Regression of Cardiac Hypertrophy in Spontaneously Hypertensive Rats by Enalapril and the Expression of Contractile Proteins

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Several experimental models involving the development of cardiac hypertrophy in adult rats are characterized by the reexpression of the fetal isoform of myosin heavy chain (V3). To determine whether a similar adult-to-fetal shift in the expression of the thin-filament proteins occurs during cardiac hypertrophy, we have examined the expression of the isoforms of myosin, tropomyosin, and troponin T in the left ventricle of young spontaneously hypertensive rats (SHR) with and without treatment using enalapril, an angiotensin converting enzyme inhibitor. Phosphorylation of tropomyosin, which is predominant in the fetal state, was also analyzed. Twelve-week-old SHR were treated with enalapril for 2, 5, 8, and 9 weeks followed by withdrawal of treatment for 9 weeks. Control SHR, without drug treatment, were weight- and age-matched. After 9 weeks of enalapril treatment, mean arterial blood pressure was reduced (from 166 ± 11 to 89 ± 5 mm Hg), and left ventricular weight/body weight ratio was regressed (from 2.53 ± 0.14 to 1.96 ± 0.05 g/kg) to normotensive levels. During the 9-week treatment period, the percent V3 decreased in SHR substantially from 35 ± 3% to 13 ± 1%. There was a significant correlation between the left ventricular hypertrophy and the percent V3 myosin expression in the SHR during regression (r = 0.697, p < 0.001). However, only the adult isoforms of tropomyosin and troponin T were detected in the SHR with or without enalapril treatment, and the level of tropomyosin phosphorylation remained constant irrespective of the degree of left ventricular hypertrophy. These results suggest that the adult-to-fetal switch in the expression program of myosin isoforms that accompanies the development of left ventricle hypertrophy is not adopted by the thin-filament proteins, tropomyosin and troponin T.

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Generally, left ventricular hypertrophy develops in response to a chronic pressure load in rats.1–3 Cardiac hypertrophy is a quantitative as well as a qualitative process; the former is reflected in the total nucleic acid and protein synthesis and the latter in the selective synthesis of different protein isoforms. Myosin has been studied widely in the hypertrophied heart. There are two isoforms of myosin heavy chains, α and β, in the left ventricle coded by different genes.4 Under non-denaturing conditions, myosin exists as either homodimers, V1 (αα) and V3 (ββ), or heterodimers, V2 (αβ), which are separable on non-denaturing polyacrylamide gels.5 In the adult rat, the α form is preferentially expressed, whereas β is the major form in the fetal heart.1 In several models of rat cardiac hypertrophy (e.g., aortic coarctation, Goldblatt hypertension, and spontaneously hypertensive rats [SHR]), the β fetal myosin isoform is reexpressed at the expense of the α form.6,7

The maximum shortening velocity of muscle in general correlates positively with the steady-state, actin-activated myosin adenosine triphosphatase (ATPase) activity.6 In rats, the maximum shortening velocity of cardiac muscle is markedly reduced in the hypertrophied heart, which expresses preferentially the V3 to V1 myosin isoform9; this is consistent with in vitro observations that V3 has lower ATPase activity than V1.10 Thus, the adult-to-fetal switch in the expression of myosin isoforms in cardiac hypertrophy can account for, in part, the difference in contractility between the normal and hypertrophied hearts in rats. However, other factors may contribute to the altered contractile properties of the hypertrophied heart.11,12

Possible candidates are the thin-filament contractile proteins, tropomyosin, troponin, and actin. Unlike myosin, relatively little is known about these proteins in the hypertrophied heart.
Tropomyosin in mammalian striated muscle consists of two isoforms, α and β. In rat skeletal muscle, the β isoform predominates in the fetal muscle, which is slowly replaced by the α isoform during development until about equal amounts of the two isoforms are expressed in the adult. In rat cardiac muscle, however, only the α isoform, which is coded by the same gene as in the skeletal muscle, has been detected at the protein level in the fetal and adult heart. Recently, it has been reported that messenger RNA (mRNA) of β-tropomyosin is found in the left ventricle of rats during the early phase of hypertrophy development induced by aortic coarctation. If one considers β-tropomyosin to be a fetal isoform (i.e., skeletal muscle expression program), these data suggest that an adult-to-fetal switch in the expression program for tropomyosin occurs at least at the mRNA level; however, the relative amounts of the α- and β-tropomyosin mRNA and expression of the β isoform at the protein level in the hypertrophied heart were not analyzed. The level of tropomyosin phosphorylation varies dramatically during development of the heart. In the fetal rat heart, the level of phosphorylation is about 70% but declines to less than 30% in the adult heart.

