Effects of Total Replacement of Atrial Myosin Light Chain-2 With the Ventricular Isoform in Atrial Myocytes of Transgenic Mice

Corinn M. Pawloski-Dahm, PhD; Guojie Song, MD, PhD; Darryl L. Kirkpatrick, BS; Joe Palermo, PhD; James Gulick, MS; Gerald W. Dorn II, MD; Jeffrey Robbins, PhD; Richard A. Walsh, MD

Background—In contrast to their well-known and critical role in excitation-contraction coupling of vascular smooth muscle, the effects of the myosin light chains on cardiomyocyte mechanics are poorly understood. Accordingly, we designed the present experiment to define the cardiac chamber–specific functional effects of the ventricular isoform of the regulatory myosin light chain (MLC2v).

Methods and Results—Postnatal transgenic cardiac-specific overexpression of MLC2v was achieved by use of the α-myosin heavy chain promoter. Enzymatically disaggregated atrial and ventricular mouse myocytes were field-stimulated at multiple frequencies, and mechanical properties and calcium kinetics were studied by use of video edge detection and FURA 2-AM, respectively. MLC2v overexpression resulted in complete replacement of the atrial with the ventricular isoform of the regulatory myosin light chain at the steady-state mRNA and protein levels in the atria of transgenic mice. Mechanical properties of transgenic atrial myocytes were enhanced to the level of ventricular myocytes of control animals in association with modest decreases in the amplitude of the calcium transient.

Conclusions—MLC2v modulates chamber-specific contractility by enhanced calcium sensitivity and/or improved cross-bridge cycling of the thin and thick filaments of the cardiomyocyte. (Circulation. 1998;97:1508-1513.)

Key Words: genes • myocytes • myosin

It has long been recognized that contraction of the heart is dependent on the force generated by the interactions between the thick and thin filaments of the cardiac sarcomere. Detailed structural studies have demonstrated that force generation in muscle cells is due to cross-bridge cycling between thin-filament actin and thick-filament myosin prompted by ATP hydrolysis.1–3 Myosin is a hexameric molecule composed of two heavy-chain proteins and two pairs of distinct light-chain proteins. There are two classes of MLCs, and one of each is associated with each heavy chain. Both types of MLC are usually encoded by a multiple gene family, giving rise to a number of isoforms in each class that are regulated in a tissue- and cardiac chamber–specific fashion during development and pathological processes. The two heavy chains each form a head region that contains the ATP binding site and an α-helical tail region, whereas MLC1 and MLC2 are situated in the neck region of the myosin heavy chain proteins. Data from more recent structural studies provide evidence that it is small conformational changes in the light chain binding regions that are responsible for the actual movement of smooth muscle myosin with the release of ADP.1–3 Although the structural relationship of the MLC proteins to the contractile apparatus of muscle is becoming clearer, the functional role of the light chain proteins and their isoforms in muscle contraction is incompletely understood. In particular, less is known regarding the role of the cardiac MLCs in excitation-contraction coupling than is the case for these proteins in skeletal and smooth muscle.

MLC2 is also called the regulatory light chain, because phosphorylation of this protein controls contraction in smooth muscle. In skeletal muscle, phosphorylation of MLC2 is thought to have a modulatory role in both the rate and magnitude of force generation.4–9 In contrast, little is known about the role of MLC2 and its phosphorylation in cardiac myocyte shortening, although it has been demonstrated that cardiac MLC2 phosphorylation produces a dramatic increase in the sensitivity of tension development to increasing extracellular Ca 2+ concentrations.10 In addition, Damron et al11 reported that increased phosphorylation of MLC2 with endothelin or arachidonic acid treatment produced a positive inotropic effect, which they interpreted as being consistent with an increase in calcium sensitivity; however, calcium transient data were not reported in that study. Thus, the role
of MLC2 in a phosphorylated or dephosphorylated state in cardiac muscle contraction is unclear. The cardiac regulatory MLCs exist in chamber-specific isoforms for the atria (MLC2a) and ventricles (MLC2v). Although these isoforms arise from distinct genes and are altered in pathological states, assigning different functional roles for the encoded proteins has not been possible.

Recently, Palermo et al.12 produced a transgenic mouse with cardiac-specific postnatal overexpression of MLC2v. Although large increases in mRNA for MLC2v were seen in both the atria and ventricles of the transgenic mouse heart, no difference was observed in the total MLC2 protein in either compartment. However, ectopic expression of MLC2v in the atria resulted in the total replacement of the atrial isoform of MLC2 by MLC2v. A similar phenomenon has been reported for this laboratory unless otherwise noted.18–20 Transgenic mice produced a transgenic mouse with postnatal cardiac-specific overexpression of MLC2v.

