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Report

Small Amounts of α-Myosin Heavy Chain Isoform Expression Significantly Increase Power Output of Rat Cardiac Myocyte Fragments

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Myocardial performance is likely affected by the relative expression of the two myosin heavy chain (MyHC) isoforms, namely α-MyHC and β-MyHC. The relative expression of each isoform is regulated developmentally and in pathophysiological states. Many pathophysiological states are associated with small shifts in the relative expression of each MyHC isoform, yet the functional consequence of these shifts remains unclear. The purpose of this study was to determine the functional effect of a small shift in the relative expression of α-MyHC. To this end, power output was measured in rat cardiac myocyte fragments that expressed ≈12% α-MyHC and in myocyte fragments that expressed ≈0% α-MyHC, as determined in the same cells by SDS-PAGE analysis after mechanical experiments. Myocyte fragments expressing ≈12% α-MyHC developed ≈52% greater peak normalized power output than myocyte fragments expressing ≈0% α-MyHC. These results indicate that small amounts of α-MyHC expression significantly augment myocyte power output.

Myocardial performance demonstrates plasticity through protein isoform switches.[1] One cardiac protein, whose isoform expression influences myocardial performance, is myosin heavy chain (MyHC), the molecular motor that drives myocardial contraction.[2] Two functionally diverse MyHC isoforms are expressed in mammalian myocardium, α-MyHC and β-MyHC. The two MyHC isoforms display 93% amino acid identity,[3] yet α-MyHC exhibits two to three times faster actin-activated ATPase activity[4] and actin filament sliding velocity.[5] Similarly, myocyte fragments that express exclusively α-MyHC generate nearly three times greater peak normalized power than myocytes expressing only β-MyHC.[6]

The relative expression of each MyHC isoform varies throughout mammalian development[7] and in pathophysiological conditions such as hypothyroidism,[7] diabetes,[8] hypertension,[4] and heart failure.[9,10] In rodents, pathophysiological conditions are associated with the downregulation of α-MyHC and the concomitant upregulation of β-MyHC expression.[4,7,8] Interestingly, recent evidence has demonstrated a similar downregulation of α-MyHC protein expression and upregulation of β-MyHC expression in failing human hearts.[9,10] These studies have shown that normal adult human hearts express a small but detectable amount of α-MyHC protein, whereas failing human hearts express exclusively β-MyHC. The functional consequence of such a small shift in MyHC isoform expression, however, is unclear. Therefore, the purpose of this study was to determine if a small amount of α-MyHC protein expression is sufficient to augment single cardiac myocyte function. To this end, power output was measured in single myocyte fragments that expressed small amounts of α-MyHC and in fragments that expressed ≈0% α-MyHC (ie, 100% β-MyHC). The relative expression of α-MyHC and β-MyHC was determined for each myocyte by SDS-PAGE analysis and silver staining after mechanical measurements, thereby providing a direct correlation between relative MyHC isoform expression and myocyte fragment function.

Materials and Methods

Experimental Animals

Male thyroidectomized Sprague-Dawley rats (160 to 220 g) were obtained from Harlan (Madison, Wis). It has been demonstrated that 3 weeks after thyroidectomy, rat myocardium expresses exclusively β-MyHC, whereas the expression of other myofibrillar proteins appears unaltered.[6,11,12] To obtain myocytes that expressed small amounts of α-MyHC, thyroidectomized rats were killed 2 to 2.5 weeks after surgery. To obtain myocytes that expressed exclusively β-MyHC, thyroidectomized rats were killed 3 to 5 weeks after surgery. The care and use of animals were in accordance with guidelines established by the Animal Care and Use Committee of the University of Missouri.

Cardiac Myocyte Preparation and Functional Measurements

Single skinned cardiac myocyte fragments were obtained by mechanical disruption of hearts from rats as described previously.[13] Dimensions of the myocyte fragment preparations are listed in Table 1. The solutions and protocol for force-velocity and power-load measurements have been previously described.[13] After mechanical attachment, myocyte fragment preparations were transferred from relaxing solution into maximal Ca^{2+}-activating solution (pCa 4.5). Once steady-state isometric force was attained, a series of force clamps was performed to determine isotonic shortening velocities. Ca^{2+}-activated force remained similar throughout the series of load clamps (force after the load clamps was 0.92±0.09 [mean±SD] of the force before load clamps).

SDS-PAGE and Silver Staining

After the mechanical measurements, the relative expression of each MyHC isoform was determined using SDS-PAGE and silver staining as previously described.[6,12] Silver staining intensity was determined to be linear over the range of ≈0.5 ng to at least 30 ng MyHC protein, which is within the range expected when single-myocyte MyHC isoform expression changes from ≈0% to 100% of either isoform.

