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Research Article

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Myosin Heavy Chain Messenger RNA and Protein Isoform Transitions during Cardiac Hypertrophy

Interaction between Hemodynamic and Thyroid Hormone–induced Signals

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Abstract

Expression of the cardiac myosin isozymes is regulated during development, by hormonal stimuli and hemodynamic load. In this study, the levels of expression of the two isoforms (α and β) of myosin heavy chain (MHC) during cardiac hypertrophy were investigated at the messenger RNA (mRNA) and protein levels. In normal control and sham-operated rats, the α-MHC mRNA predominated in the ventricular myocardium. In response to aortic coarctation, there was a rapid induction of the β-MHC mRNA followed by the appearance of comparable levels of the β-MHC protein in parallel to an increase in the left ventricular weight. Administration of thyroxine to coarctated animals caused a rapid deinduction of β-MHC and induction of α-MHC, both at the mRNA and protein levels, despite progression of left ventricular hypertrophy. These results suggest that the MHC isozyme transition during hemodynamic overload is mainly regulated by pretranslational mechanisms, and that a complex interplay exists between hemodynamic and hormonal stimuli in MHC gene expression.

Introduction

In several models of cardiac hypertrophy, an increase in myocardial mass has been shown to be associated with a decrease in active tension and velocity of shortening (reviewed in reference 1). Because of the close correlation observed between the maximum velocity of contraction with the specific activity of myosin ATPase (2), the early search for biochemical correlates of cardiac hypertrophy centered around the attempts to relate myosin ATPase activity to physiological function. The relationship of decreased mechanical performance to decreased myosin ATPase activity was reported in several animal species in various models of hemodynamic load (reviewed in reference 3). In addition, the changes in myosin ATPase activity have been shown to be associated with altered contractile performance in various pathophysiological states, including physical training, hyper- and hypothyroidism, and other hormonal changes (3).

Differences in myosin ATPase activity were later found to be due to the existence of the distinct myosin isozymes called V1, V2, and V3, in order of decreasing electrophoretic mobility and ATPase activity (4). These three isozymes, however, consist of two heavy chains (α and β) associated with identical light chains; V1 is the $\alpha\alpha$ homodimer and has the highest ATPase activity; V3 is the $\beta\beta$ homodimer with the lowest ATPase activity; while V2 is believed to be the $\alpha\beta$ heterodimer (5). Thyroid hormone induces V1 myosin and deinduces V3 in all species so far studied (reviewed in reference 6). Hemodynamic overload on rat (7, 8) and rabbit (9) ventricles and human atria (10, 11) has been shown to result in the transition of myosin toward V3, thus providing, at least in part, a biochemical basis for the decrease in contractility associated with hypertrophy (12). In the ventricles of larger animals, in which normal ventricular myosin is predominantly V3, little or no isozyme transition occurs in response to hemodynamic overload (13). Immunological studies and peptide mapping suggested that the α- and β-myosin heavy chains (MHCs)[1] differ in primary structure (reviewed in references 6 and 14). More recently, complimentary DNA (cDNA) and genomic DNA cloning experiments have demonstrated that they are coded by two separate genes (15–17) that are linked 4 kilobases apart in the rat genome (18).

With the availability of gene-specific DNA probes, it is now possible to address the question of how the MHC isozyme transitions observed during cardiac hypertrophy are regulated. In this study, we examined the effect of aortic coarctation (CoA) on α- and β-MHC gene expression at the messenger RNA (mRNA) and protein levels in the rat. The close correlation observed between the relative levels of α- and β-MHC mRNAs and their corresponding proteins suggests that the MHC isozyme transition induced by pressure overload is mainly regulated by pretranslational mechanisms. In addition, thyroid hormone, when given to coarctated animals in high enough doses, is able to reverse at the pretranslational level the MHC isozyme transition produced by pressure overload.

Methods

Animals and surgical procedures. The study consisted of two groups of rats. The first group included male Wistar rats that were 6–8 wk old and weighed ~ 180 g at the time of operation. Surgical procedures were carried out under pentobarbital anesthesia, 40 mg/kg i.p. CoA was performed by placing a partially occluded weck hemoclip around the upper

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1. *Abbreviations used in this paper:* CoA, aortic coarctation; MHC, myosin heavy chain; nt, nucleotide; T_3, tri-iodothyronine; T_4, thyroxine.

part of the abdominal aorta as described (8). The mortality rate of this procedure was ~ 60% in this study, but almost all surviving rats developed significant degrees of cardiac hypertrophy. Most of the death occurred during early postoperative period. The major causes of death included leg ischemia, acute congestive heart failure, sudden death, and acute renal failure. Sham-operated controls (n = 10) underwent an identical procedure except for placement of the hemoclip. The abdominal incision was closed and the animals were allowed to recover. Subgroups (n = 18) of operated animals received intraperitoneal injection of L-thyroxine (Sigma Chemical Co., St. Louis, MO) 10–20 μg/d for the duration of the experiment as specified in the text. 63 out of 150 operated rats survived the procedures and were killed at predetermined days after the operation (2, 4, 6, 8, 11, 13, 15, and 32 d). Sham-operated animals were also killed after different time intervals postoperatively; however, their results were pooled since the different parameters measured (see below) did not vary significantly with time. The body weight was measured and the heart was rapidly excised. The atria, great vessels, and right ventricular free walls were removed. The left ventricles were opened, rinsed in cold saline, blotted dry, weighed, frozen in liquid nitrogen, and stored at −80°C.

The second group of animals included six male rats, 4–5 mo old and weighing 350–400 g. In these rats, three sham operated and three with CoA, pressure measurements were carried out before the surgical procedure and at the time of killing. Fluid-filled catheters (0.61 mm in diameter) were inserted into the right carotid artery and right jugular vein. Pressures were recorded in the central aorta, right atrium, and main pulmonary artery. The animals were killed 12 wk postoperatively. The atria, right ventricular free walls, and left ventricles were harvested separately for mRNA analysis.

Calculation of the percent hypertrophy score. The degree of hypertrophy was estimated by calculation of the percent hypertrophy score, which was defined as: (exp LVW − Th. LVW) × 100/Th. LVW, where exp LVW is experimental left ventricular weight, and Th. LVW is theoretical left ventricular weight derived from the regression curve of left ventricular weight versus body weight established previously in 87 sham-operated rats.

