Myosin Heavy Chain Isoform Expression in the Failing and Nonfailing Human Heart

Setsuya Miyata, Wayne Minobe, Michael R. Bristow, Leslie A. Leinwand

Abstract—In the heart, the relative proportions of the 2 forms of the motor protein myosin heavy chain (MyHC) have been shown to be affected by a wide variety of pathological and physiological stimuli. Hearts that express the faster MyHC motor protein, α, produce more power than those expressing the slower MyHC motor protein, β, leading to the hypothesis that MyHC isoforms play a major role in the determination of cardiac contractility. We showed previously that a significant amount of αMyHC mRNA is expressed in nonfailing human ventricular myocardium and that αMyHC mRNA expression is decreased 15-fold in end-stage failing left ventricles. In the present study, we determined the MyHC protein isoform content of human heart samples of known MyHC mRNA composition. We demonstrate that αMyHC protein was easily detectable in 12 nonfailing hearts. αMyHC protein represented 7.2±3.2% of total MyHC protein (compared with ≈35% of the MyHC mRNA), suggesting that translational regulation may be operative; in contrast, there was effectively no detectable αMyHC protein in the left ventricles of 10 end-stage failing human hearts. (Circ Res. 2000;86:386-390.)

Key Words: myosin ▪ heart failure ▪ isoforms

In conjunction with 2 pairs of nonidentical light chains, 2 myosin heavy chain (MyHCs) constitute the functional myosin motor molecule. Two isoforms of MyHC (α and β) are expressed in mammalian heart. Myosin consisting of αMyHC has a higher ATPase activity than myosin composed of βMyHC,1 and in the rodent heart, contractile velocity correlates with the relative amount of each MyHC. Hearts expressing αMyHC have more rapid contractile velocity than hearts expressing βMyHC, which allows greater economy in force generation because the tension-time integral for force per cross-bridge cycle is greater.2,3 The MyHC composition of the ventricular myocardium of rodents has been reported to be >90% αMyHC,4–6 whereas that of humans has been reported to be >95% βMyHC.7–13 In the rodent heart, thyroid hormone elevation and exercise have been shown to increase αMyHC, whereas thyroid depletion, aging, cardiomyopathy, and pressure overload have been shown to increase βMyHC (see Swynghedauw14). Because the normal human heart was previously thought to be entirely βMyHC,7–13 stimuli that might induce isoform shifts toward βMyHC in human heart disease were thought to be irrelevant.

The MyHC composition of human heart was originally investigated with immunohistochemistry, because of the difficulty in electrophoretic separation of the human αMyHC and βMyHC,9 or by peptide mapping.7,10 Immunohistochemistry can show the spatial expression of each isoform, but it is not quantitative because 2 different antibodies cannot be directly compared.15,16 The results of immunohistochemical analysis were quite varied, with reports ranging from <5% to 88% of myocytes expressing αMyHC.8,11,12 We showed that αMyHC mRNA was expressed at considerable levels in the nonfailing human left ventricles (LVs) and was substantially decreased in end-stage failing human LVs.17,18 The proportion of total MyHC mRNA that is αMyHC mRNA was ≈30% in nonfailing LVs and was reduced by 15-fold, to ≈2%, in end-stage LVs. Because of the potential functional significance of altered MyHC composition in contractility and to gain insight into the molecular mechanisms of changes in gene expression in heart failure, we quantified αMyHC and βMyHC proteins in human nonfailing and failing LVs according to a recently reported gel electrophoretic method.19 We demonstrate that αMyHC protein is detectable in nonfailing LVs but is virtually undetectable in failing LVs.

Materials and Methods

Patients from whom hearts were provided through the Colorado Transplant Program are described in Tables 1 and 2.17 LVs were obtained from 12 control organ donor candidates (5 men and 7 women, mean age 36.8±16.9 years) and 10 patients undergoing heart transplantation (8 men and 2 women, mean age 44.9±19.3 years).

