Strong Binding of Myosin Modulates Length-Dependent Ca\textsuperscript{2+} Activation of Rat Ventricular Myocytes

Daniel P. Fitzsimons, Richard L. Moss

Abstract—Reductions in sarcomere length (SL) and concomitant increases in interfilament lattice spacing have been shown to decrease the Ca\textsuperscript{2+} sensitivity of tension in myocardium. We tested the idea that increased lattice spacing influences the SL dependence of isometric tension by reducing the probability of strong interactions of myosin crossbridges with actin, thereby decreasing cooperative activation of the thin filament. Single ventricular myocytes were isolated by enzymatic digestion of rat hearts and were subsequently rapidly skinned. Maximal tension and Ca\textsuperscript{2+} sensitivity of tension (ie, pCa\textsubscript{50}) were measured in the absence and presence of N-ethylmaleimide–modified myosin subfragment 1 (NEM-S1) at both short and long SLs. NEM-S1, a strong-binding non–tension-generating derivative of the myosin head, was applied to single skinned myocytes to cooperatively promote strong binding of endogenous myosin crossbridges. Compared with control myocytes at SL of \(\approx 1.90\ \mu\)m, application of NEM-S1 markedly increased submaximal Ca\textsuperscript{2+}-activated tensions and thereby increased Ca\textsuperscript{2+} sensitivity; ie, pCa\textsubscript{50} increased from 5.40±0.02 to 5.52±0.02 pCa units in the presence of NEM-S1. Furthermore, NEM-S1 treatment reversibly eliminated the SL dependence of the Ca\textsuperscript{2+} sensitivity of tension, in that the \(\Delta pCa_{50}\) between short and long lengths was 0.02±0.01 pCa units in the presence of NEM-S1 compared with a \(\Delta pCa_{50}\) of 0.10±0.01 pCa units in control myocytes. From these results we conclude that the decrease in the Ca\textsuperscript{2+} sensitivity of tension at short SL results predominantly from decreased cooperative activation of the thin filament due to reductions in the number of strong-binding crossbridges. (Circ Res. 1998;83:602-607.)

Key Words: Ca\textsuperscript{2+} sensitivity ■ muscle length ■ ventricular myocyte

Within normal physiological limits, alterations in ventricular end-diastolic volume result in marked changes in cardiac output. Since the heart normally operates on the positive slope of the pressure-volume relationship, increases in end-diastolic volume induce the heart to increase either stroke volume, ejection pressure, or both in a way that closely matches myocardial work to the load on the heart. This intrinsic ability of the heart to alter ventricular peak systolic pressure due to beat-to-beat variations in end-diastolic volume constitutes the basis for the well-known Frank-Starling relationship. The present study was performed to investigate molecular mechanisms underlying this relationship.

Twitch tension and Ca\textsuperscript{2+} sensitivity of tension (ie, pCa\textsubscript{50}) in cardiac muscle preparations are known to decrease as sarcomere length (SL) is shortened within its working range (ie, from \(\approx 2.30\ \mu\)m to \(\approx 1.80\ \mu\)m).\textsuperscript{1,2} The regulation of myocardial contraction requires the binding of Ca\textsuperscript{2+} to a low-affinity site on troponin C, which permits strong interactions of myosin crossbridges with actin.\textsuperscript{3} However, experimental evidence has shown that Ca\textsuperscript{2+} alone is unable to fully activate the thin filament: complete activation, in terms of force and the kinetics of force development, results from synergistic actions of Ca\textsuperscript{2+} and strong-binding myosin crossbridges.\textsuperscript{4-8}

Although a number of possible mechanisms have been proposed to account for the SL dependence of myocardial tension generation,\textsuperscript{1,2} evidence suggests that the SL dependence is primarily mediated by changes in interfilament lattice spacing.\textsuperscript{9-12} As SL is reduced, interfilament lattice spacing increases, causing greater lateral separation between the thick and thin filaments.\textsuperscript{13,14} One likely consequence of increased lattice spacing is a reduced probability that myosin crossbridges will bind to actin and generate force. This would depress force generation at short SL directly as a result of fewer strongly bound crossbridges and perhaps indirectly because of reduced cooperative activation of the thin filament by strong-binding crossbridges.