MHC mRNA analysis. Total cellular RNA was extracted from the myocardium by the hot phenol procedure (19) and was stored at −20°C in ethanol. The DNA probe used was the 3′ end PstI fragment of pCMHC5, a cDNA clone specific for the β-MHC gene (15, 18). This 347-nucleotide (nt) long single-stranded probe, 3′ end-labeled with ^{32}P, contains 180 nt of common coding sequence at carboxyl end of the α- and β-MHCs, in addition to the entire 3′ untranslated sequence of the β-MHC gene, which diverges completely from the α-MHC gene. It also contains 43 nt of oligo-dT and -dG tails. This probe can be used to detect both α- and β-MHC mRNAs specifically by S1 nuclease mapping analysis (20).

The probe was hybridized in DNA excess to 20 μg of total RNA in 80% formamide for 16 h at 42°C. S1 nuclease digestion was done with 150 U of enzyme (New England Nuclear, Boston, MA) for 1 h at 25°C and the digestion products were separated on 7% polyacrylamide 8.3 M urea-sequencing gel as described (21). The relative amounts of α- and β-MHC mRNA were quantitated by counting the radioactivity of the corresponding bands excised from the gels.

Myosin isozyme analysis. Crude tissue extracts were obtained from samples of myocardium weighing ~ 100 mg, crashed in liquid nitrogen, and extracted at 4°C with 4 vol of a slightly modified Guba's solution as described (8). Electrophoretic separation of myosin isozyme was performed in 4% polyacrylamide gel in the presence of 2 mM sodium pyrophosphate, 2 mM EDTA, 10% vol/vol glycerol, and 0.01% vol/vol 2-mercaptoethanol at pH 8.5 as previously described (8). It has been demonstrated that identical results are obtained either with the myosin in crude extracts or with a purified one (7). The intensity of each band was quantitated by densitometry, and the relative amounts of α- and β-MHCs were calculated by assuming that V1, V2, and V3 correspond to αα, αβ, and ββ, respectively (5).

Serum thyroid hormone levels. In all thyroxine (T_4)-treated and randomly selected untreated animals, 0.5 ml of blood was drawn at the time of operation and killing. Serum concentrations of T_4 and tri-iodothyronine (T_3) were measured in duplicate by radioimmunoassay (Clinical Assays, Cambridge, MA).

Statistical analysis. The results for different groups of animals are expressed as mean±standard error. The statistical significance was determined by analysis of variance and Newman-Keuls test for multiple sample comparison, and unpaired Student's t test for two-group comparison. The regression line was calculated using the method of least squares. Statistical significance was considered to be $P < 0.05$.

Results

Left ventricular hypertrophy caused by CoA produces a shift of MHC isoforms both at the mRNA and protein levels. We examined the effect of acute and chronic CoA in the rat at three different levels: (*i*) degree of hypertrophy as represented by the percent hypertrophy score, (*ii*) the MHC isozyme distribution at the mRNA level, and (*iii*) at the protein level. To establish temporal correlations among these variables, animals were killed at 2, 4, 6, 8, 11, 13, 15, and 32 d after operation and the above three parameters were measured simultaneously. This model of hypertrophy was chosen because acute pressure overload appears to produce cardiac hypertrophy more rapidly than volume overload or various hypertension models in rats (8).

Fig. 1 shows the time course of the hypertrophy score in this animal model. The hypertrophic response is already evident (24±2%, $P < 0.05$ compared with sham) at 2 d after the operation and reached a peak around the 11th postoperative day (49±8%).

To obtain biochemical correlates of cardiac hypertrophy, total cellular RNA was isolated from the left ventricles of the same animals shown in Fig. 1. The relative levels of the mRNAs coding for the α- and β-MHCs were quantitated by S1 nuclease mapping, which allows the detection of closely related gene products with high specificity and sensitivity (21). Fig. 2 *A* shows a representative S1 mapping analysis of the α- and β-MHC mRNAs, using as a probe the 3′ end–labeled PstI fragment of pCMHC5, a cDNA clone specific for the rat β-MHC. With this probe, the β-MHC mRNA was shown to yield a fully protected 304-nt long fragment, while the α-MHC mRNA produces a 180-nt long partially protected fragment (20). In normal adult and sham-operated controls, the α-MHC mRNA predominated, whereas a significant accumulation of the β-MHC mRNA was detected in the coarctated animals. Full protection of the probe

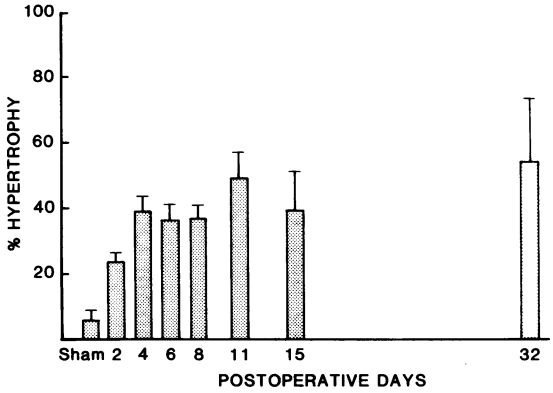

Figure 1. Time course of percent hypertrophy scores. The number in abscissa indicates postoperative days. The sham-operated animals were pooled into one group (see Methods). The numbers of samples in each group are: sham, 10; 2 d, 6; 4 d, 6; 6 d, 4; 8 d, 5; 11 d, 3; 15 d, 8; and 32 d, 3.

also indicates that the β-MHC mRNA induced by hemodynamic load is identical to the β-MHC mRNA present in the fetal and hypothyroid ventricles and slow skeletal muscle at its 3′ untranslated sequences (Fig. 2 *A* and reference 20). These results demonstrate that β-MHC mRNA induced by coarctation is coded by the same β-MHC gene that is expressed in fetal and hypothyroid ventricles and slow skeletal muscle (18).

We next examined whether these changes in the relative levels of the α- and β-MHC mRNAs would be reflected in the corresponding protein levels. Fig. 2 *B* shows a representative pyrophosphate gel electrophoresis of the myosin extracted from the left ventricles of the same animals shown in Fig. 2 *A*. Three bands, V1, V2, and V3, were detected in the coarctated animal, whereas only one band (V1) is visible in this sham-operated control. The close correlation between the corresponding mRNA (Fig. 2 *A*) and protein (Fig. 2 *B*) levels is readily apparent.