RNA was extracted and analyzed with quantitative polymerase chain reaction as previously described and reported.17,18

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TABLE 1. Clinical Characteristics and αMyHC cDNA and Protein Ratios of 12 Organ Donors Without a Heart Failure History

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Gender</th>
<th>Cause of Coma</th>
<th>Cardiovascular Abnormality</th>
<th>Duration of Life Support, h</th>
<th>DOA/DOB, μg·kg⁻¹·min⁻¹</th>
<th>Amplified αMyHC cDNA Ratio, %</th>
<th>αMyHC Protein Ratio of Amount, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFH-1</td>
<td>38</td>
<td>M</td>
<td>SIGSW</td>
<td>None</td>
<td>20</td>
<td>14/0</td>
<td>26.0</td>
<td>11.2</td>
</tr>
<tr>
<td>NFH-2</td>
<td>16</td>
<td>M</td>
<td>MVA</td>
<td>None</td>
<td>32</td>
<td>0/0</td>
<td>55.0</td>
<td>8.2</td>
</tr>
<tr>
<td>NFH-3</td>
<td>51</td>
<td>F</td>
<td>CVA</td>
<td>LV hypertrophy</td>
<td>34</td>
<td>13/0</td>
<td>10.8</td>
<td>4.2</td>
</tr>
<tr>
<td>NFH-4</td>
<td>33</td>
<td>F</td>
<td>CVA</td>
<td>None</td>
<td>37</td>
<td>5/12</td>
<td>52.6</td>
<td>7.2</td>
</tr>
<tr>
<td>NFH-5</td>
<td>49</td>
<td>F</td>
<td>CVA</td>
<td>None</td>
<td>18</td>
<td>4/0</td>
<td>24.0</td>
<td>6.5</td>
</tr>
<tr>
<td>NFH-6</td>
<td>31</td>
<td>F</td>
<td>CVA</td>
<td>DHD</td>
<td>?</td>
<td>12/12</td>
<td>35.6*</td>
<td>5.6</td>
</tr>
<tr>
<td>NFH-7</td>
<td>17</td>
<td>M</td>
<td>Trauma</td>
<td>None</td>
<td>61</td>
<td>5/0</td>
<td>25.7</td>
<td>7.0</td>
</tr>
<tr>
<td>NFH-8</td>
<td>35</td>
<td>M</td>
<td>MVA</td>
<td>None</td>
<td>69</td>
<td>1–9/0</td>
<td>42.3*</td>
<td>13.0</td>
</tr>
<tr>
<td>NFH-9</td>
<td>14</td>
<td>F</td>
<td>MVA</td>
<td>None</td>
<td>36</td>
<td>20/0</td>
<td>46.1*</td>
<td>8.5</td>
</tr>
<tr>
<td>NFH-10</td>
<td>59</td>
<td>M</td>
<td>CVA</td>
<td>None</td>
<td>?</td>
<td>0/0</td>
<td>24.7*</td>
<td>9.4</td>
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<tr>
<td>NFH-11</td>
<td>27</td>
<td>F</td>
<td>MVA</td>
<td>None</td>
<td>71</td>
<td>0/0</td>
<td>54.7</td>
<td>4.5</td>
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<tr>
<td>NFH-12</td>
<td>42</td>
<td>F</td>
<td>CVA</td>
<td>None</td>
<td>66</td>
<td>12/0</td>
<td>24.1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Mean±SD 35.13±14.69 7.21±3.20

DOA/DOB indicates dopamine/dobutamine at organ harvest; MVA, motor vehicle accident; CVA, cerebrovascular accident; SIGSW, self-inflicted gunshot wound; CAD, coronary artery disease; AV, aortic valve; MV, mitral valve; and DHD, donor heart dysfunction, with generalized hypokinesis and estimated LV ejection fraction of 0.20 to 0.25.

*RNA levels were determined with the method described previously.18 The remainder were carried out as described previously.17