Original received January 25, 2002; resubmission received April 15, 2002; revised resubmission received May 8, 2002; accepted May 10, 2002.

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(*Circ Res.* 2002;90:1150-1152.)

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Circulation Research is available at http://www.circresaha.org
DOI: 10.1161/01.RES.0000022879.57270.11

TABLE 1. Myocyte Fragment Dimensions and Force

	Length, μm	Width, μm	Sarcomere Length, μm		Maximal Ca^{2+}-Activated Force, μN
			pCa 9.0	pCa 4.5	
≈12% α-MyHC myocyte fragments (n=6)	141±28	19±3	2.23±0.06	2.21±0.05	7.21±2.1
≈0% α-MyHC myocyte fragments (n=6)	176±56	26±5	2.24±0.04	2.21±0.02	9.99±3.9

Values are mean±SD.

Data Analysis and Statistics

Myocyte preparation length traces, force-velocity curves, and power-load curves were analyzed as previously described.[13] Unpaired Student *t* tests were used to determine differences among myocyte fragment dimensions, force-velocity characteristics, and peak power between myocytes expressing ≈12% α-MyHC and ≈0% α-MyHC. $P<0.05$ was chosen as indicating significance between groups. All values are expressed as mean±SD.

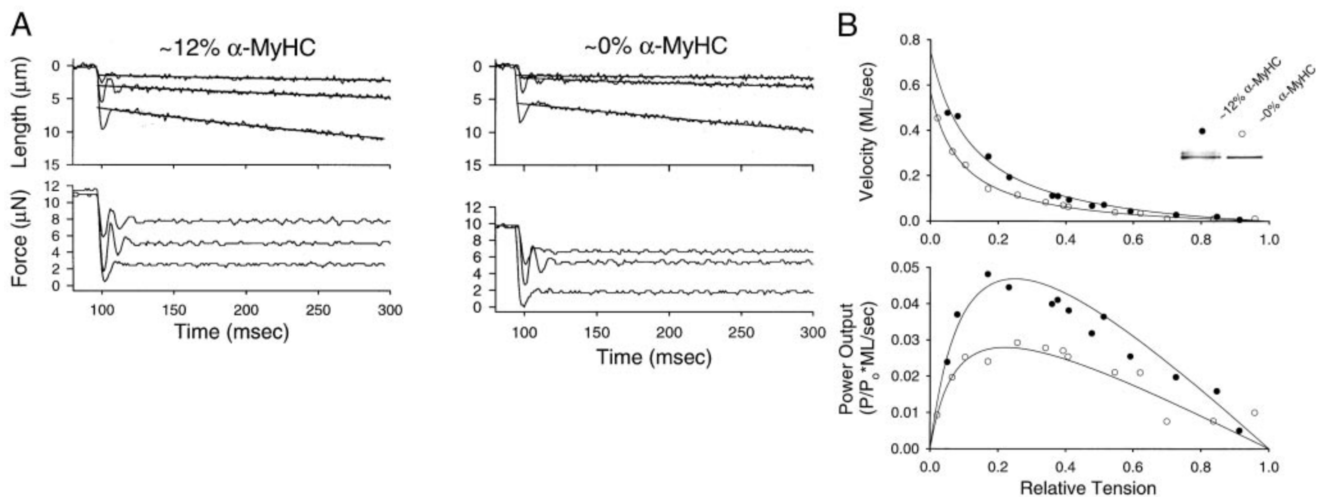
Results

The Figure shows representative length traces, force traces, normalized force-velocity, and power-load curves, as well as the corresponding silver-stained gels for a myocyte fragment that expressed ≈12% α-MyHC and a myocyte that expressed ≈0% α-MyHC. The myocyte fragment that expressed ≈12% α-MyHC generated ≈65% greater peak normalized power (0.046 versus 0.028 $P/P_o \cdot$ ML/sec) than the myocyte fragment that expressed only β-MyHC.

Force-velocity and power-load data were collected from 6 myocyte fragments that expressed small amounts of α-MyHC (11.7±2.7%) and 6 myocyte fragments that expressed almost exclusively β-MyHC (0.7±1.6% α-MyHC). Mechanical data from these myocyte fragments are presented in Table 2. The expression of ≈12% α-MyHC yielded ≈52% greater peak normalized power output (0.041±0.006 versus 0.027±0.007 $P/P_o \cdot$ ML/sec). Force-velocity relationships for myocyte fragments expressing ≈12% α-MyHC were also significantly less curved (a/P_o=0.14±0.03 for ≈12% α-MyHC versus 0.08±0.04 for ≈0% α-MyHC), and therefore F_{opt} was ≈37% greater (0.26±0.02 versus 0.19±0.04) in ≈12% α-MyHC myocyte fragments.