To better illustrate the temporal correlation between the mRNA and protein levels, the time courses of the mean levels of β-MHC mRNA and protein were superimposed in Fig. 3. In sham-operated animals, the levels of β-MHC mRNA and protein were identical (4±2%). After CoA, the rise of β-mRNA preceeded that of β-protein and reached its initial peak 2–4 d before that

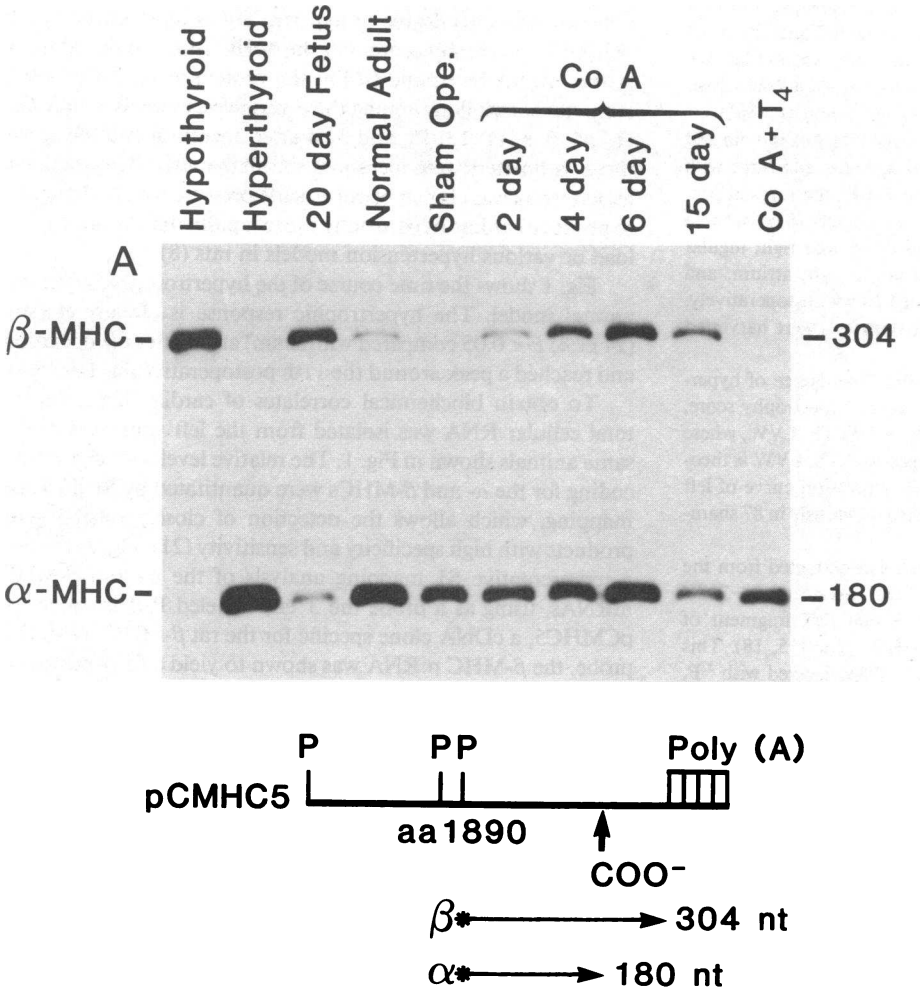

Figure 2. (*A*) S1 nuclease mapping analysis of cardiac ventricular RNAs using 3′ end PstI fragment of clone pCMHC5. With this probe, β-MHC mRNA yields 304-nt long fragment, while α-MHC mRNA produces 180-nt long fragment. Hypothyroid, thyroidectomized animals; hyperthyroid, animals infected with L-thyroxine (20 μg/d) for 2 wk; sham ope, sham-operated animal; and CoA + T_4, CoA plus L-thyroxine injection. (*B*) Myosin isozyme separation by nondenaturing gel electrophoresis. The same ventricular samples shown in Fig. 2 *A* were analyzed.

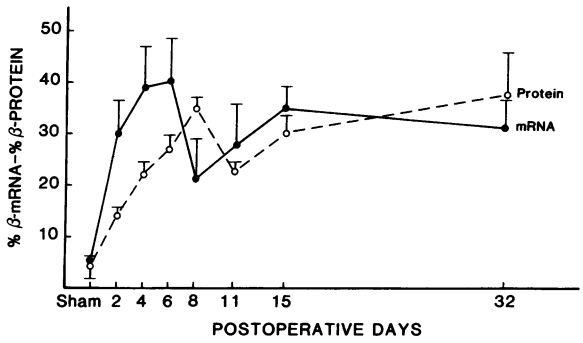

Figure 3. β-MHC gene expression during aortic coarctation. The time courses of the relative amounts of the β-MHC mRNA (*solid circles*) and corresponding protein (*open circles*) in the left ventricles are shown. The left ventricular samples analyzed were the same as those shown in Fig. 1.

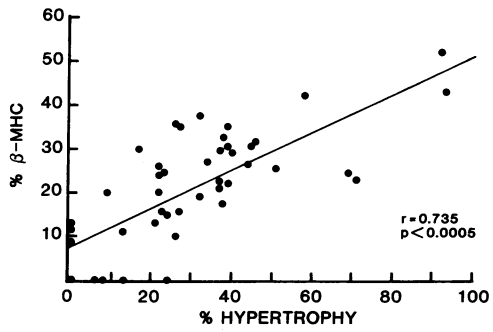

Figure 4. Correlation between percent β-MHC protein and percent hypertrophy scores.

of the protein. The peak values for β-MHC mRNA (41±8%, $P < 0.001$ compared with sham) and protein (35±3%, $P < 0.001$ compared with sham) are similar (P = NS, mRNA vs. protein). There was a trend toward transient decline in percent β-mRNA at 8 d, and the percent protein also showed a similar tendency 3 d later; however, these did not reach statistical significance ($P < 0.1$) by analysis of variance. In the chronic stage, 32 d after operation, the mean values of the β-mRNA (31±6%) and protein (37±9%) are similar (P = NS, mRNA vs. protein). Although we did not directly measure the rate of synthesis and degradation of the α- and β-MHC proteins, the close correlation observed between the relative levels of mRNAs and the corresponding proteins strongly suggests that the MHC isozyme transition during hemodynamic overload is mainly regulated by pretranslational mechanisms. The lag time between the two parameters seen during the early stages could be accounted for by the different half-lives of MHC mRNA (50–60 h) (20, 22) and protein (7–9 d) (23). Translational and posttranslational mechanisms, if present, do not appear to play a major role in the production of the MHC phenotypes in this model of cardiac hypertrophy.