Selected Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>MLC</td>
<td>myosin light chain</td>
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<tr>
<td>MLC1</td>
<td>essential myosin light chain</td>
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<tr>
<td>MLC2</td>
<td>regulatory myosin light chain</td>
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<tr>
<td>MLC2a</td>
<td>atrial isoform of regulatory myosin light chain</td>
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<tr>
<td>MLC2v</td>
<td>ventricular isoform of regulatory myosin light chain</td>
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Mechanical Properties

For measurements of morphological and mechanical properties of isolated myocytes, cells were placed in a well on the stage of an inverted microscope and were perfused continuously with oxygenated physiological buffer. Two platinum electrodes connected to a Grass model S9 stimulator were placed on either side of an identified healthy-appearing, rod-shaped myocyte with clearly visible striations and no evidence of blebbing. Myocytes were field-stimulated at varying frequencies (0.25, 0.5, and 1.0 Hz; 5-ms pulse duration) for at least 40 seconds per pacing rate. Cell images were acquired continuously through a charge-coupled device (model GP-CD60) and recorded on videotape. With a video motion edge detector (Crescent Electronics), these videotaped images were then captured on a Gould chart recorder from which percent shortening (+dL/dt) and relengthening (−dL/dt) were quantified by comparison with a calibrated micrometer on the microscope stage.

Calcium Measurements

Once left ventricular cells were isolated, half were used for mechanical studies and the other half for the cardiac kinetic studies. In contrast, for the assessment of atrial cell function, the entire left atrium was required, so separate mice were used for mechanical and calcium measurements. Disaggregated myocytes were placed in FURA 2-AM (ventricular cells, 7.5 μmol/L) and atrial cells, 2.5 μmol/L) and incubated at 37°C for ~15 minutes in the dark. After FURA loading of cells was completed, the cells were then suspended in physiological buffer as described above. Cytosolic free calcium was measured in mouse myocytes by ratio imaging of 340 to 380 nm fluorescence of FURA 2 (emission wavelength = 510 nm) with a photo scan dual-beam spectrofluorometer (Photon Tech, Inc) coupled to an Olympus IMT-2 UV fluorescent microscope with UV transparent optics. Cells underwent a pacing protocol similar to that performed in the mechanical studies, and baseline and peak intracellular calcium transients were measured in response to changes in stimulation frequencies.

Results

Morphological and Mechanical Properties of Mouse Ventricular Myocytes

Ventricular myocytes isolated from transgenic mice were morphologically indistinct from control ventricular myocytes

Statistical Analyses

At least three cells were examined per mouse, per chamber (atrium and ventricle), and the values were averaged for mechanical parameters and Ca²⁺ kinetics. Statistical analysis is based on the number of animals rather than the number of cells. Data are expressed as mean±SEM and are analyzed by two-way ANOVA followed by the Student-Newman-Keuls test for individual post hoc comparisons. Morphological data were analyzed by unpaired t test. If data were not normally distributed or failed equal variance tests after log₁₀ transformations, they were analyzed by nonparametric statistics (ie, either Kruskal-Wallis for ANOVA designs or Mann-Whitney rank sum test for comparison between two groups of data). A value of P<.05 was set as the criteria for statistical significance.
Representative analog tracings of myocyte shortening (Fig 1) demonstrate that the extent of ventricular myocyte shortening measured in these cells was not different between transgenic and control mice at any of the three stimulation frequencies. However, the rates of shortening ($+dL/dt$) and relengthening ($-dL/dt$) produced by electrical stimulation of the myocytes at each pacing rate were diminished in the transgenic ventricular myocytes compared with control cells. These findings are confirmed by the composite data as illustrated in Table 1 and Fig 2. Thus, with no detectable difference in total MLC2v protein levels between mice, transgenic ventricular myocytes demonstrated similarities in percent shortening but depressed rates of contraction and relaxation.

### Morphological and Mechanical Properties of Mouse Atrial Myocytes

Morphological and mechanical properties of atrial myocytes isolated and studied from transgenic mice with cardiac-specific ectopic replacement of the atrial with the ventricular isoform of MLC2 in the heart were significantly shorter than atrial myocytes similarly isolated from nontransgenic littermates (Table 1). Compared with ventricular myocytes, atrial myocytes were shorter and thinner in both groups (Table 1). However, no differences were seen in cell width-to-length ratios between either atrial and ventricular myocytes or atrial cells isolated from control versus those from transgenic mice. When electrically stimulated at incremental pacing frequencies, control atrial myocytes exhibited a much attenuated percent shortening compared with transgenic atrial or control ventricular myocytes (Table 1; Figs 1 and 2). Similarly, rates of shortening and relengthening in control atrial myocytes were much less than those in either transgenic atrial or control ventricular cells (Table 1, Fig 2). These findings were similar at all three stimulation frequencies. Furthermore, comparisons of contractile properties within groups were not significantly different with increasing pacing rates. In contrast to control atrial myocytes, the atrial myocytes isolated from the mice in which MLC2v protein
had completely replaced the atrial isoform of MLC2 demonstrated contractile properties that were similar to those of nontransgenic ventricular myocytes (Fig 1).