Discussion

Considerable evidence suggests that MyHC isoform expression is a primary determinant of myocardial performance.[2,6,11,12] The role of MyHC in determining myocardial performance has been underscored by the large number of mutations of the β-MyHC gene that have been described in patients afflicted by hypertrophic cardiomyopathy[14] and dilated cardiomyopathy.[15] More than 50 different mutations have been reported, and most of them reside in the head or motor region of the molecule, specifically in the nucleotide and actin binding domains.[14] Likewise, despite a high degree of amino acid identity between α-MyHC and β-MyHC, there seems to be important sites of difference within regions of the rod, tail-hinge, lever arm, and head region.[3,16] The structural differences between the two molecules are manifested functionally in the myocardium and have led to the hypothesis that the relative expression of the two MyHC isoforms is critical in determining the contractile performance of the heart. The effect of a small amount of relative α-MyHC expression on myocardial function, however, has been elusive. Our results demonstrate that low relative expression of α-MyHC (≈12%) is sufficient to augment skinned myocyte fragment function and further support the hypothesis that the



Effects of small amounts of α-MyHC expression on loaded shortening and power output in skinned rat myocyte fragments. A, Representative length traces during force clamps of a myocyte fragment that expressed ≈12% α-MyHC and a myocyte fragment that expressed ≈0% α-MyHC. B, Normalized force-velocity and power-load relationships obtained during maximal Ca^{2+} activations of the myocyte fragment that expressed ≈12% α-MyHC (●) and the myocyte fragment that expressed ≈0% α-MyHC (○). The gel inset shows the MyHC bands for these 2 myocyte fragments. Loaded shortening velocities were faster and power output was greater over most relative loads in the ≈12% α-MyHC myocyte fragment, with peak normalized power being ≈65% greater in the ≈12% α-MyHC myocyte fragment.

TABLE 2. Force-Velocity and Power Output Characteristics

	Absolute Peak Power, μW/mg	Normalized Peak Power, $P/P_o \cdot$ ML/sec	a/P_o	F_{opt}	V_{max}, ML/sec
≈12% α-MyHC myocyte fragments (n=6)	1.12±0.35*	0.041±0.006*	0.14±0.03*	0.26±0.02*	0.67±0.12
≈0% α-MyHC myocyte fragments (n=6)	0.60±0.22	0.027±0.007	0.08±0.04	0.19±0.04	0.61±0.12

Values are mean±SD. P_o indicates isometric force; P, force during isotonic shortening; P/P_o, relative load; and ML/sec, isotonic shortening velocity in muscle lengths per second.

*$P<0.05$; significant difference versus ≈0% α-MyHC myocytes.

relative expression of the two MyHCs is critical in determining the contractile performance of the myocardium.

The functional differences between myocytes expressing a small amount of α-MyHC and myocytes expressing only β-MyHC reported in the present study may be largely attributed to differences in crossbridge cycling rates. It is well established that α-MyHC exhibits faster actin-activated ATPase activity,[4] faster rates of force development,[11] and increased unloaded shortening velocity.[11] Further, single skinned myocytes that express exclusively α-MyHC generate nearly three times greater peak normalized power than myocytes expressing only β-MyHC.[6] We were interested in whether limited (≈12% or less) expression of α-MyHC would affect cardiac myofibrillar power-generating capacity, especially because recent studies have shown variations in MyHC expression of this range between normal and failing human hearts.[9,10] Power output was greater at all relative loads in myocytes expressing small amounts of α-MyHC and peak normalized power output increased ≈52%. Interestingly, model simulations have also predicted that small amounts of α-MyHC will markedly accelerate the rate of twitch force production.[17] Taken together, these results imply that expression of just small amounts of faster cycling crossbridges (ie, α-MyHC) will have significant functional impact during contractions against loads that the myocardium encounters in vivo. Because stroke volume is largely determined by the rate of loaded myocardial shortening, our results suggest that small relative α-MyHC expression is significant to augment stroke volume. This may be important especially in times of stress such as during exercise, which necessitates cardiac reserve.

Acknowledgments

This work was supported by National Heart, Lung, and Blood Institute Grant HL-57852 (K.S.M.) and a predoctoral fellowship granted by the Heartland Affiliate of the American Heart Association (T.J.H.).

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KEY WORDS: myosin heavy chain ■ cardiac myocytes ■ power output ■ sarcomeric proteins ■ myocardial performance