The causes of the tendency toward bimodal response in the MHC isozyme transition observed both at the mRNA and protein levels are not clear. It is tempting to speculate that the initial rapid response is due to a sudden rise in systolic ventricular wall stress, which then returns toward normal with the development of hypertrophy, thus resulting in a decrease in β-MHC gene expression at 8 d after coarctation. However, this hypothesis does not explain the reelevation of the percent β-MHC mRNA at 15 d and thereafter, when wall stress would be close to normal. Additional studies with precise hemodynamic data would be necessary to further clarify this phenomenon.

To examine the correlation between the morphological and biochemical parameters during cardiac hypertrophy, the values of β-MHC protein were plotted against hypertrophy scores. As shown in Fig. 4 there is a positive linear correlation between these two parameters ($r = 0.735$, $P < 0.0005$), which suggests that the hypertrophic process and the MHC isozyme shift occur simultaneously in these acute and subacute stages of overload. A similar correlation between the percent V3 and percent hypertrophy in several models of cardiac hypertrophy was previously demonstrated in rats killed at chronic stages (8).

Effect of CoA on MHC gene expression in the atria and right ventricles. The MHCs expressed in the atria and right ventricle are encoded by the same genes expressed in the left ventricles (20). We next examined whether the changes in MHC gene expression produced by CoA are limited to the left ventricle or extend to the right ventricle and atrium. In a separate set of experiments, hemodynamic measurements and MHC mRNA analyses were performed to relate these two parameters. Larger rats (350–400 g, 4–5 mo old) were used for ease of catheterization, and were killed 12 wk after operation to avoid the possible acute and subacute effects of surgical stress.

The mean aortic pressure in these animals (Table I *A*) showed an increase of 48±5 mmHg compared with the sham controls (P = 0.005). The mean pulmonary artery pressure increased from 13±1 to 18±1 mmHg ($P < 0.025$). The right atrial pressure did not change significantly. In these rats (6–7 mo old at the

Table I A. Effect of CoA on MHC Gene Expression in the Atria and Right Ventricle: Pressures (mmHg)

		Mean AO	Mean PA	Mean RA
Sham (n = 3)	Pre	110±6	ND	ND
	Post	115±5	13±1	1±0
12 wk CoA	Pre	108±3	ND	ND
(n = 3)	Post	163±5	18±1	2±1

Table I B. Effect of CoA on MHC Gene Expression in the Atria and Right Ventricle: Percent β-MHC mRNA

	LV	RV	Atria
	%	%	%
Control (n = 3)	22	24	5
12 wk CoA (n = 3)	77	75	10

Pressure measurements and MHC mRNA analysis were done on the same animals. (*A*) Mean AO, mean aortic pressure; mean PA, mean pulmonic pressure; mean RA, mean right atrial pressure; pre, before operation; post, at the time of killing; and 12 wk CoA, 12 wk of CoA. (*B*) Note: The samples were pooled in each category and, therefore, no standard error was given to the percent numbers. LV, left ventricular free wall; RV, right ventricular free wall; and atria, both right and left atria.

time of killing) the percent β-MHC mRNA in the ventricles of the sham-operated controls was higher (Table I *B*) than that of younger animals, in agreement with the previous findings of the effect of aging on myosin isozyme transition (24). 12 wk after the operation, there was a significant induction of β-mRNA (above 70%) in the right and left ventricles of coarctated animals, while it was not induced significantly in the atria. Although we did not measure the β-MHC protein in these 12-wk postoperative animals, similar percentages of V3 myosin in both left and right ventricles were observed previously in chronically coarctated rats that were free of signs of congestive heart failure (8).

These results demonstrate that the right ventricle can undergo a degree of MHC isozyme transition similar to that seen in the left ventricle despite a relatively small increase in the mean pulmonary artery pressure. However, when the differences in the wall thickness and ventricular geometry between the right and left ventricles are taken into account, it is possible that the stress imposed on the individual myocyte in these animals might have been similar in both ventricles. A detailed hemodynamic study would be necessary to further examine this issue.

The absence of significant MHC isozyme shift in the atria is consistent with the notion that increased hemodynamic load is the major determinant of the MHC isozyme transition, since no significant change in pressure was observed in the right atrium. It has been shown that human atria undergo the α- to β-MHC transition in various pathological conditions (10, 11). However, it remains to be determined whether rat atrium also undergoes MHC isozyme transition in response to hemodynamic overload. It merits mention that thyroid hormone manipulation triggers little response on the MHC genes in the rat atrium, in contrast to its marked effect on the expression of the same genes in the ventricles (25, 26).

Thyroid hormone can overcome the effect of coarctation on MHC gene expression. Thyroid hormone is a potent regulator of the cardiac as well as skeletal muscle MHC gene family (25). In the ventricle, hyperthyroidism induces the α-MHC gene and deinduces the β. Indeed, the developmental transitions of the ventricular myosins have been shown to coincide with the postnatal surge of thyroid hormone (27).

We measured circulating T_4 and T_3 levels to examine whether the induction of the β-MHC gene during CoA may possibly be associated with a decrease in thyroid hormone levels. As shown in Table II, T_4 and T_3 levels were significantly lower in the coarctated rats at 2 and 4 d after operation than in nonoperated controls. This phenomenon might have been due to the surgical stress itself, as the decrease in thyroid hormone levels in a variety of stressful conditions has been well documented (reviewed in reference 28). However, this transient decline in thyroid hormone levels would not account for the α- and β-myosin transition observed in the coarctated animals, since the β-MHC gene remained expressed even after thyroid hormone levels returned to the control levels (eight postoperative days and thereafter).

We next explored whether high doses of thyroid hormone could overcome the effect of pressure overload on MHC gene expression. When L-thyroxine (20 μg/d) was administered 32 d after coarctation, there was a rapid deinduction of the β-MHC mRNA within 4 d (Fig. 5 *A*) followed by a decrease in corresponding protein (Fig. 5 *B*). Furthermore, when L-thyroxine (10 μg/d) was administered daily starting 4 d before the operation and continued 4 and 13 d after the coarctation, the inducation of the β-MHC gene was prevented at both the mRNA (Fig. 6 *A*) and protein levels (Fig. 6 *B*) despite the fact that these animals had similar or higher hypertrophy scores (up to 97%) than those with coarctation alone. These results are in agreement with the previous report indicating that thyroid hormone prevented the decrease in myosin ATPase activity associated with CoA (29). Chronic treatment (12 wk) with physiological replacement dose of L-thyroxine (1.5 μg/100 g per d), on the other hand, did not prevent the induction of β-MHC gene produced by CoA (30). These results indicate that hemodynamic overload causes the induction of the β-MHC gene in the presence of physiological levels of thyroid hormone, but this effect can be overcome at the pretranslational level by higher amounts of the hormone (see Table II).

