Modulation of Crossbridge Kinetics by Myosin Isoenzymes in Skinned Human Heart Fibers

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Skinned fibers from the normal human heart with the β-myosin heavy chain (ventricular fibers) revealed both a higher force generation per cross section and a higher Ca\(^{2+}\) sensitivity than skinned fibers with the α-myosin heavy chain (atrial fibers). The relation between isometric ATPase activity and isometric tension of atrial fibers was higher than that of ventricular fibers. Since the ATPase–tension relation equals the rate constant for the transition from force-generating into non–force-generating crossbridge states (g\_app\), myosin heavy chain isoenzymes seem to have different crossbridge turnover kinetics. Modulation of g\_app by myosin heavy chain isoenzymes could explain the different contractile behavior of atrial and ventricular fibers. g\_app was independent of Ca\(^{2+}\). (Circulation Research 1991;68:614–618)

In the steady state, isometric force (F) of a muscle fiber can be described by the equation:\(^1\):

\[
F = F' n_{tot} f_{app}/(f_{app} + g_{app})
\]

(1)

where F’ is the force generation per crossbridge, \(n_{tot}\) is the total amount of cycling crossbridges per half sarcomere, \(f_{app}\) is the rate constant for the transition from non–force-generating into force-generating crossbridge states, and \(g_{app}\) is the rate constant for the transition of force-generating into non–force-generating crossbridge states. The expression \(f_{app}/(f_{app} + g_{app})\) represents the fraction of cycling crossbridges in the force-generating states. For activation levels up to approximately 20% of maximal tension, Ca\(^{2+}\) seems to enhance \(n_{tot}\) (recruitment\(^2\)). At higher activation levels, however, Ca\(^{2+}\) increases tension by changing crossbridge turnover kinetics,\(^3\) namely by increasing \(f_{app}\).\(^1\) Similarly, phosphorylation of myosin phosphorylatable light chains increases \(f_{app}\) and, therefore, isometric tension.\(^4-6\) The level of isometric ATPase activity during isometric steady-state tension can be described by the equation:\(^1\):

\[
\text{ATPase} = n_{tot} s f_{app} g_{app}/(f_{app} + g_{app})
\]

(2)

where s is the number of half sarcomeres. Tension cost is obtained by dividing Equation 2 by Equation 1, which yields \(g_{app}/F' s/F'\). Since under isometric steady-state conditions s is constant and F’ of α- and β-myosin heavy chain (MHC) is considered to be equal (see “Discussion”), the relation of ATPase activity and tension is, therefore, proportional to g\_app. From Equation 1, it can then easily be derived that isometric tension as well as Ca\(^{2+}\) sensitivity of muscle fibers can be modulated by changing g\_app; an increasing value of g\_app would predict a decrease in force and Ca\(^{2+}\) sensitivity, whereas a decreasing value would cause the opposite effect. This prediction is illustrated as a simple simulation in Figure 1: n (the fraction of crossbridges in the force-generating state \(f_{app}/(f_{app} + g_{app})\)) is plotted against \(f_{app}\) (arbitrarily taking maximum \(f_{app}\) as 5 s\(^{-1}\)) at two different values of g\_app (namely, 1 s\(^{-1}\) and 2 s\(^{-1}\)); this relation between force (proportional to n) and Ca\(^{2+}\) (which determines \(f_{app}\)) at high g\_app value is shifted to the left. Interestingly, Ca\(^{2+}\) sensitivity of skinned atrial fibers rich in α-MHC and atrial-specific myosin light chains (A-MLCs) is lower than that of ventricular fibers with the β-MHC and ventricular-specific MLC (V-MLC) of human and pig hearts.\(^7\) We tested the hypothesis that the different Ca\(^{2+}\) sensitivity of skinned atrial and ventricular fibers is indeed associated with a different value for g\_app. Therefore, both isometric ATPase activity and isometric tension of skinned atrial and ventricular fibers of a normal human heart were determined to obtain the relation between ATPase and force at different Ca\(^{2+}\) concentrations. To avoid influences of different myosin P light chain phosphorylation levels, which affect Ca\(^{2+}\) sensitivi-
activity obtained under relaxation conditions. Isometric tension of fibers was determined by an optoelectronic force transducer system. Relaxation solution contained (mM) imidazole 20, Na₂ATP 10, NaN₃ 5, EGTA 5, MgCl₂ 12.5, phospho(enol)pyruvate 5, NADH 0.6, and P₁,P₃-di(adenosine-5')pentaphosphate (myokinase inhibitor) 0.2, together with 100 units/ml pyruvate kinase and 125 units/ml lactate dehydrogenase, pH 7.0 at 25°C. The contraction solution contained CaEGTA (5 mM) instead of EGTA. Free Ca²⁺ concentration was determined by calculator programs designed for experiments in skinned muscle cells.¹²