Two troponin T isoforms, 43 kDa and 41 kDa, have been identified in the rat left ventricle. The smaller isoform is present in trace amounts in the fetal heart but increases after birth with concomitant decrease of the larger form, which becomes undetectable in the adult heart. Different isoforms of troponin C and troponin I have also been identified in fetal and adult cardiac muscle in a variety of vertebrates.

The objectives of the present study are 1) to determine whether the observed adult-to-fetal switch in the expression of myosin isoforms during the development of cardiac hypertrophy in SHR also applies to the expression of thin-filament proteins, tropomyosin and troponin T; 2) to determine whether there are any increases in the level of phosphorylation of tropomyosin during the development of cardiac hypertrophy; and 3) to determine whether enalapril, an angiotensin converting enzyme inhibitor effective in lowering blood pressure and regressing ventricular mass in SHR, has any effect on the expression of the contractile protein isoforms. To our knowledge, this is the first report on the expression of myosin and thin-filament proteins during the development and regression of cardiac hypertrophy in SHR.

Methods

Animals and Experimental Design

Thirty 12-week-old SHR (Charles River Laboratories, Montreal, Canada) were treated with enalapril by inclusion of the drug in the water at a rate of 25 mg/kg/day. Concentrations were adjusted weekly to match the body weight/consumption ratio as described by Korner et al. Groups of rats were treated for 2, 5, 8, or 9 weeks with enalapril. A separate group of rats was treated with enalapril for 9 weeks followed by withdrawal of treatment for 9 weeks. Control untreated SHR were age- and weight-matched. Direct blood pressure was measured using implanted aortic catheters as described by Head and Adams. After death, the hearts were removed and blotted, and the left ventricles were excised and weighed. Left ventricular hypertrophy was expressed as the ratio left ventricular weight/body weight (g/kg). The left ventricles were frozen immediately in liquid N₂ and stored at −70°C until analyzed for myosin, tropomyosin, and troponin T.

Protein Extracts and Gel Electrophoresis

Myosin. The frozen tissue from the left ventricle was homogenized in 10% glycerol, 20 mM Na₂HPO₄, pH 8.8, centrifuged, and the supernatant analyzed for the V₁, V₂, and V₃ heavy chain isoforms by electrophoretic separation in 4% nondenaturing pyrophosphate polyacrylamide gels at 2°C with a circulating bath as described by Hoh et al., except that a slab gel system was used instead of tubes for easy comparison of band patterns in multiple samples.

Tropomyosin. Left ventricular muscle was homogenized in 10 vol 9.5 M urea, 4% ampholine (pH range 4–6), 5% β-mercaptoethanol, and 2% nonidet P40. The homogenate was subjected to two-dimensional isoelectric focusing/sodium dodecyl sulfate (SDS) gel electrophoresis as described by Heeley et al. A pH range of 4–6 with 4% ampholine was used. This gel system can separate the phosphorylated and nonphosphorylated α- and β-tropomyosin into four distinct spots.

Troponin T. The left ventricular tissue was homogenized in 10 vol low salt buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 1 mM dithiothreitol), solubilized in SDS sample buffer, heated at 100°C for 5 minutes, and separated on SDS-polyacrylamide gel electrophoresis. Proteins were transferred to Immobilon (Millipore Corp., Bedford, Mass.) and immunoblotted with mouse anti-troponin T antiserum (a gift from Dr. Jim J.-C. Lin, Department of Biology, University of Iowa, Iowa City) as described by Jin and Lin, except that alkaline phosphatase conjugated goat anti-mouse immunoglobulin G was used. The relative amount of the protein isoforms was analyzed by either a Laser densitometer (LKB, Sweden) for one-dimensional gels or by an image analyzer for two-dimensional gels after Coomassie blue staining. Results were expressed as the mean±SEM. Student’s t test was used to compare two groups of data at each time point. Regression lines were calculated using the method of least squares. A value of p<0.05 was considered statistically significant.