The specific aim of the present study was to investigate the hypothesis that lower tensions at short SL arise from reduced cooperative activation of the thin filament by strongly bound crossbridges. To test this idea, N-ethylmaleimide–modified myosin subfragment 1 (NEM-S1), a strong-binding non–tension-generating derivative of the crossbridge head,\textsuperscript{3} was applied to single skinned ventricular myocytes to cooperatively promote strong binding of endogenous myosin crossbridges. If the lower Ca\textsuperscript{2+} sensitivity of tension at short SL is due to reduced cooperative activation of the thin filament, the Ca\textsuperscript{2+} sensitivity of tension should be similar at both short (ie, \(\approx 1.90\ \mu\)m) and long (ie, \(\approx 2.25\ \mu\)m) SLs when NEM-S1 is present in the activating
solutions. In the present study, we report that NEM-S1 treatment nearly eliminates the SL dependence of the Ca\textsuperscript{2+} sensitivity of tension normally observed in ventricular myocytes. NEM-S1 increased submaximal Ca\textsuperscript{2+}-activated tensions and increased Ca\textsuperscript{2+} sensitivity of tension, especially at short SL. A preliminary report of the present study was presented at the 1997 Biophysical Society Meeting.\textsuperscript{15}

Materials and Methods

Solutions

Compositions of perfusate used in the preparation of ventricular myocytes and relaxing and activating solutions used in the mechanical measurements were as follows (mmol/L): (1) Ca\textsuperscript{2+} Ringer’s solution: NaCl 118, HEPES 25, glucose 11, pyruvate 5, KCl 4.8, NaH\textsubscript{2}PO\textsubscript{4} 2.4, MgCl\textsubscript{2} 1.2, and CaCl\textsubscript{2} 1, pH 7.4 at 25°C; (2) relaxing solution: KCl 100, imidazole 20, MgATP 4, EGTA 2, and free Mg\textsuperscript{2+} 1, pH 7.0 at 25°C; and (3) activating solutions: KCl 79.2, imidazole 14.5, EGTA 7, MgCl\textsubscript{2} 5.3, ATP 4.74, and free Ca\textsuperscript{2+} ranging from 1 mmol/L (ie, pCa 9.0) to 32 mmol/L (ie, pCa 4.5), pH 7.0 at 15°C with anionic strength of 180 mmol/L. The computer program of Fabiato\textsuperscript{16} (1988) was used to calculate the final concentration of each metal, ligand, and metal-ligand complex based on stability constants described by Godt and Lindley\textsuperscript{17} (1982).

Single Skinned Ventricular Myocytes

Mechanical measurements were performed on single skinned ventricular myocytes from 2- to 3-month-old female Sprague-Dawley rats weighing 200 to 224 g. Single ventricular myocytes were obtained by enzymatic digestion, as described previously, with all solutions at 37°C and bubbled with 100% O\textsubscript{2}. Briefly, the heart was rapidly removed from an anesthetized rat and placed in warm (37°C) NaCl 118, HEPES 25, glucose 11, pyruvate 5, KCl 4.8, NaH\textsubscript{2}PO\textsubscript{4} 2.4, MgCl\textsubscript{2} 1.2, and CaCl\textsubscript{2} 1, pH 7.4 at 25°C; and (2) relaxing solution: NaCl 118, HEPES 25, glucose 11, pyruvate 5, KCl 4.8, NaH\textsubscript{2}PO\textsubscript{4} 2.4, MgCl\textsubscript{2} 1.2, and CaCl\textsubscript{2} 1, pH 7.4 at 25°C; and (3) activating solutions: KCl 79.2, imidazole 14.5, EGTA 7, MgCl\textsubscript{2} 5.3, ATP 4.74, and free Ca\textsuperscript{2+} ranging from 1 mmol/L (ie, pCa 9.0) to 32 mmol/L (ie, pCa 4.5), pH 7.0 at 15°C with anionic strength of 180 mmol/L. The computer program of Fabiato\textsuperscript{16} (1988) was used to calculate the final concentration of each metal, ligand, and metal-ligand complex based on stability constants described by Godt and Lindley\textsuperscript{17} (1982).