Table II. Serum Thyroid Hormone Levels (Mean±SE)

	n	T_4	T_3
		μg/dl	*ng/dl*
Preoperative control	17	2.65±0.15	69±6 (n = 5)
2 d post-op sham	1	1.23	45
2 d post-op CoA	3	1.25±0.20*	44±4‡
4 d post-op sham	1	0.74	40
4 d post-op CoA	3	1.39±0.27*	51±8§
8 d post-op CoA	4	2.51±0.50	61±8
2 wk post-op CoA	3	2.47±0.30	ND
32 d post-op sham	1	2.55	84
32 d post-op CoA	3	2.35±0.12	75±3
32 d CoA + T_4 1 d	4	6.34±1.07*	97±5‡
32 d CoA + T_4 4 d	3	10.02±0.85*	129±4*

n, number of samples.
* $P < 0.0005$ (compared with preoperative control.)
‡ $P < 0.01$.
§ $P < 0.05$.

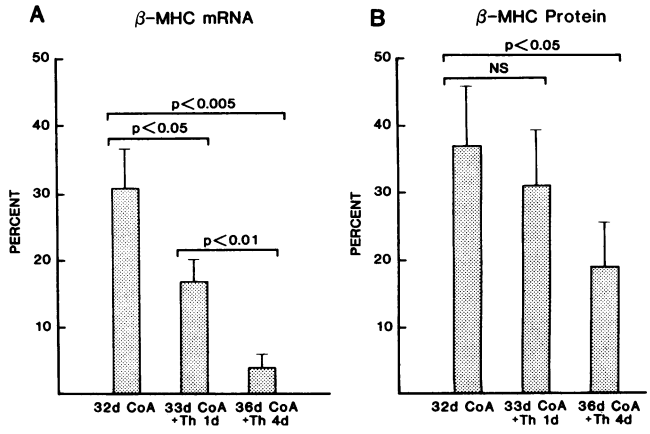

Figure 5. Effect of thyroid hormone on MHC gene expression in coarctated rats. (*A*) β-MHC mRNA. (*B*) β-MHC protein. 33 d CoA + Th 1 d, CoA for 32 d followed by L-thyroxine injection (20 μg/d) for 1 d (n = 4). 36 d CoA + Th 4 d, CoA for 32 d followed by L-thyroxine injection (20 μg/d) for 4 d (n = 3).

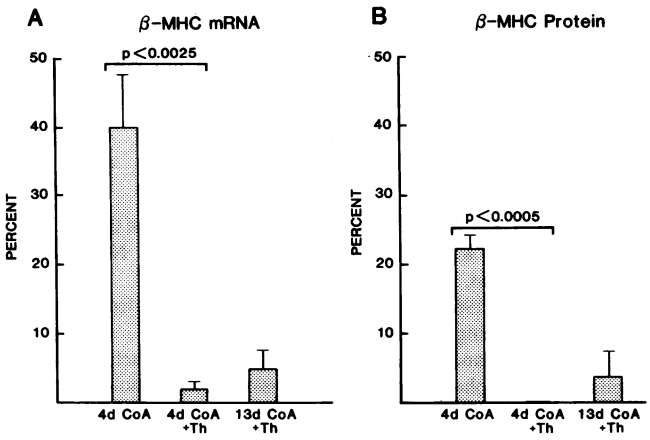

Figure 6. Effect of CoA *plus* thyroid hormone on MHC gene expression. (*A*) β-MHC mRNA. (*B*) β-MHC protein. 4 d CoA + Th, CoA plus L-thyroxine injection (10 μg/d) for 4 d (n = 3). 13 d CoA + Th, CoA plus L-thyroxine injection (10 μg/d) for 13 d (n = 5). Daily T_4 injection was started 4 d before the operation in these experiments, since administration of thyroid hormone starting at the day of operation resulted in 100% mortality of the animals.

Discussion

Cardiac adaptation to work overload involves both quantitative and qualitative changes in its constitutive contractile units. Previously, it has been postulated that, when faced with increased hemodynamic loads, the first adaptational mechanism is to increase the ventricular mass by addition of new sarcomeres. When this process reaches its maximum, the α- to β-MHC isozyme switch, shown to be a thermoeconomically beneficial process (31), occurs as the second adaptational process (reviewed in reference 32). The results presented here, however, demonstrate that these two processes do not occur sequentially. Instead, they occur simultaneously as the rapid rise in β-MHC mRNA and its protein is seen concomitant with the hypertrophic response (Figs. 1 and 3).

Theoretically, a change in the MHC isozyme can take place by several different mechanisms: (*i*) changes in the transcriptional rate of each gene, (*ii*) selective changes in the mRNA stability, (*iii*) preferential translation of a particular mRNA, and (*iv*) changes in protein stability. The observed close correlation between the relative levels of MHC mRNAs and the corresponding proteins strongly suggests that the MHC isozyme transition during hemodynamic overload is mainly regulated by pretranslational mechanisms. Translational and posttranslational mechanisms need not be invoked to account for the observed MHC phenotypes in this model of hypertrophy.

From the data presented here, it is not possible to conclusively determine whether changes in the α- and β-MHC mRNA levels are due to changes in transcriptional rate or in mRNA stability. We and others have previously shown that the cardiac MHC isozyme transition during normal development and thyroid hormone manipulation is regulated by changes in the levels of the respective mRNAs (20, 33), which in turn appear to be transcriptionally regulated. Furthermore, the preliminary results of nuclear run-off experiments suggest that thyroid hormone regulates the α- and β-MHC genes at the level of transcription, and not by changes in mRNA half-life (34). Although it remains to be proven whether the same holds true during hemodynamic overload, the observed severalfold accumulation of the β-MHC mRNA within 2 d is unlikely to derive solely from an increase in its stability. Indeed, the fact that an animal that died of acute congestive heart failure 12 h after coarctation had already accumulated significant amounts (40%) of the β-MHC mRNA (data not shown) suggests that a very rapid increase in the cytoplasmic accumulation of β-MHC mRNA could occur in response to a sudden increase in hemodynamic loads.