Samples for protein gel electrophoresis (15 to 200 mg) were prepared as described by Caforio et al.20 from tissue that had no visible fat and no connective tissue. Briefly, samples were homogenized in low-salt buffer (20 mmol/L KCl, 2 mmol/L KH₂PO₄, 1 mmol/L EGTA, pH 6.8, 1 mmol/L PMSF, 100 μL N,N-dimethyl formamide). The samples were then centrifuged at 5000 rpm for 10 minutes at 4°C in a JA-17 rotor (Beckman Instruments). Pellets were suspended in high-salt buffer (40 mmol/L Na₄P₂O₇, 1 mmol/L MgCl₂, 1 mmol/L EGTA, pH 9.5) and then centrifuged at 15 000 rpm for 30 minutes at 4°C. Laemmli’s buffer was added to each sample, and then each sample was boiled.21 The preparation and composition of the gel were carried out as described by Reiser and Kline.19 Gel samples (0.25 to 1 μg) were loaded in a 3-μL volume onto 15-well gels. The stacking and separating gels (0.75 mm thick) consisted of 4% and 8% acrylamide, respectively; the stacking gels were run in a Hoeffer Scientific SE600 instrument at 5°C. The gels were run at a constant voltage of 200 V for 30 hours. The gels were fixed and silver stained as described in Blough et al.22 A gel documentation system (Bio-Rad) was used to scan the stained gels. The abundance of αMyHC and βMyHC was determined with quantitative reverse transcription–polymerase chain reaction as previously described.17,18 Protein samples were subjected to electrophoresis as described earlier and transferred to 0.2-μm nitrocellulose. The blots were blocked in 10% nonfat dry milk in PBS for 2 hours at room temperature. Blots were incubated with a monoclonal antibody against sarcosmeric MyHC (F59)23 or F88.12F8 (Alexis Biochemicals) at a dilution of 1:500 or 1:5000, respectively, in 5% BSA overnight at 4°C. After primary antibody incubations, 3 washes in PBS were followed by an incubation in the secondary antibody, peroxidase-conjugated goat anti-mouse IgG (Jackson Laboratories) diluted 1:5000 in 10% nonfat dry milk in PBS for 2 hours at room temperature. The blots were then washed 3 times in 0.05% NP-40/PBS. Immuno-reactive bands were visualized by using the Renaissance Western Blot Chemiluminescence Reagent (NEN Life Sciences).

TABLE 2. Clinical Characteristics and αMyHC cDNA and Protein Ratios of 10 Patients With End-Stage Heart Failure

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Gender</th>
<th>Diagnosis</th>
<th>Surgical/Transluminal Intervention</th>
<th>Medical Treatment</th>
<th>Amplified αMyHC cDNA Ratio, %</th>
<th>αMyHC Protein Ratio of Amount, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-1</td>
<td>64</td>
<td>F</td>
<td>IDC</td>
<td>None</td>
<td>Dxn</td>
<td>8.0</td>
<td>0.0</td>
</tr>
<tr>
<td>F-2</td>
<td>60</td>
<td>M</td>
<td>IDC</td>
<td>AICD</td>
<td>ACEI</td>
<td>1.1</td>
<td>0.0</td>
</tr>
<tr>
<td>F-3</td>
<td>24</td>
<td>M</td>
<td>IDC</td>
<td>None</td>
<td>Dxt</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>F-4</td>
<td>53</td>
<td>M</td>
<td>IDC</td>
<td>None</td>
<td>Ca-blkgr</td>
<td>0.6</td>
<td>0.0</td>
</tr>
<tr>
<td>F-5</td>
<td>18</td>
<td>F</td>
<td>IDC</td>
<td>None</td>
<td>NA</td>
<td>6.1</td>
<td>0.0</td>
</tr>
<tr>
<td>F-6</td>
<td>31</td>
<td>M</td>
<td>IDC</td>
<td>None</td>
<td>NA</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>F-7</td>
<td>60</td>
<td>M</td>
<td>CAD</td>
<td>CABG×6</td>
<td>Dxn</td>
<td>1.2</td>
<td>0.0</td>
</tr>
<tr>
<td>F-8</td>
<td>64</td>
<td>M</td>
<td>CAD</td>
<td>CABG×5, PTCA×1</td>
<td>Dxt</td>
<td>1.8</td>
<td>0.0</td>
</tr>
<tr>
<td>F-9</td>
<td>54</td>
<td>M</td>
<td>CAD</td>
<td>PTCA×3</td>
<td>CABG</td>
<td>2.4</td>
<td>0.0</td>
</tr>
<tr>
<td>F-10</td>
<td>18</td>
<td>M</td>
<td>IDC</td>
<td>None</td>
<td>NA</td>
<td>8.8*</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Mean±SD 2.2±2.95 0.075±0.24

IDC indicates idiopathic dilated cardiomyopathy; Dxn, digoxin; ACEI, ACE inhibitor; Dxt, diuretics; Ca-blkgr, Ca²⁺ channel antagonists; EF, ejection fraction; CABG, coronary artery bypass graft surgery; PTCA, percutaneous transluminal coronary angioplasty; AICD, automatic implantable cardiac defibrillator; +, administrated; –, not administrated; and NA, not available.
Results and Discussion

