Protocollagen Proline Hydroxylase Activity in Rat Heart During Experimental Cardiac Hypertrophy

By Seppo Lindy, Heikki Turto, and Jouni Uitto

ABSTRACT

Cardiac hypertrophy was produced in rats by constricting the abdominal aorta subdiaphragmatically. The weight of the left ventricle was significantly elevated 2 days after aortic constriction and was 19% higher than in sham-operated animals at 11 days. The activity of protocollagen proline hydroxylase (PPH), an enzyme participating in collagen biosynthesis, and the hydroxyproline content of the heart muscle were determined. PPH activity was increased at 2 days after aortic constriction and declined thereafter, being still above the control level at 11 days. The hydroxyproline content of the left ventricle was significantly increased at 11 days in hypertrophied heart muscle compared to controls. The present results suggest that in cardiac hypertrophy an early connective tissue activation occurs. Later, this leads to connective tissue accumulation. The increased collagen content may give support to the cardiac muscle contracting against systolic overload.

KEY WORDS

- aortic constriction
- connective tissue
- left ventricular hypertrophy
- collagen
- hydroxyproline

Much data has recently accumulated concerning the biochemical changes in heart muscle in experimental cardiac hypertrophy (for review see refs. 1, 2). During the first few days the process leading to hypertrophy is characterized by increased RNA synthesis (3, 4), enhanced protein synthesis (5-7), and elevated thymidine incorporation into DNA (8-10). Using radioautography, increased thymidine incorporation has been shown to occur mainly in fibroblasts (8-10). In a later phase, increased collagen content has been found in the hypertrophied heart muscle (10-13).

In the present work, we studied connective tissue metabolism of rat heart by determining the collagen content, measured as hydroxyproline, and the activity of protocollagen proline hydroxylase (PPH) during left ventricular hypertrophy caused by aortic constriction. PPH is an enzyme participating in the biosynthesis of collagen, and consequently assay of its activity may give further information about the capacity for connective tissue formation in heart hypertrophy.

Methods

Under ether anesthesia, the abdominal aorta of male Sprague-Dawley rats, weighing about 200 g, was constricted subdiaphragmatically by tying a silk ligature around the aorta and a needle with an outer diameter of 1.0 mm. The needle was then removed leaving the aorta constricted to the diameter of the needle. Control animals were sham-operated without the ligature. The rats were fed ordinary laboratory diet and given water ad libitum.

The rats were killed under ether anesthesia at 2, 4, 7, and 11 days after the operation. The heart was removed, and the atria and the right ventricle were dissected and discarded; the remaining left ventricle and septum were weighed. A sample of about 200 mg was taken from the left ventricular wall, weighed, and homogenized with an Ultra-Turrax homogenizer in 0.14M NaCl (1 part in 20 volumes) three times for 5 seconds. The homogenate was centrifuged at 15,000 g for 30 minutes at 4°C.
For the assay of PPH activity, 300 μliters of the supernatant fluid were incubated with 14C-labeled protocollagen substrate (50,000 dpm), 50 mM Tris-HCl buffer (pH 7.8 at 24°C), 2 mM ascorbic acid, 0.5 mM α-ketoglutarate, 0.08 mM FeSO₄, and 0.05 mg/ml catalase, in a final volume of 4.0 ml (14, 15). After 60 minutes of incubation at 37°C, the samples were hydrolyzed in 6M HCl for 6 hours at 136°C, and total 14C-radioactivity (16) and 14C-hydroxyproline (17) were assayed in the hydrolysate. The activity of PPH was expressed as dpm 14C-hydroxyproline formed per hour per gram wet weight of the tissue. Protocollagen substrate was prepared from tibias of 10-day-old chick embryos as described earlier (14, 18) and divided into aliquots of 50,000 dpm containing about 0.3 μg of protocollagen.

The amount of hydroxyproline was determined in the heart samples at 7 and 11 days as follows. Part of the homogenate was precipitated with 10% trichloroacetic acid (TCA), and the precipitate was washed twice with 5% TCA and then dissolved in 1M NaOH. From the alkali digest, an aliquot was hydrolyzed in 6M HCl for 6 hours at 136°C. Hydroxyproline was measured from the hydrolysate according to the method of Kivirikko et al. (19).

The results were evaluated using Student's t-test. The differences were regarded as significant at \( P < 0.05 \).

Results

The cofactor requirements and subcellular distribution of PPH in rat heart were investigated. These were similar to those found in other animal or human tissues (20, 21). The enzyme in rat heart required ascorbate, α-ketoglutarate, and ferrous iron as cofactors, and 79% of the enzyme activity was found in the 100,000 g supernatant fluid of the tissue homogenate. In addition, the amount of 14C-hydroxyproline formed from the 14C-proline-labeled protocollagen was proportional to the amount of 15,000 g supernatant protein up to 0.7 mg (Fig. 1). Consequently, subsequent experiments were carried out with 0.1–0.4 mg of protein from the 15,000 g supernatant fluid of the tissue homogenate. These control experiments were performed to assure that adequate amounts of enzyme protein were used in the determinations of PPH activity.

Cardiac hypertrophy was verified by calculating the ratio of left ventricular weight to body weight. Two days after the operation, the value in animals with aortic constriction was 9% higher than that in the controls and increased further to 20% above the control level at 7 days (Table 1).

Figure 2 presents PPH activity in the left ventricle after coarctation of the aorta. The PPH activity was significantly elevated at 2 days, being 139% higher than in the controls.