The issue of whether or not the relative increase in β-MHC mRNA and protein, during CoA, is accompanied by an absolute decrease in α-MHC mRNA and protein cannot be resolved here conclusively. Northern blot analysis using a probe common to both α- and β-MHC mRNAs detected no significant increase in the MHC mRNA concentration during hypertrophy when normalized to equal amounts of total cellular RNAs (data not shown). However, nearly 90% of the total cellular RNA is ribosomal RNA which increases in proportion to mRNA with cardiac hypertrophy (35). Therefore, the MHC mRNA concentration relative to total cellular RNA may remain constant even though its absolute amount increases during hypertrophy (36).

Since the sham-operated controls and the atria of the coarctated animals did not exhibit MHC isozyme shifts, it seems reasonable to assume that the observed changes in MHC gene expression during hypertrophy are caused by increased afterload, either directly or indirectly. Note, however, that increased afterload is not the only consequence of CoA. This manipulation might cause a rise in circulating catecholamine levels as a stress response to surgical trauma and/or activation of the renin–angiotensin system by reduced renal blood flow due to the suprarenal coarctation. Norepinephrine (37, 38) and possibly angiotensin-II could, in principle, directly stimulate myocardial cell hypertrophy independent of their hemodynamic effects.

The effect of norepinephrine on cardiac cell growth in culture has been shown to be mediated by stimulation of α_1-adrenergic receptor (38), which couples to the hydrolysis of membrane phosphatidylinositol followed by release of inositol triphosphate (39) and activation of protein kinase–C (40). Furthermore, phorbol esters, direct activators of protein kinase–C, can cause hypertrophy when given to cultured neonatal cardiac cells (41). However, it is not known whether norepinephrine or activation of protein kinase—C per se plays any role in mediating the effect of pressure overload on production of cardiac hypertrophy in vivo (42). In addition, norepinephrine does not appear to cause MHC isozyme transition in cultured neonatal cardiocytes (reference 43 and our unpublished observation).

In various models of cardiac hypertrophy in vivo, systolic and diastolic wall stress have both been implicated as major determinants of the degree and pattern of hypertrophy during pressure and volume overload (44). Previous studies using isolated heart preparations demonstrated that increased wall tension alone can directly stimulate protein synthesis (45). Furthermore, α-amanitin–sensitive RNA polymerase II activity, taken as an index of mRNA synthesis, was reported to be increased in isolated cardiac nuclei subjected to hydrostatic pressure (46).

These observations, in combination with the results presented in this report, suggest that increased hemodynamic load is likely to be the major determinant mediating the observed changes in MHC gene expression at the pretranslational level, although we cannot fully exclude the possible contributions of some hormonal factors in our experimental model. In addition to its effect on MHC gene expression, CoA also induces the mRNAs coding

for the skeletal α-actin and β-tropomyosin genes which are normally expressed in the ventricles of the fetal and neonatal but not the adult animals (47, 48). Moreover, recent studies have demonstrated the presence of an increased amount of B (fetal) creatine kinase (49), and fetal-like myosin light chain 1 (50) in hypertrophied human ventricles. Therefore, induction of genes encoding fetal contractile protein isoforms may be a general phenomenon in response to hemodynamic overload. However, little is known as to the precise mechanisms of how hemodynamic stimuli are converted into biochemical signals that lead to changes in gene expression.

Thyroid hormone is perhaps the most well-defined modulator of expression of the MHC multigene family (25). It has been shown that the developmental transition (β to α) of the rat ventricular MHC isozymes coincides with the postnatal surge in circulating thyroid hormone level (27). In the present study, however, changes in circulating thyroid hormone levels did not account for the observed changes in the MHC gene expression. The observation that the skeletal α-actin and β-tropomyosin genes, which are induced by hemodynamic overload, do not respond to thyroid hormone manipulation (47) further supports the notion that the induction of the β-MHC gene during CoA involves different mechanisms from those triggered by thyroid hormone. Conversely in coarctated animals, high levels thyroid hormone can "override" the pretranslational effect of biochemical signals induced by hemodynamic loads. This action of thyroid hormone is probably independent of its effects in vivo on hemodynamic or adrenergic factors, since the β- to α-MHC isozyme transition induced by this hormone occurs in cultured myocardial cells both at the protein (43, 51) and mRNA (our unpublished observation) levels. Although thyroid hormone appears to regulate the expression of the MHC genes mainly at the transcriptional level (34), it is yet to be demonstrated whether its regulation is direct, presumably mediated by T_3-nuclear receptor bound to the regulatory regions of the MHC genes, or indirect, acting through the modulation of secondary intracellular signals.

It has been shown that many other pathophysiological states, including exercise (52, 53), diabetes (54, 55), starvation (56), high carbohydrate diet (56), adrenalectomy (56), dwarfism (57), and gonadectomy (58), can also modulate the cardiac MHC isozyme distribution. It remains to be seen whether these stimuli act independently on the expression of the MHC genes or whether their effects are mediated by a common biochemical signal. It is of interest that these seemingly divergent pathophysiological states, including hemodynamic and various hormonal manipulations, appear to have a common feature; that is, they all can influence energy metabolism of the myocardium. Further work is needed, however, to elucidate the precise molecular mechanisms of MHC gene regulation and of cardiac hypertrophy.

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References

1. Spann, J. F. 1983. Contractile and pump function of the pressure-overload heart. *In* Myocardial Hypertrophy and Failure. N. R. Alpert, editor. Raven Press, New York. 19–38.

2. Barany, M. 1967. ATPase activity of myosin correlated with speed of muscle shortening. *J. Gen. Physiol.* 50:197–218.

3. Sheuer, J., and A. K. Bahn. 1979. Cardiac contractile proteins: adenosine triphosphatase activity and physiological function. *Circ. Res.* 45:1–12.

4. Hoh, J. F. Y., P. A. McGrath, and H. T. Hale. 1978. Electrophoretic analysis of multiple forms of rat cardiac myosin. Effect of hypophysectomy and thyroxine replacement. *J. Mol. Cell. Cardiol.* 10:1053–1076.

5. Pope, B., J. F. Y. Hoh, and A. Weeds. 1980. The ATPase activities of rat cardiac myosin isoenzymes. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 118:205–208.

6. Morkin, E., I. L. Flink, and S. Goldman. 1983. Biochemical and physiological effects of thyroid hormone on cardiac performance. *Prog. Cardiovasc. Dis.* 25:435–464.

