Antithetical Accumulation of Myosin Heavy Chain but Not $\alpha$-Actin mRNA Isoforms During Early Stages of Pressure-Overload–Induced Rat Cardiac Hypertrophy

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Myocardial response to a hemodynamic overload involves changes in the expression of isogenes encoding myosin heavy chain (MHC) and actin: $\beta$-MHC/$\alpha$-MHC and skeletal/cardiac $\alpha$-actin mRNA isoform ratios are increased. It is not known whether these changes are due to increased accumulations of the two neosynthesized transcripts, $\beta$-MHC and skeletal $\alpha$-actin, or whether the mRNA isoforms normally present, $\alpha$-MHC and cardiac $\alpha$-actin, are concomitantly decreased. To answer these questions, using dot-blot hybridizations, primer extension, and exonuclease VII mapping assays, we have analyzed the content of sarcomeric MHC and actin mRNAs in the poly(A$^+$) RNA in left ventricles of 23–24-day-old rats 18 and 24 hours after a pressure overload induced by stenosis of the thoracic aorta. The results showed a 1.9-fold increase in poly(A$^+$) RNA after the stenosis. Skeletal/cardiac $\alpha$-actin mRNA isoforms were already increased fivefold (from 0.19 to 0.99) at 18 hours, and this was exclusively due to a 5.5-fold increase in skeletal $\alpha$-actin mRNA. At 24 hours, this ratio was increased ninefold (from 0.14 to 1.22), and this was due to a 4.3-fold increase in the level of skeletal $\alpha$-actin mRNAs ($p<0.001$) and a 1.9-fold decrease of cardiac $\alpha$-actin mRNA ($p<0.001$), restoring the same proportion of sarcomeric actin mRNA in sham-operated and operated rats. A significant increase in the $\beta$-MHC/$\alpha$-MHC mRNAs (9.6-fold, from 0.05 to 0.49) was only observed at 24 hours, because of a striking 3.5-fold decrease ($p<0.001$) in the level of $\alpha$-MHC mRNA, resulting in a 2.6-fold decrease ($p<0.001$) in total sarcomeric MHC mRNA. These results strongly suggest that, in the early stages of a hemodynamic overload, the genes encoding MHC isoforms are regulated in an antithetical manner, whereas the actin pair is not, since the expression of the skeletal $\alpha$-actin gene was upregulated and that of the cardiac $\alpha$-actin gene was unaffected. Thus, the different sarcomeric components in the same heart are not equivalent targets for the mechanisms activated by changes in external cardiac load. *(Circulation Research 1993;72:857–864)*

Key Words • cardiac hypertrophy • $\alpha$-actin • myosin heavy chain • regulation • rats • mRNA isoforms

Chronic increases in work load that are imposed on the adult heart profoundly modify both the size and the structure of cardiac myocytes. These cells are terminally differentiated and have lost their ability to proliferate. Therefore, in response to hemodynamic overload, they hypertrophy without dividing, and simultaneously, the type and/or the content of key proteins for cardiac contraction and relaxation change (see References 1 and 2 for review). This myocardial response to work overload is due to a complex reprogramming of the expression of cardiac genes, characterized in most cases by the reexpression of a fetal phenotype. The factors and mechanisms involved in these changes are still largely unknown (see References 3 and 4 for review). The first and the most studied genes were those encoding the two major contractile proteins, myosin and actin. In rats, the two isogenes normally expressed during embryonic development, i.e., $\beta$-myosin heavy chain ($\beta$-MHC) and skeletal $\alpha$-actin, are reactivated during the hypertrophic response of the ventricular tissue. In humans, the expression of the $\beta$-MHC isogene is induced in the hypertrophied atrium; however, there are few changes during ventricular hypertrophy, because the normal adult ventricle is already composed mainly of $\beta$-MHC and skeletal $\alpha$-actin. The subset of MHC and actin isogenes expressed in the cardiac myocyte ($\alpha$- and $\beta$-MHC, skeletal and cardiac $\alpha$-actin) has now become an excellent model system for studying the mechanisms that regulate cardiac gene expression during normal growth, hypertrophy, and senescence, because 1) the induction of the $\beta$-MHC is physiologically relevant, since it results in a slower and more economic cardiac contraction; 2) MHC and actin belong to different sarcomeric structures, and this allows one to follow the distinct re-
sponses of different components of the same and highly integrated muscular structure; 3) the tools are available to study these isogenes both at the mRNA and at the protein levels; and 4) their expression can be studied in both in vitro models of cell culture and in vivo models of hemodynamic overload.