Intracellular Ca$^{2+}$ Measurements in Mouse Ventricular Myocytes

The differences in rates of contraction and relaxation between transgenic and control ventricular myocytes could not be explained by differences in intracellular Ca$^{2+}$ kinetics (Table 2). Baseline and peak Ca$^{2+}$ levels obtained during electrical pacing of myocytes were not different between ventricular cells isolated from control and transgenic mice. In addition, the times of 50% (T50) and 80% (T80) Ca$^{2+}$ signal decay were similar between groups. Altering the pacing frequencies affected neither the intergroup group relationships nor intragroup group comparisons of the Ca$^{2+}$ kinetics.

Intracellular Ca$^{2+}$ Measurements in Mouse Atrial Myocytes

Baseline Ca$^{2+}$ levels and Ca$^{2+}$ signal amplitudes for atrial myocytes paced at three pacing rates are shown in Table 2. As was seen with ventricular myocytes isolated from transgenic mice, atrial myocytes from these mice demonstrated baseline Ca$^{2+}$ signals similar to atrial cells taken from nontransgenic mice. The amplitude of the Ca$^{2+}$ signals was slightly but significantly lower in the transgenic atrial myocytes than in control cells. Furthermore, although baseline signals were not different between ventricular and atrial myocytes, the amplitude of the Ca$^{2+}$ signals produced by electrical pacing of the cells was significantly lower in the atrial than in the ventricular myocytes in both groups of mice.

Discussion

The present studies report, for the first time, the mechanical properties and calcium kinetics of atrial myocytes derived from the mouse heart. These data demonstrate that isolated unloaded mouse atrial myocytes contract to a lesser extent and at slower rates than do isolated ventricular cells. However, total replacement of the atrial isoform of MLC2 by the ventricular isoform in the atria of the mouse results in atrial cells that contract and relax at greater rates and to a greater extent than do isolated atrial cells from control mice. In fact, the transgenic atrial myocytes demonstrate contractile properties similar to normal ventricular cells. These studies also

![Figure 2. Group data for mechanical properties of ventricular (vent) and atrial myocytes isolated from control (c) and transgenic (tg) mice with cardiac-specific overexpression of MLC2v. A, Extent of cell shortening (% Shortening). B, Rate of shortening (+dL/dt, μm/s). C, Rate of relengthening (−dL/dt, μm/s). Data are mean±SEM. *P<.05 vs c; #P<.05 vs vent.](http://circ.ahajournals.org/)

<table>
<thead>
<tr>
<th>TABLE 2. Calcium Kinetics of Isolated Ventricular and Atrial Myocytes With Changes in Pacing Rates</th>
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<tr>
<td>Pacing Rate, bpm</td>
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<td>Ventricular Myocytes</td>
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<td>0.64±0.12</td>
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<td>0.60±0.08</td>
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<tr>
<td>60</td>
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<tr>
<td>0.46±0.05†</td>
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<tr>
<td>Atrial Myocytes</td>
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<tr>
<td>15</td>
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<tr>
<td>0.78±0.10†</td>
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<tr>
<td>0.75±0.08†</td>
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<tr>
<td>0.53±0.09†</td>
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<td>C</td>
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<td>Tg</td>
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<td>0.64±0.12†</td>
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C indicates control, n=4; Tg, Transgenic, n=4. Values are mean±SEM. *P<.05 vs C. †P<.05 vs ventricular myocytes.
The atrial-to-ventricular switch of MLC2 occurs during postnatal development of the ventricle in response to the accompanying changes in intra-atrial pressures. On the basis of the present studies, the postnatal MLC isoform switch in the left ventricle of the neonate may facilitate ejection against the increased systemic arterial pressure that occurs at parturition. In addition, several studies have demonstrated expression of the ventricular isoform of MLC2 in the atria of hypothyroid hearts of both humans and experimental animals in response to pathological conditions. Wanker et al found MLC2v in atrial samples from patients with a variety of cardiomyopathies, and the level of ventricular isoform expression correlated with the severity of heart failure. Likewise, Cummins reported that the degree of pressure-overload hypertrophy in humans is the most significant factor influencing ventricular MLC2v isoform expression in the human atria. Just as in the developing neonatal ventricle, it was hypothesized that the changes in chamber pressures were responsible for this isoform switch in myopathic atria. However, Kumar et al demonstrated that the atria from the spontaneously hypertensive rat had greater levels of MLC2v mRNA expression than did atria from age-matched normotensive Wistar-Kyoto rats that preceded the development of both hypertension and cardiac hypertrophy. These studies are inconsistent with the hypothesis that the atrial-to-ventricular switch of MLC2v in the atria occurs solely as a result of hemodynamic factors. Therefore, although there may be some relationship between cardiomyopathy and the atrial-to-ventricular MLC2 switch in the atria, it remains unknown whether this phenomenon plays a role in or is a consequence of the pathological condition. Furthermore, it is unknown how this switch affects atrial as well as ventricular function. Data from the present studies suggest that enhanced atrial expression of MLC2v in the diseased heart may be a compensatory mechanism to maintain and enhance the left atrial contribution to ventricular filling.