Experimental Apparatus and Myocyte Attachment

An experimental apparatus similar to one described previously\textsuperscript{18} was used to attach and record the activation and relaxation of single skinned ventricular myocytes. The entire experimental apparatus was mounted on a temperature-controlled Langendorff coronary perfusion apparatus (Instrument) and a force transducer (model 403, Cambridge Technology; sensitivity, 20 mV/mg; resolution, <50 μg; resonant frequency, 300 Hz). The output signal from the force transducer was amplified 10-fold and then fed to an oscilloscope (model 310, Nicolet Instruments) for storage on magnetic disk and subsequent analysis. The piezoelectric translator was driven by a power supply/amplifier (model BOP 1000 M, Kepco Instruments) to induce length changes for measurements of tension. The force transducer and piezoelectric translator were each mounted on a 3-way micromanipulator (Narishige Instruments) to allow precise positioning of the attached myocyte relative to the objective. SL

and cell width during activation and relaxation were recorded on videotape using a video camera (model WV-BL600, Panasonic) and VHS recorder (model HR-S6600U, JVC). Myocytes were considered too compliant and were discarded from data analysis if SL varied by >0.20 μm between relaxed and maximally activated conditions for a given initial SL.

Approximately 200 μL of a suspension of skinned ventricular myocytes was placed on a glass coverslip. A rod-shaped ventricular myocyte was selected for attachment by scanning the myocyte suspension through the microscope. The tips of the steel pins were coated with adhesive and gently lowered onto both ends of the myocyte. The silicone adhesive was allowed to cure for 45 to 60 minutes, at which time the steel pins were lifted from the surface of the coverslip, thereby suspending the myocyte between the force transducer and translator. The success rate of attachment was nearly 100% with this procedure. Once firmly attached to the steel pins, the myocyte was transferred to a glass-bottomed steel chamber containing a solution of pCa 9.0. SL was adjusted to either 1.90 or 2.25 μm. Temperature of the various pCa solutions was maintained at 15°C with the use of thermoelectric devices placed on one edge of the steel plate.

NEM-S1

Myosin subfragment 1 (S1) was purified from rabbit fast-twitch skeletal muscle and modified with N-ethylmaleimide (NEM) as described by Swartz and Moss.\textsuperscript{3} Addition of NEM-S1 has been shown to significantly increase actomyosin ATPase activity in the presence of Ca\textsuperscript{2+}.\textsuperscript{19,20} However, NEM-S1 exhibits no apparent intrinsic ATPase activity.\textsuperscript{19} It is believed that NEM-S1 acts in a manner similar to unmodified S1 in the absence of ATP by forming rigor bridges with actin. Thus, alkylation of cysteine residues in the myosin crossbridge by NEM modification results in a derivative of myosin that strongly binds regulated thin filaments in the presence of ATP and the absence of Ca\textsuperscript{2+}.\textsuperscript{3} The concentration of NEM-S1 was estimated at 280 nm (with light-scattering correction performed at 320 nm) with the use of a mass absorbivity value of 0.75 and a molecular weight of 118 000 for S1. Before use, the NEM-S1 stock was dialyzed overnight against a solution of 20 mmol/L imidazole, pH 7.0, and 1 mmol/L dithiothreitol and then filtered through a 0.45 μm polyvinylidene difluoride membrane filter (Millipore). A working solution of NEM-S1 was prepared just before use by mixing equal volumes of an NEM-S1 stock solution and a 2× stock of pCa 9.0 solution. NEM-S1 concentration was adjusted to 6 μmol/L by adding the appropriate amount of 1× stock pCa 9.0 solution.