Although the increases in the β-MHC/α-MHC and skeletal/cardiac α-actin mRNAs have been independently well characterized in a variety of experimental models of hemodynamic overload,3-9 a direct comparison of the results for these two gene families has been rare, and relatively little is known about the precise mechanisms involved in the early stages of hypertrophy for the establishment of the new phenotype. It has been shown that, after coartation of the ascending aorta, skeletal α-actin mRNAs are detectable by in situ hybridization earlier than are β-MHC mRNAs (4 hours versus 24 hours)10 and that, after abdominal aortic constriction, the level of skeletal α-actin mRNA is already high at days 1 and 2.9,11 Since the overall mRNA content of the myocyte markedly increases after the imposition of a work overload,12 the increased mRNA isoform (isoRNA) ratios could potentially result from either increases in the β-MHC and skeletal α-actin mRNAs or decreases in α-MHC and cardiac α-actin mRNAs or both, and these responses may or may not be simultaneously triggered for the two gene families. The present study was undertaken to test these hypotheses. To do this, we have determined the amount of each of these transcripts in the total poly(A⁺) RNA population during the first 24 hours after stenosis of the thoracic aorta of 23-24-day-old rats. The actin isomRNA ratio was determined by primer extension assay, and the MHC isomRNA ratio was determined by an exonuclease VII mapping assay using an oligonucleotide probe. The levels of poly(A⁺) RNA and of sarcomeric α-actin and MHC mRNAs were determined by dot-blot hybridization techniques.

**Materials and Methods**

**Animals and Surgical Procedure**

Female Wistar rats (Iffa-Credo) that were 23-24 days old and weighed approximately 50 g at the time of the operation were used in this study. The surgical procedure was carried out under Brietal anesthesia (3 mg/kg i.p.), and respiratory assistance was maintained throughout the operation. Aortic stenosis was performed by placing a partially calibrated occluded Weck hemochip around the ascending thoracic aorta between the base of the heart and the right carotid artery.10,13 The opening surface of the clip was checked by a microscope and was 0.95 mm². Sham-operated control rats underwent an identical procedure except for placement of the hemochip. Sham-operated and operated rats were killed 18 and 24 hours after the operation. The body weight was measured, and the heart was rapidly excised. Right and left atria, extraneous tissue, and large vessels were then dissected from the base of the heart, and the right ventricular free wall was separated from the remaining portion of the heart. Left ventricular (including interventricular septum) samples were weighed and then blotted dry, frozen in liquid nitrogen, and stored at −70°C to −80°C. The degree of left ventricular hypertrophy was calculated from the ratio of left ventricular mass/body mass normalized to that of control (sham-operated) rats killed at the same stage: \([\text{left ventricular mass/body mass}} -\text{(control left ventricular mass/control body mass)}\)−1. To obtain control left ventricles of animals only expressing the β-MHC gene, 23-24-day-old female Wistar rats (Iffa-Credo) were thyroidectomized and were maintained during 6 weeks in the laboratory, after which time there was a complete shift in expression from the α- to the β-MHC isoform.14

**RNA Preparation**

Total RNA was extracted from left ventricles, liver, and fast and slow skeletal muscle of normal animals according to the procedure described in Chirgwin et al.,15 in which the RNA is separated from the guanidium thiocyanate homogenate by ultracentrifugation through a cesium chloride gradient. Liver RNAs and skeletal and thyroidectomized-rat ventricular RNAs were used as negative and positive controls, respectively. The RNA concentration was determined by standard spectrophotometric techniques. One absorbancy unit at 260 nm in a 1-cm light path cuvette was assumed to be equal to 40 μg/ml RNA. The quality of RNA was assessed by visual inspection of ethidium bromide–stained denaturing agarose gel. RNAs were stored at −20°C as a suspension in 70% ethanol containing 0.3 M potassium acetate at pH 5.5.