The present studies demonstrate that mechanical properties of mouse atrial myocytes that ectopically express MLC2v are similar to ventricular myocytes. It appears that the only biochemical difference between the nontransgenic and transgenic left atria is the total replacement of the atrial MLC2 isoform by the ventricular MLC2 isoform. Transgenic atrial MLC2v has a higher basal phosphorylation level than MLC2v in the ventricles. In addition, there was also no difference in the degree of phosphorylation between the wild-type and transgenic atrial MLC2. Thus, the altered mechanical properties of the transgenic atrial myocytes appear to be unrelated to any change in the level of phosphorylation brought about by the atrial-to-ventricular MLC2 isoform switch. In addition, there are no differences in the myosin heavy chain isoforms, the major determinant of myosin ATPase activity, in the calcium-cycling proteins (the sarcoplasmic reticulum ATPase and phospholamban) or in α-actin isoform composition between the atria of wild-type and transgenic mice. We therefore consider it a reasonable hypothesis that the morphological differences (shorter atrial myocytes) as well as mechanical differences (greater percent shortening and faster rates of shortening and relengthening) in the transgenic atrial myocytes are a direct consequence of the regulatory MLC isoform replacement. The cell length differences could not have been observed or predicted from previously performed in vitro motility assays and are difficult to explain on the basis of current understanding of structural relationships of myosins in cardiac muscle. However, these data imply that MLC2 plays an important role in determining the contractile properties of the cardiac chambers.

Depressed mechanical function of nontransgenic atrial myocytes compared with ventricular cells might be predicted from the calcium kinetic studies. Increased intracellular Ca2+ levels and lower T50 and T80 in the ventricular myocytes compared with atrial myocytes support the mechanical data that unloaded ventricular cells contract faster and to a greater extent than do atrial cells. However, compared with nontransgenic atrial cells, transgenic atrial myocytes exhibit slightly lower electrically stimulated increases in intracellular Ca2+, with no differences in T50 or T80. Thus, the increase in atrial myocyte contractility in transgenic mice compared with wild-type atrial myocyte shortening resulting from replacement of the atrial with the ventricular isoform of the regulatory MLC cannot be explained on the basis of altered calcium kinetics. It appears that the ventricular MLC isoform switch enhances atrial cardiomyocyte calcium sensitivity of the myofilament and/or facilitates more effective actin-myosin cross-bridge development and cycling.

On the basis of the biochemical analyses of the transgenic ventricles (ie, no difference in MLC2v protein expression between control and transgenic), no difference in mechanical properties would be predicted between the left ventricular myocytes of these groups. There was no difference in the gross morphology of the ventricular myocytes. However, modest but statistically significant slower rates of shortening and relengthening of transgenic ventricular myocytes were observed. The reasons for depressed mechanical function in the transgenic ventricular myocytes are not readily apparent. These differences cannot be explained on the basis of altered phosphorylation status, myosin heavy chain isoform composition, calcium-cycling proteins, or α-actin isoform composition between wild-type and transgenic ventricles. It has been postulated that heart rate plays a role in the activity of MLC kinase, the enzyme responsible for phosphorylation of MLC2. However, neither conscious heart rates nor phosphorylation status differed between wild-type and transgenic mice. One possibility for the mildly depressed function of the transgenic ventricular myocytes is that there may be other biochemical changes not yet established, either as a result of the enhanced mechanical properties of the atria or simply endogenous to this transgenic line. What is clear from the present experiments is that the depressed mechanical proper-
ties of the transgenic ventricular myocytes are not due to changes in intracellular calcium handling.

In conclusion, these data demonstrate that total replacement of the atrial isoform of MLC2 with the ventricular isoform in the left atrium of the mouse results in atrial myocytes with mechanical and morphological properties similar to those of ventricular cells, despite modestly diminished intracellular calcium transients. These studies suggest that MLC2 isoforms in cardiac tissue are central to the differential contractility of compartmentalized heart muscle. Further study of these transgenic mice might lead to an even greater understanding of the role of MLC2v in cardiac muscle contraction under normal conditions, as well as the role of its expression in the atria observed in cardiomyopathies.

Acknowledgment

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References

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