Tension-pCa Relationships

Ca\textsuperscript{2+}-activated isometric tension was measured in solutions of varying pCa (ie, −log[Ca\textsuperscript{2+}]). Maximal tension (P\textsubscript{o}) and Ca\textsuperscript{2+} sensitivity of tension (pCa\textsubscript{o}) were measured as functions of SL between 1.90 and 2.25 μm. Steepness of the tension-pCa relationship for Ca\textsuperscript{2+}-activated tensions of <50% P\textsubscript{o} (ie, the Hill coefficient, n\textsubscript{H}) was quantified from Hill plot transformations of the tension-pCa data. We focused on this portion of the curve, since the tension-pCa relationship is biphasic, with most of the cooperative activation of the thin filament apparent at tensions of <50% P\textsubscript{o} and little additional cooperative activation at tensions above half maximal.\textsuperscript{3}

For control myocytes (n=6), 2 tension-pCa relationships were obtained, first at a short SL (ie, ~1.90 μm) and then at a long SL (ie, ~2.25 μm). For NEM-S1-treated myocytes (n=6), 3 tension-pCa relationships were obtained. An initial tension-pCa relationship was characterized at short SL (ie, ~1.90 μm) before incubation with NEM-S1 (pre–NEM-S1). While at the short SL, the myocytes were incubated for 15 minutes in pCa 9.0 solution containing 6 μmol/L NEM-S1, and a second tension-pCa relationship was then obtained. A final tension-pCa relationship was obtained in the presence of NEM-S1 after lengthening the myocyte to a long SL (ie, ~2.25 μm).

Tension-pCa relationships were always obtained first at short SL (ie, ~1.90 μm), followed by lengthening the myocyte to a long SL (ie, ~2.25 μm), since early experiments showed that multiple activations at long SL made it very difficult to return the myocyte to a shorter SL and consistently retain uniform striation spacing. Tension-pCa relationships were obtained by first maximally activating the myo-
cytes at pCa 4.5 and then transferring the myocytes to a series of submaximal pCa solutions between pCa 6.0 and pCa 5.0. At each pCa, steady tension was allowed to develop, and the myocyte was then rapidly slackened to determine total tension. NEM-S1–treated myocytes were first activated in solutions without NEM-S1 and were then relaxed in a pCa 9.0 solution containing 6 μmol/L NEM-S1. NEM-S1–treated myocytes were always paired with same-day control myocytes to account for any animal-to-animal variability. The Ca2+-activated tension at a given pCa was calculated as the difference between the total tension generated and the Ca2+-independent tension obtained by slackening the myocyte while in a solution of pCa 9.0. To determine any decline in tension-generating capability, the myocyte was maximally activated at the beginning and end of each protocol at a given SL. P0 for successive submaximal activations was interpolated between the 2 P0 measurements. Ca2+-activated tensions (ie, P) obtained in submaximal pCa solutions were expressed relative to P0 obtained at the same SL (ie, P/P0). Tension-pCa relationships were obtained at short SL (5.5 ± 0.03, n2 = 3.18 ± 0.39). Figure 1 shows the mean tension-pCa relationships for control myocytes (n = 6) at short and long SLs, respectively. Mean pCa50 was 5.41 ± 0.02 at short SL, whereas lengthening the myocyte significantly increased the mean pCa50 to 5.51 ± 0.02 (P < 0.05). This SL dependence of Ca2+ sensitivity is consistent with that reported previously by McDonald and Moss11 in single cardiac myocytes. The steepness of the tension-pCa relationship for Ca2+ activated tensions of <50% P0 (ie, the Hill coefficient, n2) provides an index of the apparent molecular cooperativity of tension development. Increasing SL in the range investigated did not significantly alter the steepness of the tension-pCa relationships at least within the variability of the data.