**Primer Extension and Exonuclease VII Mapping Assays**

Primer extension and exonuclease VII mapping assays allow the simultaneous identification and quantification of sarcomeric α-actin (skeletal and cardiac) and MHC (α and β) isomRNAs, respectively. Primer extensions were performed as previously described.16-18 The principle of the exonuclease VII mapping technique using an oligonucleotide that we have recently described19 is the same as that of S1 nuclease mapping,20 except that an exonuclease is used instead of an endonuclease. The ratios of MHC isomRNAs are determined by laser densitometry (Shimadzu CS-9000 densitometer), and this method has the advantage of yielding clear-cut results, without unspecific bands. The synthetic oligonucleotide is 67 bases long (5’-CCC GCC CCG TGT CTC TTC ATT CAG GCC CTT GGC GCC AAT GTG ACG CTT GCT GGC CCG CAG CTT GTT G-3’). It is the same as that used by Waspe et al.,20 except that we added six additional nucleotides at its 5’ end, complementary neither to α- nor to β-MHC mRNAs, to allow for the determination of the specific digestion activity of the enzyme (Figure 1A). To demonstrate that this technique yields quantitative results, we quantitated the relative amounts of α- and β-MHC mRNAs in synthetic mixtures containing known and various amounts of each of these isomRNAs. Our results (Figures 1B and 2) showed that the 100% β-MHC theoretical control (thyroidectomized-rat ventricular RNA) contained, in fact, 97% β-MHC mRNA and 3% α-MHC mRNA and that the 100% α-MHC theoretical control (23-day-old-rat ventricular RNA) contained, in fact, 91% α-MHC mRNA and 9% β-MHC mRNA. These percentages were checked by successive dot-blot hybridizations with two oligonucleotides specific to α- and β-MHC mRNAs (corresponding to 3’ untranslated nucleotides 1,285-1,327) (not shown). Figure 2 compares the
theoretical results calculated by taking into account these values and the results obtained experimentally. There was very good agreement between both values, indicating that this exonuclease VII mapping technique could be used to determine rapidly and accurately the percentages of α- and β-MHC mRNAs in a same RNA sample.

**FIGURE 1.** Exonuclease VII mapping technique. Panel A: Diagram of a single 67-base synthetic oligonucleotide (closed and open boxes), complementary to a 40-nucleotide common coding sequence present in both α-myosin heavy chain (α-MHC) and β-MHC mRNAs and to a 15-nucleotide sequence specific to β-MHC mRNA (diverging significantly from that of α-MHC mRNA) and containing in addition a 12-nucleotide-sequence complementary neither to α- nor β-MHC. This oligonucleotide was labeled with 32P and hybridized to total RNA. Exonuclease VII digests the probe differently, depending on whether it hybridizes with either α-MHC or β-MHC mRNAs, and the products are resolved on denaturing acrylamide gels. Panel B: Autoradiogram of an 8% acrylamide/7 M urea denaturing gel: 1) liver, 2) 67-base oligonucleotide probe, 3) thyroidectomized-rat heart RNA, 4–7) mixtures of thyroidectomized-rat heart RNA and 23-day-old rat RNA (60%, 40%, and 20%), and 8) 23-day-old rat RNA.

**FIGURE 2.** Graph showing exonuclease VII digestion analysis of rat α-myosin heavy chain (α-MHC) and β-MHC mRNAs. Variable amounts of cardiac RNA from thyroidectomized rats (10, 5, 2.5, 1.25, and 0.625 µg) were prepared as in Boehler et al.21 All hybridizations were performed on the same membranes, which were hybridized with each probe and then dehybridized each time in 0.1x standard saline citrate (SSC) and 0.1% sodium dodecyl sulfate at 97–100°C two times for 5 minutes. Total poly(A+) was determined with a (γ-32P)ATP 5'-end-labeled oligo(dT) (25–30-mer, Pharmacia, Inc.). Specific hybridization probes for total sarcomeric α-actin and α-MHC were, respectively, the 18-base oligonucleotide probe used for primer extension assays and a 42-base oligonucleotide probe (Eurogentec, Belgium) corresponding to 1,285–1,327 nucleotides in the untranslated 3' end of α-MHC mRNA.22 They were 5'-labeled with (γ-32P)ATP (6,000 Ci/mmol) by using T4 polynucleotide kinase with a mean specific activity of 4–6x10⁶ cpm µg⁻¹. For each of these two probes, duration and temperature of prehybridization and hybridization of each membrane (rapid hybridization buffer system, Multiprime-RPN 1517, Amersham) were 1 and 2 hours, respectively, at 55°C. For total sarcomeric α-actin, blots were then washed at room temperature (two times in 6x SSC for 15 minutes) and at 42°C (one time in 3x SSC and 0.1% sodium dodecyl sulfate for 15 minutes and then one time in 1x SSC and 0.1% sodium dodecyl sulfate for 15 minutes). For α-MHC, blots were washed at room temperature (two times in 3x SSC for 15 minutes) and at 50°C (one time in 1x SSC for 15 minutes). Finally, the membranes were hybridized to a T4 polynucleotide kinase (γ-32P)ATP 5'-end-labeled 18S ribosomal RNA 15-nucleotide probe with a specific activity of 5x10⁶ cpm µg⁻¹. The blots were exposed to x-ray films with Quanta III intensifying screens (Appligene) for 1–3 days at ~80°C, and the autoradiograms were quantitated by laser den-
Data Analysis