### Table 1

<table>
<thead>
<tr>
<th>Days after operation</th>
<th>Left ventricular wt/body wt (mg/100 g)</th>
<th>Controls</th>
<th>AC = aortic constriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>188.1 ± 4.1</td>
<td>203.6 ± 6.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>185.9 ± 13.6</td>
<td>216.8 ± 16.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7</td>
<td>187.8 ± 7.8</td>
<td>227.5 ± 9.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>11</td>
<td>184.2 ± 4.8</td>
<td>220.2 ± 8.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± sd of 7 or 8 rats. AC = aortic constriction.

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The activity then decreased but was still significantly higher than the control values at 11 days after coarctation.

The contents of hydroxyproline per unit wet weight and per total weight of the left ventricle in the heart after aortic constriction are shown in Table 2. At 7 days, the values of hydroxyproline in the left ventricle in the group with coarctation did not differ from those in the sham-operated controls. Eleven days after coarctation, no significant differences in hydroxyproline per unit wet weight of tissue could be seen between the two groups, but the total amount of hydroxyproline was significantly higher ($P < 0.01$) in left ventricles of animals with coarctation.

**Discussion**

Increased collagen content of the heart has been shown to accompany cardiac hypertrophy caused by various experimental procedures. Constriction of the pulmonary artery in cats caused right ventricular hypertrophy and collagen accumulation within 20-50 days (11). In rats, aortic constriction lasting 5 months, physical exercise for 6 weeks, and isoprenaline-thyroxine injections for 17 days have been shown to increase cardiac collagen content.

**TABLE 2**

Contents of Hydroxyproline in Hypertrophied and Control Hearts in Rats

<table>
<thead>
<tr>
<th>Days after AC</th>
<th>Hydroxyproline (μg/mg wet wt)</th>
<th>Hydroxyproline (μg/LV)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control LVH</td>
<td>Control LVH</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.308 ± 0.038</td>
<td>0.270 ± 0.082</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>0.372 ± 0.071</td>
<td>0.424 ± 0.072</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD of 7 or 8 rats. LVH = left ventricular hypertrophy; AC = aortic constriction.
this increase being of the same extent as the increase in heart weight (12). On the other hand, isoprenaline injections without thyroxine or exposure to simulated high altitude for 320 days increased collagen content without increasing the muscle mass (12). Collagen content does not increase, despite increase in heart weight, when the hypertrophy is caused by sideropenic anemia (12), possibly because collagen formation is thought to be directly related to the amount of ionizable iron in the tissue (12). The lack of collagen deposition in thyroxine-induced cardiac hypertrophy (12, 22), on the other hand, may be due to the inhibition of biosynthesis and enhancement of catabolism of collagen in hyperthyroidism (23).

That elevated collagen content in coarctation-induced cardiac hypertrophy is, at least partly, due to increased synthesis has been recently verified by measuring the incorporation of radioactive proline into collagen hydroxyproline (24). In the present work, we found increased collagen content per left ventricle at day 11 after coarctation. This confirms a similar finding presented earlier (10). However, Grove et al. (10) also found an increase in hydroxyproline per unit wet weight of tissue in the hypertrophied hearts. This difference may be due to a slower onset and less marked hypertrophy with constriction of abdominal as opposed to the thoracic aorta.

PPH activity, found to be increased in the hypertrophied heart muscle, had already increased by the second day after operation, thereby preceding the increase in collagen content. PPH is an enzyme which hydroxylates proline to hydroxyproline in procollagen, the precursor of collagen. Increase of PPH activity has been shown to parallel the accumulation of collagen in experimental granuloma (25). In silica-induced pulmonary fibrosis in rats (26), the increase in PPH activity occurred markedly earlier than the increase in collagen content, just as in the present study. Therefore, increase in PPH activity may be considered as an indicator of increased collagen-forming capacity.

Increased amounts of labeled thymidine have been found to incorporate into the nuclei of connective tissue cells (8-10), suggesting fibroblast proliferation. In fact, increased numbers of connective tissue nuclei per heart have been found after constriction; however, the concentration of connective tissue nuclei decreases (8) or remains constant (9, 10). Thus the increased PPH activity per unit wet weight of tissue found in our work cannot be explained only by the increase in the number of connective tissue cells, but increased activity is also caused by de novo synthesis of enzyme protein or by activation of inactive forms of PPH. This is supported by the finding of Zak et al. (24) that a peak in collagen hydroxyproline labeling preceded a peak in DNA labeling in rat heart muscle undergoing hypertrophy.

Collagen fibers are located in the heart between muscle fibers and along blood vessels. Because the capillary concentration in hypertrophied heart, caused by physical exercise, has been shown to decrease (27), it seems possible that collagen, found to be increased in the present work, is mainly accumulated between the muscle fibers. This is supported by the work of Schoenmackers, who found that in intact heart connective tissue constitutes 7% of the cross section compared to 20% in cardiac hypertrophy due to hypertension (28).

In hypertrophied heart, collagen may give support to the cardiac muscle, which is contracting against overload. Preceding the accumulation of collagen, increased activity of PPH was found in the cardiac muscle, suggesting increased capacity for collagen biosynthesis. This increase is not only caused by increased numbers of fibroblasts but also by de novo synthesis or activation of PPH, an enzyme involved in the biosynthesis of collagen.

References
3. FANBURG, B.L., AND POSNER, B.I.: Ribonucleic

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