The goals of the present study were to determine the amount of αMyHC protein in the nonfailing and failing human hearts and to determine the relationship between MyHC mRNA and protein levels. The clinical characteristics and the αMyHC mRNA content of the patients analyzed in the present study are shown in Tables 1 and 2. The range of αMyHC mRNA was 10.8% to 55% in nonfailing LVs and 0% to 8.8% in failing LVs. To identify αMyHC protein, immunoblot analysis was carried out with a monoclonal antibody specific to αMyHC protein. To quantify the proportion of αMyHC protein, an electrophoretic separation protocol was used, followed by silver staining and densitometry. Figure 1 is an immunoblot performed on a high-resolution gel that separates αMyHC and βMyHC proteins. The gel was probed with pansarcomeric MyHC antibody F59 and an αMyHC-specific antibody (bottom). Lane 1 represents rabbit skeletal MyHC, used as a control. It was recognized by the pansarcomeric antibody but not by the αMyHC antibody. It was recognized by the pansarcomeric MyHC antibody but not by the αMyHC-specific antibody. Lane 2, human right atrial sample; lane 3, a 1:1 mixture of right atrium and LV; lane 4, NFH-1; and lane 5, NFH-3.

![Image of immunoblot](http://circres.ahajournals.org/)

**Figure 1.** Immunodetection of αMyHC and βMyHC after high-resolution gel electrophoresis. Top, Probed with F59, which recognizes all sarcomeric MyHCs. Bottom, Probed with F88.12.F8, which is specific to αMyHC. Lane 1 indicates rabbit skeletal MyHC; lane 2, human right atrial sample; lane 3, a 1:1 mixture of right atrium and LV; lane 4, NFH-1; and lane 5, NFH-3.

Cardiac myocyte contraction accelerates MyHC synthesis by increasing the rate of translation initiation. This occurs in the absence of an increase in mRNA abundance. After 7 days of ascending aortic constriction, βMyHC protein increases from 5% to 31% of total MyHC in the absence of changes in mRNA abundance. Finally, contractile arrest of cardiac myocytes has been shown to inhibit MyHC synthesis and to decelerate MyHC degradation. Protein synthesis in the heart is regulated by changes in efficiency and capacity and has been shown to vary widely depending on the stimulus. Before drawing firm conclusions about the degree of discordance between mRNA and protein, 1 limitation of the silver-staining approach should be noted: in samples with very low amounts of αMyHC, the βMyHC band may obscure a faint αMyHC band. To complement the approach used here and to be able to determine the absolute quantities of α and βMyHC, we are in the process of developing ELISAs.

One question that arises from these observations is whether a relatively small amount of αMyHC is capable of...
changing the contractile properties of the heart. Thus far, the cases in which shifts in myosin composition have been studied have generally been at end points where the shift has been quite large. The impact of small shifts such as those described here have not yet been reported. However, with the assumption of a 3-fold difference in the velocity of shortening in muscles expressing the α and β isoforms, the decrease in αMyHC from 7.5% to <0.1% could theoretically reduce systolic function by 12.5%, although the direct relationship to velocity of contraction would have to be tested directly. In support of this hypothesis, we recently used transgenesis to express βMyHC in the adult mouse heart, which normally expresses exclusively αMyHC. When only 12% of the total MyHC is βMyHC, there is a significant decrease in systolic function and Ca$^{2+}$-activated myofibrillar ATPase activity. However, in vitro biochemical mixing experiments with varying proportions of α and β myosin show that β myosin can have a “slowing” effect on α myosin, suggesting that the impact of myosin changes in the intact heart may not reflect the behavior of purified molecules in solution. The sensitivity of the cardiac sarcomere to alterations in MyHC has been emphasized by the discovery of >50 alleles of the βMyHC gene found in patients with hypertrophic cardiomyopathy (see Bonne et al). Most of these alleles have mutations in the motor domain, and in vitro biochemical studies have shown that the motor activity is impaired in at least 3 different alleles. Somewhat surprisingly, the functional differences in the mutant alleles are less than the difference between wild-type αMyHC and βMyHC. Further investigation is required to understand the relationship of decreased myosin motor activity and the pathogenesis of hypertrophic cardiomyopathy and heart failure.

In summary, we separated human αMyHC and βMyHC proteins through electrophoresis and quantified the relative amounts of αMyHC and βMyHC in nonfailing and failing LVs. αMyHC is detectable in nonfailing LVs but effectively undetectable in failing LVs. Future experiments will be directed toward a determination of the mechanism of αMyHC mRNA decrease and the relevance of changes in MyHC gene expression to human cardiac function.

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References


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