Results for the various groups of rats are expressed as mean±SEM. The statistical significance of differences between the various groups was determined by one-way analysis of variance, and group-to-group comparison was made by Scheffe’s F test. At each postsurgical time, operated and sham-operated animals were compared by unpaired Student’s t test. The accepted level of significance was p<0.05, p<0.01, or p<0.001.

Results

Left Ventricular Hypertrophy and poly(A+)
RNA Content

Constriction of the thoracic aorta in 23-day-old rats produced a left ventricular hypertrophy of 18.1±1.6% (n=6) at 18 hours and 29.1±5.9% (n=8) at 24 hours. Figure 3 shows that poly(A+) RNA content normalized to 18S ribosomal RNA; i.e., the proportion of the mRNA population in total cardiac RNA increased significantly by 1.9-fold (p<0.001) in operated versus sham-operated rats (2.8±0.2 AU [n=8] versus 1.5±0.2 AU [n=9]) at 24 postsurgical hours. This is consistent with the increase of 1.7 reported by de la Bastie et al12 on a model of abdominal aortic stenosis.

mRNA Isoform Ratios of Sarcomeric α-Actin and MHC

Figure 4 shows two autoradiograms obtained from the analysis of liver, skeletal muscle, and cardiac RNA samples by the primer extension (panel A) and exonuclease VII mapping (panel B) assays. No signals were detected from liver RNAs, indicating the specificity of both assays for sarcomeric actins and MHCs. Figure 4A (primer extension) shows one signal with an apparent length of 186 nucleotides from rat fast skeletal muscle RNA and two signals with the cardiac RNAs, one of which comigrates with that from skeletal muscle and a second one that has an apparent length of 195 nucleotides; these two signals represent skeletal and cardiac α-actin mRNAs, respectively.17 Figure 4B (exonuclease VII mapping) shows four bands between 59 and 62 nucleotides corresponding to β-MHC mRNA from rat soleus muscle RNA and five bands between 43 and 47 nucleotides corresponding to α-MHC mRNA from 23-day-old rat left ventricular RNA. As can be readily distinguished in this figure, the intensities of signals for α-skeletal actin and for β-MHC increase in the RNA from operated rats as compared with sham-operated rats, in agreement with previous results.5-9

All data from sham-operated and constricted animals are listed in Table 1. They show in operated versus sham-operated rat left ventricles an increase in the proportion of skeletal α-actin mRNA to total sarcomeric α-actin of 310% (p<0.001) at 18 hours and 460% (p<0.001) at 24 hours. As a consequence, the ratio of skeletal/cardiac α-actin isoMHCs increases fivefold (from 0.19 to 0.99) at 18 hours and ninefold at 24 hours. β-MHC mRNA increases by 290% at 18 hours (p=NS) and by 680% (p<0.001) at 24 hours, which corresponds to a threefold (from 0.04 to 0.12) and a 9.8-fold (from 0.05 to 0.49) increase, respectively, of β-MHC/α-MHC. Although β-MHC mRNA increased clearly at 18 hours in some animals, the data were scattered, which is probably why the 290% increase is not statistically significant. These results extend the scope of previous results show-
ing that skeletal α-actin is detectable by in situ hybridization earlier than β-MHC.10