Effects of NEM-S1 Treatment

Previous experiments on skinned skeletal muscle fibers demonstrated that incubation with NEM-S1 potentiated submaximal Ca2+-activated force in a time- and concentration-dependent manner.1 A steady plateau in the elevation of submaximal tension was reached after 15 minutes in a solution of 6 μmol/L NEM-S1, and longer incubations yielded no further effects. Maximal Ca2+-activated tension was unaffected by concentrations of NEM-S1 up to 6 μmol/L, but increases in NEM-S1 concentration above this level resulted in a progressive reduction in maximal force, presumably due to NEM-S1–mediated competitive inhibition of endogenous crossbridge binding.5 Similar results in cardiac myocytes were observed in the present study: maximal Ca2+-activated tension was unaffected as the concentration of NEM-S1 was increased up to 6 μmol/L. However, at 10 μmol/L NEM-S1 maximal Ca2+-activated tension was reduced by nearly 20% relative to that obtained at 6 μmol/L. Therefore, to investigate the effects of strong-binding crossbridges on the length dependence of submaximal tension, before each activation ventricular myocytes were incubated for 15 minutes in a solution of pCa 9.0 and 6 μmol/L NEM-S1. Control experiments in which myocytes were incubated with 6 μmol/L unmodified S1 (ie, no NEM modification) showed no effects of S1 addition on Ca2+ sensitivity of tension because of the rapid rate of cycling of unmodified exogenous S1.

Figure 2 shows the cumulative tension-pCa data from NEM-S1–treated myocytes (n = 6): (1) at short SL before NEM-S1 treatment, (2) at short SL in the presence of NEM-S1, and (3) at long SL in the presence of NEM-S1. Mean pCa50 at short SL before NEM-S1 treatment was 5.40 ± 0.01, which increased to 5.52 ± 0.02 after NEM-S1 treatment (ΔpCa50 = 0.12 ± 0.02, P < 0.05). In fact, treatment of myocytes with 6 μmol/L NEM-S1 at short SL increased Ca2+ sensitivity to a mean value (5.52 ± 0.02) similar to that observed in control myocytes at the longer SL (5.51 ± 0.02, Table). However, NEM-S1 did not completely abolish the SL dependence of Ca2+ sensitivity, since lengthening the NEM-S1–treated myocytes to a longer SL increased the pCa50 to 5.54 ± 0.02 (ΔpCa50 = 0.02 ± 0.01, P < 0.05). This small, but significant, augmentation of Ca2+ sensitivity is not surprising, since increasing SL in this range would be expected to promote net formation of strongly bound force-generating crossbridges as a result of the increased probability of crossbridge attachment.11 Nevertheless, these data show that NEM-S1 treatment of single skinned myocytes nearly eliminated the SL dependence of the Ca2+ sensitivity of tension.
Figure 2. Ca\(^{2+}\) sensitivity of tension of skinned cardiac myocytes before and after NEM-S1 treatment at short and long SLs. Values are mean±SE from 6 experiments. Ca\(^{2+}\) -activated tensions (ie, P) obtained in submaximal pCa solutions are expressed relative to maximal tension (ie, \(P_0\)) obtained at the same SL. Tension-pCa curves were fit by computer, and mean values of pCa\(_{50}\) and \(n_2\) were from Hill plot analysis (see Materials and Methods). Tension-pCa relationships were obtained from the following: (1) pre-NEM-S1–treated myocytes at short SL (○, pCa\(_{50}\)=5.40±0.02, \(n_2=3.59±0.29\)), (2) NEM-S1–treated myocytes at short SL (△, pCa\(_{50}\)=5.52±0.02, \(n_2=3.18±0.23\)), and (3) NEM-S1–treated myocytes at long SL (▲, pCa\(_{50}\)=5.54±0.02, \(n_2=2.47±0.11\)).