Statistics

Values are expressed as mean±SEM. Statistical comparisons were performed by Student’s t test. pCa–tension relations were fitted by the Hill equation¹³:

\[
Y = \frac{[\text{Ca}^{2+}]^n}{[\text{pCa}_{50}]} + [\text{Ca}^{2+}]^H
\]

where Y is the fractional force, pCa₅₀ is the Ca²⁺ concentration giving half-maximal activation, and H is an index of cooperativity.

Results

Results of simultaneous measurements of isometric ATPase and tension are shown in Figure 2, left panel. In these original experiments, force generated at maximal Ca²⁺ activation (pCa 4.3) by atrial and ventricular fibers was 1,193 mg/mm² and 2,774 mg/mm², respectively. ATPase activity of the same fibers was 76.4 μM NADH/(s/mm²) and 50.62 μM NADH/(s/mm²), respectively. On average, force generated by atrial and ventricular skinned fibers was 1,430 mg/mm² and 2,150 mg/mm², respectively (means of six fibers each; SEM<10%) (Figure 3). Thus, the ATPase–force relation of atrial fibers was more than twice that of ventricular fibers (Figure 2, right panel). According to the model of Brenner,¹ the ATPase–force relation is proportional to g₉app Atrial-specific myosin (α-MHC and A-MLC), therefore, seems to have a higher rate constant for the transition of crossbridges from force-generating into non–force-generating states than ventricular myosin (β-MHC and V-MLC). The ATPase–force relation of both atrial and ventricular fibers was independent of Ca²⁺ (Figure 2, right panel).

Since force of a muscle depends on crossbridge kinetics, tension generation and Ca²⁺ sensitivity of atrial fibers (high g₉app) should be lower than that of ventricular fibers (low g₉app) (see also Equation 1). To test this hypothesis, we determined the Ca²⁺-dependent isometric tension of skinned atrial and ventricular fibers per cross section (Figure 3). At all Ca²⁺ concentrations, ventricular fibers generated more tension than atrial fibers (see also Figure 2, left panel). Although this behavior can be predicted from Equation 1 and from the results shown in Figure 2, right panel, different amounts of connective tissue,
contractile material, and values for \( F' \) (force per crossbridge) could also account for the different tension outputs of atrial and ventricular fibers. If, however, isometric tension is normalized to maximal force obtained at full \( \text{Ca}^{2+} \) activation (\( \text{Ca}^{2+} \) sensitivity), atrial fibers were less sensitive to \( \text{Ca}^{2+} \) than ventricular fibers (Figure 4). \( \text{pCa}_{50p} \), calculated according to the Hill equation,\(^{13} \) was 5.17 and 5.25 for atrial and ventricular fibers, respectively (\( p<0.05 \)). According to the crossbridge model presented at the beginning of this article, this observation is independent from the total amount of contractile material and from different values for \( F' \) of atrial and ventricular myosin and is in agreement with different crossbridge kinetics of cardiac myosin isoenzymes (variation of \( g_{\text{app}} \)).

**Figure 2.** Left panel: Original recordings of the simultaneous measurements of ATPase activity (decrease of NADH fluorescence; upper tracings) and isometric tension (lower tracings) of an atrial and a ventricular fiber of the normal human myocardium. The time-dependent decrease of NADH fluorescence was used as a measure for ATPase activity. Downward deflections in the tension recordings are due to solution changes. Right panel: The isometric ATPase–tension relation of skinned atrial (\( \alpha \)-myosin heavy chain [●]) and ventricular (\( \beta \)-myosin heavy chain [○]) fibers of the normal human heart (two fibers each) is plotted versus free \( \text{Ca}^{2+} \) (given as pCa). ATPase activity is expressed as \( \mu \text{M NADH/(s-mm)}^2 \) (decrease of NADH concentration per time per fiber volume, where \( s \) is the number of half sarcomeres). Force is expressed as mg/mm\(^2 \) (force per fiber cross section). The ATPase–tension relation gives the dimension \( \mu \text{M NADH/(s-mm-mg)} \).