Sarcomeric α-Actin Transcripts

Densitometric analysis of the hybridization signal from each animal revealed that the content of sarcomeric α-actin in the poly(A') RNA population significantly increased at 18 hours (2.17±0.29 AU [n=6] versus 1.25±0.11 AU [n=7], p<0.05, Figure 5A) in operated rats. This 1.74-fold increase was exclusively due to a 5.5-fold increase of skeletal α-actin mRNA in poly(A') RNA (1.10±0.19 AU [n=6] versus 0.20±0.02 AU [n=7], p<0.001, Figure 5B); the level of cardiac α-actin mRNA remained unchanged (Figure 5C). At 24 hours, the sarcomeric α-actin mRNA level in poly(A') RNA returned to values similar to those of sham-operated rats (1.55±0.18 AU [n=8] versus 1.55±0.14 AU [n=8], Figure 5A). This was due to a decrease in the proportion of cardiac α-actin mRNA (0.69±0.07 AU [n=8] versus 1.34±0.11 [n=8], p<0.001, Figure 5C); the skeletal α-actin mRNA remained elevated as compared with the level in sham-operated animals (0.86±0.12 AU [n=8] versus 0.20±0.04 [n=8], p<0.001, Figure 5B). Poly(A') RNA increased by 1.9-fold at this time (see Figure 3); thus, the decrease of 49% in the cardiac α-actin mRNA that we found at 24 hours indicates that the cardiac α-actin gene is not activated by the hemodynamic load. In summary, at 18 hours, the fivefold increase in skeletal/cardiact α-actin isomRNAs (Table 1) is due to an increased accumulation of skeletal α-actin mRNA, and at 24 hours, the ninefold increase in this ratio (Table 1) results both from an increase in skeletal α-actin mRNA content and a decrease in cardiac α-actin mRNA. If we now consider these isomRNA changes relative not to poly(A') RNA but to total cardiac RNA (18S ribosomal RNA), our results show that the level of skeletal α-actin mRNAs increases 8.2-fold, whereas that of cardiac α-actin mRNAs remains constant. This indicates that the skeletal α-actin gene expression is earlier and more strongly upregulated than that of other cardiac genes, whatever the mechanism, transcriptional and/or posttranscriptional. In contrast, cardiac α-actin gene expression does not change after the pressure overload.

Sarcomeric MHC Transcripts

Figure 6 shows no obvious changes at 18 hours concerning the accumulation of α-MHC, β-MHC, and total sarcomeric MHC mRNAs. At 24 hours, there is a significant decrease of 71% in the level of α-MHC mRNAs in poly(A') RNA (0.47±0.04 AU [n=8] versus 1.64±0.12 AU [n=8], p<0.001, Figure 6C) in operated versus sham-operated rats. This decrease is not compensated by a statistically nonsignificant increase of β-MHC mRNAs (Figure 6B); as a consequence, the level of sarcomeric MHC mRNAs significantly decreases (0.66±0.07 AU [n=8] versus 1.70±0.13 AU [n=8], p<0.001, Figure 6C). If, as for the isoactins, we consider the results relative to total cardiac RNA, the level of β-MHC mRNA increases 5.1-fold while that of α-MHC mRNA decreases 1.8-fold. This indicates that the α-MHC gene expression is downregulated, whereas the β-MHC gene expression is upregulated.

### Table 1. Skeletal/Cardiac α-Actin and β-Myosin Heavy Chain Cardiac α-Actin mRNA Isoform Ratios in Operated and Sham-Operated Rat Left Ventricle in Early Stages of Thoracic Aorta Stenosis

<table>
<thead>
<tr>
<th>Postsurgical hours</th>
<th>Skeletal/total sarcomeric actin</th>
<th>β-MHC/total sarcomeric MHC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Stenosis</td>
</tr>
<tr>
<td>18</td>
<td>15.9±1.5% (n=7)</td>
<td>49.8±3.3%* (n=6)</td>
</tr>
<tr>
<td>24</td>
<td>11.9±1.8% (n=8)</td>
<td>54.9±1.4%* (n=8)</td>
</tr>
</tbody>
</table>

MHC, myosin heavy chain; sham, sham-operated rats; stenosis, rats with surgically induced aortic stenosis. Values are mean±SEM. Results are the percentages of α-skeletal/sarcomeric actin mRNA and of β-MHC/sarcomeric MHC mRNA.

*p<0.001 vs. sham.

### Figure 5

Bar graphs showing sarcomeric α-actin mRNA accumulation in poly(A') RNA of operated (aortic stenosis [st]) vs. sham-operated (sh) rat left ventricle during the early stages of thoracic aorta stenosis. Data are represented for sh and st rats groups and are expressed in mean±SEM arbitrary units. *p<0.05 and ***p<0.001 by unpaired t test.
and complex response to the mechanical, hormonal, and/or neurohumoral stimuli triggered by the pressure overload (see References 3 and 4 for review).