Results

All of the following changes described are significant. In Figures 2–5, with the exception of the 12-week time point (two rats), all points represent measurements from at least four rats. In Figures 6A and 6B, each point represents one measurement (i.e., one rat).
Isoform Patterns of Myosin, Tropomyosin, and Troponin T

Figure 1 shows typical patterns of left ventricular myosin, tropomyosin, and troponin T isoforms from 1) 21-week-old SHR that have been treated with enalapril for the previous 9 weeks, 2) control untreated SHR, 3) fetal normotensive rats, and 4) adult normotensive rats. Myosin V3 is the predominant form in the enalapril-treated SHR and WKY rats, whereas V2 made up 35% of the total myosin isoforms in the untreated control SHR and over 90% in the fetal hearts from normotensive rats (Figure 1A). As shown in Figure 1B, only the α-tropomyosin isoform is found in all the left ventricular tissue in normotensive fetal and adult rats and in SHR with or without enalapril treatment. No β-tropomyosin was detected even when the gel was overloaded with tissue extracts (i.e., even if the β-isoform was expressed, it must be present in an insignificant amount relative to the α-isoform). In the normotensive fetal heart, the level of tropomyosin phosphorylation was about 65%, but the level was much lower in the normotensive adult heart (~30%). However, there was no significant change in the level of phosphorylation in the SHR (~30%) irrespective of enalapril treatment. Figure 1C shows the presence of both 43 and 41 kDa isoforms of troponin T in the fetal heart, whereas only the 41 kDa form is detected in the hearts of the adult WKY rats. In the SHR, only the adult isoform (41 kDa) is detected whether the heart is hypertrophied or regressed to normal size with enalapril treatment.

Expression of Myosin, Tropomyosin, and Troponin T Isoforms and Level of Tropomyosin Phosphorylation

Percent myosin V3 increased with age for the untreated SHR, from 21±1% in the 12-week-old to 47±2% in the 30-week-old rats (Figure 4). Treatment of the SHR with enalapril for 9 weeks decreased the expression of V3 dramatically from 35±3% in untreated 21-week-old SHR to 13±1% in enalapril-treated SHR of the same age. After withdrawal of enalapril treatment for 9 weeks, the percent expression of V3 in the SHR was 30±2% in the enalapril-treated SHR compared with 47±2% in the untreated rats.

Correlation Between Left Ventricular Hypertrophy and Protein Expression

A strong correlation was found between the percent V3 and the degree of left ventricular hypertrophy for the SHR treated with enalapril (n=23, 1990).
r=0.697, p<0.001), as shown in Figure 6A. In contrast, the correlation was insignificant for the control untreated rats (n=23, r=0.16) (data not shown); this is not unexpected as the degree of hypertrophy in the untreated rats increased only slightly with age, from 12 to 30 weeks (Figure 3). However, the percent of V₃ increased at a much faster rate for the same time period (Figure 4). These findings suggest that for the untreated SHR with established cardiac hypertrophy (age 12–30 weeks), increase in the expression of myosin V₃ in the left ventricle is attributable to aging associated with the duration of hypertrophy. A strong correlation between percent V₃ and age of the untreated rats was indeed found (n=24, r=0.852, p<0.001) (Figure 6B). The correlation between the level of tropomyosin phosphorylation and degree of left ventricular hypertrophy was not significant (n=23, r=0.045) (figure not shown).

Discussion

Reexpression of the fetal isoform (V₃) of myosin heavy chain at both mRNA and protein levels accompanying left ventricle hypertrophy is well documented using various rat models (e.g., aortic coarctation, renal artery stenosis, and in SHR).1-6 However, relatively little is known about the thin-filament contractile proteins in cardiac hypertrophy. Using the aortic coarctation rat model, two studies17,25 have shown that mRNA of fetal actin and tropomyosin isoforms are reexpressed during the early phase of induced hypertrophy (2–7 days after the operation), but the protein levels were not analyzed. It has also been shown that cardiac hypertrophy induced in rats by renal artery stenosis does not change the troponin T isoform expression profile.19 In the present study, we did not detect the fetal protein isoforms of tropomyosin (β) and troponin T (41 kDa) in hypertrophied rat left ventricles of SHR, although myosin V₃ was expressed at a much higher level in the untreated SHR than in the normotensive counterparts. These results indicate that, at least in the SHR model, the adult-to-fetal switch in the expression program of the fetal myosin isoform accompanying development of left ventricular hypertrophy is not adopted by the thin-filament pro-
teins, tropomyosin and troponin T. It is likely that different expression programs for the thick- and thin-filament proteins also occur in other models of cardiac hypertrophy induced by pressure overload, as we have found that the pattern of left ventricular tropomyosin expression is not altered in adult rats subjected to aortic coarctation (T.J. Childs and A.S. Mak, unpublished results).