**Figure 3.** Graph showing isometric tension generation per cross section (in mN/mm\(^2 \)) of skinned atrial (●) and ventricular (○) fibers of the normal human (H.) heart at different \( \text{Ca}^{2+} \) concentrations (in pCa). Values are mean±SEM of six fibers each.

**Figure 4.** Graph showing \( \text{Ca}^{2+} \)-tension relation of skinned atrial (●) and ventricular (○) fibers of the normal human (H.) heart at different \( \text{Ca}^{2+} \) concentrations. Isometric tension of the fibers from Figure 3 is expressed as percentage of maximal tension obtained at full \( \text{Ca}^{2+} \) activation (pCa 4.5). Values are mean±SEM of six different fibers each.
Discussion

The major finding of the present study is that human cardiac fibers with different myosin isoenzymes had different values for the relation between isometric ATPase and tension. As assumed in the crossbridge model of Brenner,1 this relation is proportional to \( \gamma_{\text{app}} \). Myosin crossbridges that are present in atrial and ventricular fibers thus appear to have different turnover kinetics. This seems to be reasonable since atrial and ventricular fibers of normal humans are composed exclusively of \( \alpha \)-MHC and \( \beta \)-MHC, respectively, with different sets of MLCs.14–16 Similar to skinned skeletal muscle fibers, \( \gamma_{\text{app}} \) of both cardiac myosin isoenzymes was independent of \( \text{Ca}^{2+} \). Strictly, the isometric ATPase/tension ratio equals \( \gamma_{\text{app}}/F' \). Under isometric conditions, \( s \) is constant. It cannot be ruled out that atrial and ventricular myosin isoenzymes have different values of \( F' \). However, different values of \( F' \) would change absolute tension at a given \( \text{Ca}^{2+} \) concentration but not the normalized tension/\( \text{Ca}^{2+} \) ratio (\( \gamma_{\text{app}} \) sensitivity). According to Equation 1 (also see Figure 1), different values for \( \gamma_{\text{app}} \) of atrial- and ventricular-specific myosin isoenzymes could sufficiently explain both the higher tension development per cross section and sensitivity of the \( \text{Ca}^{2+} \)-tension relation of ventricular compared with atrial skinned fibers.

The positive correlation between the amount of \( \alpha \)-MHC present in the fibers and the maximal shortening velocity of muscle fibers17,18 suggests different crossbridge kinetics of MHC isoenzymes under both isometric and isotonic conditions. Maximum shortening velocity, however, is a very special parameter for evaluating muscle contractility, since it mainly depends on the detachment rate constant of crossbridges producing negative force (\( g_2 \) in Huxley’s theory19). Nevertheless, it appears as if \( \alpha \)-MHCs transferred with a higher rate from force-generating into non–force-generating states than \( \beta \)-MHCs in positions producing both positive and negative tension. Thus, MHC isoenzymes determine energetic and contractile behaviors of a muscle fiber. The increased economy of tension development of intact papillary muscles with predominantly \( \beta \)-MHC20,21 may be explained by this mechanism. Whether the different sets of MLCs of atrial and ventricular myosin affect crossbridge kinetics cannot be decided and needs further investigation. However, it is already shown that economy of tension development may be modulated by MHC isoenzymes alone.20,21 Different regulatory proteins present on the thin filament of atrial and ventricular sarcomeres might also influence \( \text{Ca}^{2+} \) sensitivity of both fiber types. Yet, no differences of troponin I or tropomyosin subunits have been detected in the atrium and ventricle of adults.22 Results concerning different troponin T subunits of adults are controversial (compare Reference 23 with Reference 24). We suggest, therefore, that the isometric contraction of the cardiac muscle could be modulated by differential expression of MHC isoenzymes having different crossbridge turnover kinetics (\( \gamma_{\text{app}} \)).

The significance of regulation of muscle contraction by modulation of crossbridge kinetics is furthermore documented by the effects of \( \text{Ca}^{2+} \) and phosphorylation of the myosin \( P \) light chain6–8,26: they enhance \( f_{\text{app}} \), thereby increasing isometric tension and \( \text{Ca}^{2+} \) sensitivity (see Equation 1).

It cannot be ruled out, however, that variation of \( n_{\text{app}} \) with \( \text{Ca}^{2+} \) (recruitment), especially at low activation levels, affects the force–\( \text{Ca}^{2+} \) relation of atrial and ventricular fibers, too: at low \( \text{Ca}^{2+} \) the difference in relative force of ventricular and atrial fibers is higher than could be predicted from a variation of \( \gamma_{\text{app}} \) only (compare Figure 4 with Figure 1).

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References

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