7. Lompré, A.-M., K. Schwartz, A. D'Albis, G. Lacombe, N. V. Thiem, and B. Swynghedauw. 1979. Myosin isoenzyme redistribution in chronic heart overload. *Nature (Lond.).* 282:105–107.

8. Mercadier, J. J., A.-M. Lompré, C. Wisnewsky, J. L. Samuel, J. Bercovici, B. Swynghedauw, and K. Schwartz. 1981. Myosin isoenzyme changes in several models of rat cardiac hypertrophy. *Circ. Res.* 49:525–532.

9. Litten, R. Z., B. J. Low, and N. R. Alpert. 1982. Altered myosin isozyme patterns from pressure overloaded and thyrotoxic hypertrophied rabbit hearts. *Circ. Res.* 50:856–864.

10. Tsuchimochi, H., M. Sugi, M. Kuro-o, S. Ueda, F. Takaku, S. Furuta, T. Shirai, and Y. Yazaki. 1984. Isozymic changes in myosin of human atrial myocardium induced by overload: immunohistochemical study using monoclonal antibodies. *J. Clin. Invest.* 74:662–665.

11. Gorza, L., J. J. Mercadier, K. Schwartz, L. E. Thornell, S. Sartore, and S. Schiaffino. 1984. Myosin types in the human heart. An immunofluorescence study of normal and hypertrophied atrial and ventricular myocardium. *Circ. Res.* 54:694–702.

12. Schwartz, K., Y. Lecarpentier, J. L. Martin, A.-M. Lompré, J. J. Mercadier, and B. Swynghedauw. 1981. Myosin isoenzyme distribution correlates with speed of myocardial contraction. *J. Mol. Cell. Cardiol.* 13:1071–1075.

13. Mercadier, J. J., P. Bouveret, L. Gorza, S. Schiaffino, W. A. Clark, R. Zak, B. Swynghedauw, and K. Schwartz. 1983. Myosin isoenzymes in normal and hypertropied human ventricular myocardium. *Circ. Res.* 53:52–62.

14. Schwartz, K., A.-M. Lompré, G. Lacombe, P. Bouveret, C. Wisnewsky, R. G. Whalen, A. D'Albis, and B. Swynghedauw. 1983. Cardiac myosin isoenzymic transitions in mammals. *In* Myocardial Hypertrophy and Failure. N. R. Alpert, editor. Raven Press, New York. 345–358.

15. Mahdavi, V., M. Periasamy, and B. Nadal-Ginard. 1982. Molecular characterization of two myosin heavy chain genes expressed in the adult heart. *Nature (Lond.).* 297:659–665.

16. Sinha, M., P. K. Umeda, C. J. Kavinsky, C. Rajamanickam, H. J. Hsu, S. Jakovcic, and M. Rabinowitz. 1982. Molecular cloning of mRNA sequences for cardiac α and β form myosin heavy chains: expression in ventricles of normal, hypothyroid, and thyrotoxic rabbits. *Proc. Natl. Acad. Sci. USA.* 79:5847–5851.

17. Friedman, D. J., P. K. Umeda, A. M. Sinha, H. J. Hsu, S. Jakovcic, and M. Rabinowitz. 1984. Characterization of genomic clones specifying rabbit alpha and beta ventricular myosin heavy chains. *Proc. Natl. Acad. Sci. USA.* 81:3044–3048.

18. Mahdavi, V., A. P. Chambers, and B. Nadal-Ginard. 1984. Cardiac

α- and β-myosin heavy chain genes are organized in tandem. *Proc. Natl. Acad. Sci. USA.* 81:2626–2630.

19. Soeiro, R., H. Birnboim, and H. Darnell. 1966. Rapidly labelled HeLa cell nuclear RNA. II. Base composition and cellular location of a heterogeneous RNA fraction. *J. Mol. Biol.* 19:362–373.

20. Lompré, A.-M., B. Nadal-Ginard, and V. Mahdavi. 1984. Expresion of the cardiac ventricular α and β-myosin heavy chain genes is developmentally and hormonally regulated. *J. Biol. Chem.* 259:6437–6446.

21. Berk, A. J., and P. A. Sharp. 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease digested hybrids. *Cell.* 12:721–732.

22. Medford, R. M., H. T. Nguyen, and B. Nadal-Ginard. 1983. Transcriptional and cell cycle-mediated regulation of myosin heavy chain gene expression during muscle cell differentiation. *J. Biol. Chem.* 258: 11063–11073.

23. Rabinowitz, M. 1973. Protein synthesis and turnover in normal and hypertrophied heart. *Am. J. Cardiol.* 31:202–210.

24. Lompré, A.-M., J. J. Mercadier, C. Wisnewsky, P. Bouveret, C. Pantaloni, A. D'Albis, and K. Schwartz. 1981. Species and age dependent changes in the relative amounts of cardiac myosin isoenzymes in mammals. *Dev. Biol.* 84:286–290.

25. Izumo, S., B. Nadal-Ginard, and V. Mahdavi. 1986. All members of the myosin heavy chain multigene family respond to thyroid hormone in a highly tissue-specific manner. *Science (Wash. DC)* 231:597–600.

26. Samuel, J. L., L. Rappaport, I. Syrovy, C. Wishewsky, F. Harotte, R. G. Whalen, and K. Schwartz. 1986. Differential effect of thyroxine on atrial and ventricular isomyosins in rats. *Am. J. Physiol.* 250:H333–H341.

27. Chizzonite, R. A., and R. Zak. 1984. Regulation of myosin isoenzyme composition in fetal and neonatal rat ventricle by endogenous thyroid hormones. *J. Biol. Chem.* 259:12626–12632.

28. Ingbar, S. H. 1986. The Thyroid. J. P. Lippincott, Philadelphia, 387–406.

29. Affito, J. J., and M. A. Inchiosa. 1979. Decrease in rat cardiac myosin ATPase with aortic coarctation: prevention by thyroxine replacement. *Life Sci.* 25:353–364.

30. Matsuoka, R., B. Nadal-Ginard, and V. Mahdavi. 1984. α and β myosin heavy chain gene expression in response to systolic overload hypertrophy. *Circulation* 70(Suppl. II):II–196. (Abstr.)

31. Alpert, N. R., and L. A. Mulieri. 1982. Increased myothermal economy of isometric force generation in compensated cardiac hypertrophy induced by pulmonary artery constriction in the rabbit. *Circ. Res.* 50:491–500.