Our results concerning the isoactins are consistent with those reported at day 5 in 9-month-old Fisher rats\(^2\) and in a milder model of left ventricular pressure overload (constriction of the abdominal aorta) at day 1 in adult Sprague-Dawley rats.\(^1\) Skeletal α-actin mRNA is also specifically increased at day 15 in calf right ventricular hypertrophy.\(^2\) Thus, it seems that, whatever the strain of rat and whatever the animal species, the skeletal α-actin is a target gene for the various mechanisms triggered by work overload, whereas the cardiac α-actin gene is not. In vivo upregulation of the expression of the skeletal α-actin gene with little or no modulation of the cardiac α-actin gene occurs in two other situations of rapid cardiac growth: thyroxine injection\(^1\) and postnatal development.\(^2\) A thyroid responsive element has recently been found in the promoter region of the human skeletal α-actin gene\(^2\) between positions −173 and −149, which would account for the effect of thyroxine, but nothing is known concerning the mechanisms of the upregulation of the skeletal α-actin gene after birth. During in vitro hypertrophy induced by α1-adrenergic receptor stimulation of neonate cardiac myocytes plated at low density, skeletal α-actin is also induced preferentially to cardiac α-actin.\(^1\) Very recent experiments have demonstrated that specific upstream sequences between −2,000 and −1,300 are required for the induction of human skeletal α-actin gene expression by norepinephrine,\(^2\) and no equivalent regions have been reported on the cardiac α-actin gene. Another set of experiments using a completely different approach (transgenic mice carrying a tagged skeletal muscle actin) has shown that, in contrast to the behavior of the skeletal α-actin gene, the single wild-type cardiac actin was unable to fully compensate for the reduced amount of expression of the mutated gene.\(^3\) Taken together, these data strengthen the idea first put forward by Mayer et al\(^4\) that expression of the cardiac α-actin gene may not be sufficient for synthesis of adequate amounts of actin during rapid sarcomerogenesis. The cardiac α-actin gene would already be expressed in the rodent heart at submaximal rates, and when rapid synthesis of actin is necessary, the skeletal muscle actin is used. However, this hypothesis does not account for the high level of skeletal α-actin in adult human hearts,\(^5\) and the sequences required for species-specific expression of this gene as well as the corresponding transcription factors are as yet completely unknown.

After sarcomeric α-tropomyosin,\(^6\) cardiac α-actin is the second contractile gene shown to be apparently unaffected by hemodynamic overload. Both genes belong then to a category of apparently noninducible genes that comprises the Ca\(^{2+}\)-ATPase of the sarcoplasmic reticulum,\(^7\) the α\(_i\) isoform of the sarcolemmal Na\(^+\),K\(^+\)-ATPase,\(^8\) and the β-adrenergic receptors.\(^9\) The molecular basis for the lack of inducibility of these genes during hypertrophy is unclear, and one can only hypothesize that similar cis-regulatory elements and trans-acting factors drive the expression of this category of genes.

The present results show that the early mechanisms responsible for the modifications in the MHC pheno-

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**FIGURE 6.** Bar graphs showing sarcomeric myosin heavy chain (MHC) mRNA (total, α-MHC, and β-MHC) accumulations in poly(A\(^+\)) RNA of operated (aortic stenosis [st]) vs. sham-operated (sh) rat left ventricle during the early stages of a thoracic aorta stenosis. Data are represented for sh and st rats and are expressed in mean ± SEM arbitrary units. ***p < 0.001 by unpaired t test.

**Discussion**

The results of the present study show that the mechanisms responsible for the change of α-actin and MHC isomRNA ratios (skeletal/cardiac α-actin and β-MHC/α-MHC) differ markedly in the same ventricle in response to pressure overload: whereas the mRNAs encoding α- and β-MHC accumulate in an antithetical manner, in the isoactin pair the skeletal α-actin mRNA hyperaccumulates, but the cardiac α-actin mRNA remains unchanged, suggesting that expression of this latter isogene is not modified. Because these changes occur only in the banded animals and because, in the same experimental model, skeletal α-actin and β-MHC mRNAs accumulate in different regions,\(^10\) the hypothesis of a generalized myocytic response to acute stress can be discarded. A nonspecific response to a possible tissular hypoxia can also be discarded, since skeletal α-actin mRNA does not increase in a calf model of hypobaric hypoxia.\(^2\) Our data, as well as those previously reported for the same isogenes and for other genes studied so far, indicate the existence of a specific
type involve an antithetic regulation of the expression of the two isoforms by work overload and a marked downregulation of the expression of the α-MHC gene. This is consistent with the recent work of Gupta and Zak, who found that 1 week after banding the abdominal aorta there is a downregulation of the α-MHC with a corresponding upregulation of β-MHC gene expression, and with the preliminary observation by Wiesner et al., who demonstrated a significant decrease in α-MHC mRNA 2 days after banding of the ascending aorta. All these results indicate that the load-related signals act on both MHC isoforms in the early as well as in later stages of work overload.