That the level of tropomyosin phosphorylation is much higher in the fetal heart than in the adult emphasizes the importance of this modification in the embryonic muscle. This posttranslational modification could represent a modulatory mechanism for the fine tuning of the contractile apparatus to the particular needs of the embryonic tissue. We have observed a relatively constant level of tropomyosin phosphorylation in the SHR irrespective of the degree of left ventricular hypertrophy. It suggests that the phosphorylation mechanism prevalent in the fetal heart is not reintroduced in the adult SHR during the development of cardiac hypertrophy.

There are a few studies that evaluate myosin isoform expression with respect to associated regression of cardiac hypertrophy in renovascular hypertensive and normotensive rats using angiotensin converting enzyme inhibitors. As far as we are aware, there are no reports on myosin and thin-filament protein isoforms during regression in the SHR treated with angiotensin converting enzyme inhibitors. As reported by others using renovascular hypertensive rats, we found that regression of blood pressure and left ventricular mass in SHR by enalapril were accompanied by a decrease in myosin V3 with concomitant increase in V1. However, regression had no effect on the expression of tropomyosin and troponin T isoforms, nor on the level of phosphorylation of tropomyosin. This study suggests that in SHR during enalapril treatment, the renin-angiotensin system plays an important role, either directly or indirectly, in the regulation of myosin isoform expression, the ventricular mass, and blood pressure, or at least in their interaction. The expres-
sion of tropomyosin and troponin T isoforms and the phosphorylation of tropomyosin, however, appear to be independent of angiotensin II, left ventricular mass, and blood pressure in the SHR. Although troponin C and troponin I have not been investigated in the present study, it is of interest to determine whether the expression of troponin C and troponin I isoforms is affected during the development and regression of cardiac hypertrophy.

The amount of myosin V3 correlates significantly with the degree of hypertrophy in the SHR during regression with enalapril treatment (Figure 6A) but not in the control untreated SHR. The simplest explanation is that the left ventricular mass in the control group is already stabilized and only increases very slowly with age (Figure 3), whereas the more dramatic increase in the amount of V3 is essentially due to aging of the animals (Figure 6B). This suggests that the mechanism controlling the expression of the myosin isoforms is dependent on both aging and the early development of hypertrophy. During the enalapril treatment of SHR, the age effect is masked by the more acute process of regression of hypertrophy resulting in the observed increase in the expression of V3.

The hypertrophied hearts of SHR, with myosin in the fetal isoform pattern and the thin-filament proteins in the adult pattern, represent an interesting phenotype that functions differently from either the adult or fetal hearts of normotensive rats. Subtle differences in the structure and function of different isoforms of the same contractile protein have been reported. Myosin V3 has been shown to have lower actin-activated ATPase activity than the V1 isoform,10 which may account partially for the lower shortening velocity of the muscle tissue from hypertrophied hearts.9 In vitro experiments have shown that β-tropomyosin has a higher affinity for F-actin but a lower affinity for troponin T than α-tropomyosin.29 Phosphorylated tropomyosin has a greater propensity

**FIGURE 6.** Scatterplots showing correlation of percent myosin isozyme V3 with left ventricle weight/body weight ratio (L.V./B.W.) (panel A) in spontaneously hypertensive rats (SHR) treated with enalapril for 0 to 9 weeks (r=0.697, p<0.001), and with age (panel B) in SHR not treated with enalapril (r=0.832, p<0.001).
for head-to-tail polymerization, and strengthening of this head-to-tail interaction by troponin T is substantially reduced by phosphorylation. The two troponin T isoforms from bovine heart function similarly by phosphorylation.30-33 The two troponin T isoforms from bovine heart function similarly by phosphorylation.30-33 The structural and functional differences in the various isoforms of the same contractile protein are often subtle, a successful adaptation to the changing environment can be achieved by a well-orchestrated coexpression of the appropriate mixture of isoforms. It remains to be determined how different patterns of expression of various contractile proteins can equip the heart to adapt to different physiological demands. Therefore, determination of different patterns of protein expression in various models of cardiac hypertrophy remains an important area of research.

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