32. Wickman-Coffelt, J., W. W. Parmley, and D. T. Mason. 1979. The cardiac hypertrophy process. Analyses of factors determining pathological vs. physiological development. *Circ. Res.* 45:697–702.

33. Everett, A. W., A. M. Sinha, P. K. Umeda, S. Jakovcic, M. Rabinowitz, and R. Zak. 1984. Regulation of myosin synthesis by thyroid hormone: Relative change in the α- and β-myosin heavy chain mRNA levels in rabbit heart. *Biochemistry.* 23:1596–1600.

34. Umeda, P. K., J. E. Levin, A. M. Shinha, L. L. Cribbs, D. S. Dawling, D. J. Ende, H.-J. Hsu, E. Dizon, and S. Jakovcic. 1986. Molecular anatomy of cardiac myosin heavy chain genes. *In* Molecular Biology of Muscle Development. C. Emerson, D. Fischman, B. Nadal-Ginard, and M. A. W. Siddiqui, editors. Alan R. Liss, Inc., New York. 809–823.

35. Swynghedauw, B., J. M. Moalic, P. Bouveret, J. Bercovici, D. de la Bastie, and K. Schwartz. 1984. Messenger RNA content and complexity in normal and overloaded rat and human heart. *Eur. Heart J.* 5(Suppl. F):211–217.

36. Cutilleta, A. F. 1984. Myosin heavy chain mRNA during the development and regression of mycardial hypertrophy. *Eur. Heart J.* 5(Suppl. F):193–197.

37. Lacks, M. M. 1977. Norepinephrine, the producer of myocardial cellular hypertrophy and/or necrosis and/or fibrosis. *Am. Heart J.* 94: 349–399.

38. Simpson, P. C., and A. McGrath. 1983. Norepinephrine stimulated hypertrophy of cultured rat myocardial cells in an alpha-1 adrenergic response. *J. Clin. Invest.* 22:732–735.

39. Berridge, M. J., and R. F. Irvine. 1984. Inositol triphosphate: a novel second messenger in cellular signal transaction. *Nature (Lond.).* 312:315–321.

40. Nishizuka, Y. 1984. The role of protein kinase-C in cell surface signal transduction and tumor promotion. *Nature (Lond.).* 308:693–698.

41. Simpson, P. C., and J. S. Karliner. 1985. Regulation of cardiac myocyte hypertrophy by a tumor-promoting phorbol ester. *Clin. Res.* 33:2:229*A*. (Abstr.)

42. Cooper, G., R. L. Kent, C. E. Uboh, E. W. Thompson, and T. A. Marino. 1985. Hemodynamic versus adrenergic control of cat right ventricular hypertrophy. *J. Clin. Invest.* 75:1403–1414.

43. White, N., T. Tsao, and P. Simpson. 1986. Differential regulation of myosin isoenzymes in alpha-1 and thyroid hormone stimulated hypertrophy in cultured neonatal rat heart muscle cells. *Clin. Res.* 34:16*A*. (Abstr.)

44. Grossman, W. 1980. Cardiac hypertrophy: useful adaptation or pathologic process? *Am. J. Med.* 69:576–584.

45. Kira, Y., P. J. Kochel, E. E. Gordon, and H. E. Morgan. 1984. Aortic perfusion pressure as a determinant of cardiac protein synthesis. *Am. J. Physiol.* 246:C247–C258.

46. Schreiber, S. S., M. Orax, M. A. Rothchild, and F. Reff. 1978. The effect of hydrostatic pressure on isolated cardiac nuclei: stimulation of RNA II polymerase activity. *Cardiovasc. Res.* 12:265–268.

47. Izumo, S., B. Nadal-Ginard, and V. Mahdavi. 1985. Re-expression of the genes encoding fetal contractile protein isoforms during cardiac hypertrophy. *Circulation.* 72:III–26. (Abstr.)

48. Schwartz, K., A.-M. Lompré, P. Bouveret, C. Wishewsky, P. Oliviero, S. Alonso, and M. Buckingham. 1985. Accumulation of α-skeletal actin mRNA in experimental cardiac hypertrophy. *J. Mol. Cell. Cardiol.* 17(Suppl. 3):22. (Abstr.)

49. Ingwall, J. S., M. F. Kramer, M. A. Fifer, W. Grossman, and P. Allen. 1985. The creatine kinase system in normal and diseased human myocardium. *N. Engl. J. Med.* 313:1050–1054.

50. Hirzel, H. O., C. R. Tuchschmid, J. Schneider, H. P. Krayenbuehl, and M. C. Schaub. 1985. Relationship between myosin isoenzyme composition, hemodynamics, and myocardial structure in various forms of human cardiac hypertrophy. *Circ. Res.* 57:729–740.

51. Nag, A. C., and M. Cheng. 1984. Expression of myosin isenzymes in cardiac muscle in culture. *Biochem. J.* 221:21–26.

52. Bahn, A. K., and J. Scheuer. 1975. The effects of physical training on cardiac myosin ATPase activity. *Am. J. Physiol.* 228:1178–1182.

53. Rupp, H. 1981. The adaptive changes in the isoenzyme pattern of myosin from hypertrophied rat myocardium as a result of pressure overload and physical training. *Basic Res. Cardiol.* 76:79–88.

54. Dillman, W. 1980. Diabetes mellitus induces changes in cardiac myosin of rats. *Diabetes.* 29:579–582.

55. Malhotra, A., S. Penparqkul, F. S. Fein, E. H. Sonnenblick, and J. Scheuer. 1981. The effect of streptozotocin-induced diabetes in rats on cardiac contractile proteins. *Circ. Res.* 49:1243–1250.

56. Sheer, D., and E. Morkin. 1984. Myosin isoenzyme expression in rat ventricle: effects of thyroid hormone analogs, catecholamines, glucocorticoids and high carbohydrate diet. *J. Pharmacol. Exp. Ther.* 229: 872–879.

57. Whalen, R. G., M. Toutan, G. S. Butler-Browne, and S. C. Watkins. 1985. Hereditary pituitary dwarfism in mice affects skeletal and cardiac myosin isozyme transitions differently. *J. Cell Biol.* 101:603–609.

58. Schaible, T. F., A. Malhotra, G. Ciambrone, and J. Scheuer. 1984. The effect of gonadectomy on left ventricular function and cardiac contractile proteins in male and female rats. *Circ. Res.* 54:38–49.