This study did not determine the regulatory level of the expression of isoactin and isoactin genes after a work overload. The isoform transitions for MHCs are known to be regulated pretranslationally. Because of the strong similarity at the protein level between skeletal and cardiac actins, classical immunological and biochemical techniques do not allow the quantification of both isoactins in mixtures of the two; thus, the hypothesis of an analogous pretranslational regulation was never thoroughly tested. Very recently, a transcription system using isolated rat cardiomyocytes was described that enables one to analyze isoactin and isoactin transcriptional regulation in vivo. In 23–24-day-old rats, the primary mechanisms controlling the accumulation of these gene transcripts are transcriptional, and experiments are under way to address directly the question of a transcriptional regulation in work load. Our present results concerning MHC isoforms suggest that there is an additional posttranscriptional regulation. α-MHC transcripts constitute approximately 96% of MHC transcripts in sham-operated animals (see Table 1), and it is clear that the 70% decrease in α-MHC that we have found at 24 hours cannot be compensated by the 270% increase of β-MHC, because this isoMRNA represents only approximately 4% of the total. The net result is a striking decrease of MHC transcripts (Figure 6). If regulation of MHCs was only transcriptional, this would lead to a decrease in the total number of MHC protein molecules being synthesized immediately after stenosis. Since this hypothesis is highly unlikely, our results suggest an acceleration in translational process from α-MHC transcripts to α-MHC protein during the early phase of hypertrophy. Indeed, it has been shown that the increase in ribosomal RNA content during myocyte hypertrophy is closely related to higher efficiencies of peptide chain initiation and elongation and higher capacity for protein synthesis. A postranslational regulation of α-MHC seems to occur later during the development of hypertrophy, when an increased degradation of the protein would occur in excess of its synthesis. This suggests, at least for α-MHC, that a combination of transcriptional and posttranscriptional events is required to produce or to maintain the load-induced phenotype. An alternative explanation would be that, because of their different half-lives, the α-MHC mRNA level drops earlier than that of its corresponding protein and that the amount of total MHC is maintained because of the postranscriptional regulation of the β-MHC gene recently suggested.

Many factors—mechanical, hormonal, and peptide—may play a role in changes of α-actin and MHC isogene expression during in vivo cardiac hypertrophy. The effect of specific factors was studied in several in vitro models of myocardial cell hypertrophy. In neonatal myocyte cultures, α1-adrenergic agonists are implicated in α-skeletal actin and β-MHC mRNA changes, with transcription of the β-MHC being stimulated pre- and posttranslationally by α1-adrenergic-activated β-protein kinase C. However, if there is a good agreement between in vitro and in vivo results for skeletal α-actin and β-MHC, it is the case neither for cardiac α-actin nor for α-MHC, which suggests that the α1-adrenergic agonists could be implicated during in vivo work-overload hypertrophy only if they are associated with other factor(s). Transforming growth factor-β and basic fibroblast growth factor are most probably implicated, since they both induce in vitro the same changes that we have observed and since they were both found in ventricular myocytes. The last component of this complex pattern of gene activation compatible with our results is myocyte passive stretch. Such a mechanism was already postulated by Schiaffino et al., who showed that in vivo changes in skeletal α-actin and β-MHC mRNAs appear parallel to changes in ventricular wall stress. Consistent with this are the recent demonstrations that increases in coronary pressure in isolated perfused heart induce β-MHC mRNA accumulation and that static stretch imposed on cultured neonatal cardiomyocytes upregulates skeletal α-actin and β-MHC, most probably through a stretch–response element localized outside a 628 base pair 5' flanking region of the β-MHC gene.

In conclusion, the precise mechanisms regulating the expression of the multigene families of the two major constituents of the cardiac sarcomere differ in response to changes in external cardiac load.

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