A summary of myocyte length, SL, force, pCa\(_{50}\), and Hill coefficient data from control and NEM-S1–treated myocytes at short and long SLs is presented in the Table. At short SL, NEM-S1 did not alter the steepness of the tension-pCa relationship relative to values for control myocytes at the same length, at least within the variability of the data. However, treatment with 6 \(\mu\)mol/L NEM-S1 significantly increased submaximal Ca\(^{2+}\) -activated tensions at short SL, as seen in Figure 3. At Ca\(^{2+}\) concentrations below that required for half-maximal activation in control myocytes (ie, pCa \(≥\)5.5), NEM-S1 treatment significantly increased submaximal Ca\(^{2+}\) -activated tension (\(P<0.05\)). At progressively higher Ca\(^{2+}\) concentrations (ie, pCa <5.4), NEM-S1 had negligible effects on Ca\(^{2+}\) -activated tension relative to control.

At the longer SL, NEM-S1 treatment significantly reduced the steepness of the tension-pCa relationship for Ca\(^{2+}\) -acti-

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\(n_2\) is the Hill coefficient for Ca\(^{2+}\) -activated tensions of <50% \(P_0\). Values are mean±SE.

\(^*P<0.05\) vs control at short SL; \(†P<0.05\) vs NEM-S1 at short SL; and \(‡P<0.05\) vs pre-NEM-S1 at short SL.
reversed by washout of NEM-S1. Figure 4 shows representative tension-pCa relationships from a single myocyte at short SL. Three relationships were characterized in the following order: (1) before NEM-S1 treatment, (2) after incubation with 6 μmol/L NEM-S1, and (3) after washout of NEM-S1. Washout of NEM-S1 was accomplished by transferring the myocyte to a fresh solution of pCa 9.0 without NEM-S1 for a total of 15 minutes with 3 solution changes. Figure 4 clearly demonstrates the reversibility of the NEM-S1–induced enhancement of submaximal Ca2+-activated tension in cardiac myocytes.

Discussion

NEM-S1 Nearly Eliminates SL Dependence of Myofilament Ca2+ Sensitivity of Tension in Skinned Ventricular Myocytes

Beat-to-beat variations in ventricular end-diastolic volume, with corresponding changes in SL, lead to substantial alterations in cardiac output, a phenomenon that constitutes the Frank-Starling relationship. As SL is increased within the ascending limb of the length-tension relationship, myocardial twitch tension and Ca2+ sensitivity both increase. There is an expanding body of experimental evidence that variation in myofilament lattice spacing is the principal mechanism underlying the SL dependence of myocardial contraction. With reductions in SL, myofilament lattice spacing increases, resulting in greater lateral separation between thick and thin filaments in both skinned skeletal and living cardiac muscles. This would effectively reduce the probability that myosin crossbridges would attach to actin and undergo the transition to a strongly bound force-generating state. We hypothesized that a reduction in strong-binding crossbridges as a consequence of increased lattice spacing would decrease the cooperative activation of the thin filament at short lengths, thereby reducing the apparent Ca2+ sensitivity of tension development. This hypothesis based on the initial observation by Bremer and Weber (1972) that the binding of myosin S1 to actin exhibits positive cooperativity.

In the present study, this idea was tested using a strong-binding non–tension-generating derivative of myosin S1, ie, NEM-S1, to increase the formation of strongly bound crossbridges at short SL, especially at submaximal Ca2+. In control myocytes, the Ca2+ concentration required for half-maximal activation (ie, pCa0) increased by 0.10 pCa units when SL was increased from ≈1.90 μm to ≈2.25 μm. Similarly, application of 6 μmol/L NEM-S1 at short SL increased the pCa0 by 0.12 pCa units relative to control even though SL was unchanged. In fact, the pCa0 value in the presence of NEM-S1 at short SL (ie, 5.52±0.02) was similar to the pCa0 of control myocytes at long SL (ie, 5.51±0.02). The marked increase in the Ca2+ sensitivity of tension in NEM-S1–treated myocytes at short lengths resulted from significant enhancement of submaximal Ca2+-activated tension.

