reverse to normal in the debanded group without β-APN treatment but reversed in the debanded group after β-APN treatment. Obviously, the differing results in vasodilator reserve between hearts with and without β-APN treatment were not due to myocardial hypertrophy because myocardial hypertrophy in the two banded groups regressed to similar levels. Anderson et al. studied the medial area of coronary arterial trees after normalization of hypertension by 12 weeks of hydralazine treatment in spontaneously hypertensive rats and reported that regression of medial hypertrophy accompanied improvement of coronary dilator reserve. In the present study, the ratio of medial thickness/lumen radius regressed in both of the debanded groups, but the regression did not improve coronary reserve in the banded group without β-APN treatment. Based on the present study's findings, medial hypertrophy seems not to be a determinant factor for reversibility of decreased coro-
nary dilator reserve. One of the most important factors for reversal of vasodilator reserve seems to be deposition of collagen.

In the present study, hydroxyproline concentration in the myocardial tissue and perivascular fibrous collagen significantly increased in pressure-overloaded hearts. The increased collagen partially regressed after relief of pressure overload. The regression of collagen was also observed by other investigators. Poiani et al. reported that increased collagen and elastin in pulmonary artery during hypertension produced by hypoxia returned to normal after a 7-day recovery from hypoxia. In the studies of Motz and Strauer and Mukherjee et al., increased collagen deposition in the hypertrophied myocardium regressed after treatment with a Ca\(^{2+}\) blocker or angiotensin converting enzyme inhibitor in spontaneously hypertensive rats. In the present study, the decreased vasodilator reserve due to pressure overload, however, did not improve possibly because of the remaining collagen. In the studies of Weber et al. and Mukherjee et al., the proportion of collagen types I and III as well as total collagen in myocardial tissue varied with time of pressure overload during development and regression of myocardial hypertrophy by angiotensin converting enzyme inhibitor in spontaneously hypertensive rats. It is also suggested that increased cross-linking of type I and type III collagens is important for diastolic stiffness of hypertrophied hearts. Although we did not measure the types of collagen or degree of cross-linking of collagen, the rate of collagen degradation in the vascular wall or perivascular tissue may depend on the type and structure of collagen. The persistent, stiffer collagen of type I may determine the characteristics of the coronary vascular wall. If so, the type of collagen rather than its amount determines the reversibility of the decreased coronary vasodilator reserve after relief of pressure overload.

The changes in morphologically measured perivascular collagen paralleled those in the myocardial tissue estimated by hydroxyproline measurements. The inhibition of excess collagen deposition by \(\beta\)-APN treatment might produce an increase in the diastolic relaxation rate, a decrease in stiffness of the ventricular tissue, or both. The changes in mechanical properties of the myocardium could contribute to the difference in coronary dilator reserve between the debanded groups with and without \(\beta\)-APN treatment. However, we suppose that the contribution would be minimal because the inhibition of collagen deposition did not significantly alter coronary dilator reserve in the banded groups.

Marked perivascular collagen deposition has been observed in an experimental model of volume-overloaded failing hearts and in humans without coronary arterial hypertension. Coronary vascular remodeling including medial and perivascular fibrosis occurs even in the situation of normal coronary arterial pressure. It is suggested that an elevated circulating renin-angiotensin system has an important role in collagen deposition in the interstitial and perivascular tissues. In our experimental model, aortic pressure distal to the banded position, which perfuses other organs including the kidneys, was not decreased. In addition, there was no evidence of heart failure such as an elevated end-diastolic pressure, pleural effusion, or ascites. Although we did not measure the levels of plasma renin, angiotensin II, or aldosterone, the circulating renin-angiotensin system would not be elevated. The primary stimulus for medial hypertrophy and perivascular collagen deposition in the present study would be an elevated intravascular pressure. This elevated intravascular pressure might activate the angiotensin converting enzyme in the myocardial tissue. In the study of Shunkert et al., angiotensin converting enzyme messenger RNA expression was fourfold higher than that in control hearts, and intracardiac fractional conversion from angiotensin I to II increased in hypertrophied hearts. Locally generated cardiac angiotensin II may affect the coronary vascular remodeling through promotion of smooth muscle cell growth and deposition of extracellular matrix protein and its potent vasconstrictor action on coronary circulation under resting conditions and vasodilatation.

In summary, we studied coronary dilator reserve in hypertrophied hearts before and after relief of pressure overload. Inhibition of collagen deposition by superimposition of \(\beta\)-APN treatment did not improve the decreased vasodilator reserve in pressure-overloaded hearts, but significantly improved it after relief of the overload. These data suggest that factors other than collagen deposition such as coronary vascular medial hypertrophy play an important role in the regulation of coronary dilator reserve in the stabilized phase of cardiac hypertrophy and that collagen deposition diminishes the reversibility during regression of myocardial hypertrophy.

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