From the present results, the most likely explanation for the decrease in the Ca2+ sensitivity of tension at short lengths is a decrease in strongly bound crossbridges, presumably as a consequence of an increase in lateral spacing of the myofilament lattice, which would reduce the likelihood of crossbridge interaction and reduce the cooperative activation of the thin filament. Osmotic compression has frequently been used as a tool to reduce myofilament lattice spacing at short SLs to the spacing normally seen at longer SLs. Results from a number of studies have shown that the Ca2+ sensitivity of tension and Ca2+ binding affinity of troponin C in osmotically compressed preparations at short SLs were similar to values seen under control conditions at long SLs. Alternatively, length-dependent modulation of myocardial contraction has been proposed to be due to cardiac troponin C, which acts as a unique “length sensor” in myocardium, although this hypothesis has received little experimental support from other investigators. For example, slow-twitch soleus muscle does not exhibit SL dependence of Ca2+ binding, despite the fact that the troponin C isoform in cardiac and soleus muscles is identical. Furthermore, expression of skeletal troponin C in ventricular myocytes of transgenic mice did not alter the length dependence of the Ca2+ sensitivity of tension in skinned myocytes. Thus, it is unlikely that cardiac troponin C alone acts as a length sensor in mammalian myocardium.

At the present time, we do not know the relative contributions of cooperative increases in crossbridge binding versus cooperative increases in Ca2+ binding resulting in increased Ca2+ sensitivity at long lengths. However, recent work by Fuchs and coworkers suggests that force, not numbers of crossbridges, increases Ca2+ binding to troponin C. Since NEM-S1 itself does not generate force, the shift in the tension-pCa relationship most likely arises through the increase in endogenous myosin crossbridge binding. Furthermore, skeletal muscle also exhibits length-dependent changes in the Ca2+ sensitivity of force without the apparent length-dependent changes in Ca2+ binding affinity of troponin C. Both lines of evidence favor our interpretation that the apparent Ca2+ sensitivity of tension decreases at short length as a result of decreased cooperativity in crossbridge binding.

Length-dependent changes in the kinetics of crossbridge interaction (ie, the transition from weakly to strongly bound
crossbridge states) would also be expected to alter the number of strongly bound crossbridges and would thus be expected to influence the activation state of the thin filament. 5 McDonald et al. 9 (1997) have recently demonstrated that crossbridge interaction kinetics are slowed at short lengths in both skinned slow- and fast-twitch skeletal muscle fibers, an effect that also appears to be mediated by length-dependent changes in interfilament lattice spacing. Additional molecular mechanisms that may also influence the length dependence of Ca2+ sensitivity in skeletal muscle fibers includes fiber type–specific expression of troponin C isoforms (ie, slow versus fast) and the kinetics of crossbridge interaction as governed by differential expression of myosin heavy chain and light chain isoforms.

Mechanisms Responsible for the Length-Twitch Tension Relationship in Living Cardiac Muscle

Although it is becoming increasingly apparent that myofilament lattice spacing is the predominant mechanism underlying the SL dependence of myocardial contraction, other mechanisms must act synergistically with interfilament lattice spacing to determine the dynamic length-tension relationship in cardiac muscle. For example, the form of the length-tension relationship in maximally activated skeletal 34 and cardiac 35 muscle preparations is most likely due to alterations in the degree of thick- and thin-filament overlap. However, at submaximal levels of Ca2+, tension at short length is depressed primarily because of the lattice spacing–dependent reductions in the activation of the thin filament. 11,12 Another potential mechanism that may contribute to the SL dependence of myocardial contraction is the progressive decrease in the extent of Ca2+ release from the sarcoplasmic reticulum at short lengths. 32,33 A reduction in sarcoplasmic reticulum Ca2+ release with concomitant length-dependent modulation in the Ca2+ sensitivity of tension would be expected to markedly attenuate the development of tension at short lengths. Based on results to date, the length dependence of myocardial contraction in vivo appears to be due to changes in number of attached crossbridges as a result of SL-dependent alterations in (1) interfilament lattice spacing, (2) the degree of myofilament overlap, and (3) Ca2+ release.

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References

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