Cooperative activation in cardiac muscle: impact of sarcomere length

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Cooperative activation in cardiac muscle: impact of sarcomere length. Am J Physiol Heart Circ Physiol 282: H1055–H1062, 2002; 10.1152/ajpheart.00667.2001.—This study was undertaken to determine the impact of sarcomere length (SL) on the level of cooperative activation of the cardiac myofilament at physiological [Mg\(^{2+}\)]. Active force development was measured in skinned rat cardiac trabeculae as a function of free [Ca\(^{2+}\)] at five SLs (1.85–2.25 μm; 1 mM free [Mg\(^{2+}\); 15°C]). Only muscle preparations with minimal force rundown during the entire protocol were included in the analysis (average 7.2 ± 1.7%). Median SL was measured by on-line computer video micrometry and controlled within 0.01 μm. Care was taken to ensure a sufficient number of data points in the steep portion of the [Ca\(^{2+}\)]-force relationship at every SL to allow for accurate fit of the data to a modified Hill equation. Multiple linear regression analysis of the fit parameters revealed that both maximum, Ca\(^{2+}\)-saturated force and Ca\(^{2+}\) sensitivity were a significant function of SL (P < 0.001), whereas the level of cooperativity did not depend on SL (P = 0.2). Further analysis of the [Ca\(^{2+}\)]-force relationships revealed a marked asymmetry that, also, was not affected by SL (P = 0.2–0.6). Finally, we found that the level of cooperativity in isolated skinned myocardium was comparable to that reported for intact, nonskinned myocardium. Our results suggest that an increase in SL induces an increase in the Ca\(^{2+}\) responsiveness of the cardiac sarcomere without affecting the level of cooperativity.

The Frank-Starling law of the heart describes the interrelationship between end-diastolic volume and cardiac ejection volume, a regulatory system that operates on a beat-to-beat basis. The main cellular mechanism that underlies this phenomenon is the increase in the responsiveness of the cardiac myofilament to activating Ca\(^{2+}\) upon an increase in sarcomere length (SL), which is commonly referred to as length-dependent activation (1, 17, 22). In striated muscle, initiation of muscle contraction involves complex cooperative and allosteric interactions along the thin filament (2, 14, 25, 28). Cooperative activation of the myofilaments is manifest by a steeper relationship between activator Ca\(^{2+}\) and developed force than would be predicted solely on the basis of the number of Ca\(^{2+}\) binding sites that are present on the regulatory protein complexes of the thin filament (37, 38).

Myofilament length-dependent activation has been proposed to be due to the reduction of the thick-to-thin filament separation upon an increase in SL which, in turn, is hypothesized to increase the probability of cross-bridge formation at the longer SL (11, 12, 15, 28, 36). Such a mechanism may also be expected to lead to enhanced cooperativity of activation at a longer SL. Indeed, increases in the Hill coefficient, an index of the level of cooperative activation, with increased SL has been reported by Kentish et al. (22). Those results, however, have been obtained in “skinned” muscle preparations in which all membranous structures have been removed by detergent treatment. More recently, it has been suggested by Gao et al. (13) that the level of cooperative activation in skinned myocardium is greatly reduced compared with intact, that is, nonskinned, myocardium. Several shortcomings of both studies, however, hamper interpretation of these data. First, the study by Kentish et al. (22) was performed at a concentration of ionized Mg\(^{2+}\) in the bathing solution that was significantly above (~300%) the now known physiological cytosolic concentration of this ion in myocardium (16, 21). Furthermore, insufficient data were obtained at some SL in the critical steep region of the force-Ca\(^{2+}\) relationship to allow for accurate determination of the Hill coefficient. Second, although the study reported by Gao et al. (13) was performed at physiological [Mg\(^{2+}\)], SL was not controlled throughout the contraction. Lack of SL control has been shown to result in significant reduction of the measured Hill coefficient (22). This phenomenon is caused by uncontrolled shortening of the central sarcomeres due to compliance of the damaged ends of the isolated muscle preparation (20, 24, 41). Accordingly, the present study was undertaken to answer the following questions. First, what is the level of cooperative activation (Hill coefficient) of the cardiac myofilament at physiological [Mg\(^{2+}\)] and under conditions of strict SL control? Second, is the Hill coefficient affected by SL? Studies were...
performed using skinned rat cardiac trabeculae. SL was controlled throughout the experiment at all levels of myofilament activation. Furthermore, care was taken to ensure that data were included only from muscle preparations that exhibited minimal rundown during the entire experiment. We found a direct inverse proportional relationship between myofilament Ca$^{2+}$ sensitivity and SL. Second, the level of cooperative myofilament activation did not vary with SL. Third, the force-Ca$^{2+}$ relationship was asymmetric, that is, steeper at low [Ca$^{2+}$] than at high [Ca$^{2+}$]. This asymmetry was not affected by SL. Fourth, the level of cooperativity was comparable to that reported for intact, nonskinned myocardium. Our results suggest that activation of the cardiac myofilament is highly cooperative and that the level of cooperativity does not vary with SL.

METHODS

**Preparation of right ventricular cardiac trabeculae.** Male rats (LBNF-1 220–280 g) were anesthetized with ketamine/xylazine (80/4 mg/kg ip) followed by administration of heparin (1,000 U/kg). All procedures were in accordance with institutional guidelines. Hearts were rapidly excised and perfused retrograde with a modified Krebs-Henseleit solution as previously described (20). Thin, uniform, and unbranched right ventricular trabeculae were dissected and transferred to standard relaxing solution containing 1% Triton X-100 for a minimum of 2 h at 4°C to allow solubilization of all membranous structures. The muscles were stored at 4°C for use within 24 h of dissection.

**Solutions.** Three bathing solutions were used: a relaxing solution, a preactivating solution with low Ca$^{2+}$ buffering capacity, and an activating solution. The ionic composition of these solutions is summarized in Table 1. The solutions were calculated using the methods of Fabiato and Fabiato (9). To achieve a range of free [Ca$^{2+}$], activating and relaxing solutions were appropriately mixed assuming an apparent stability constant of the Ca$^{2+}$-EGTA complex of $10^{6.39}$. The ionic strength of the solutions was kept at 200 mM by adding an appropriate amount of potassium propionate (KProp). The pH was adjusted to 7.0 at 15°C with KOH.

Table 1. Ionic composition of skinned fiber solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>MgCl$_2$</th>
<th>Na$_2$ATP</th>
<th>EGTA</th>
<th>HDTA</th>
<th>Ca-EGTA</th>
<th>KProp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relaxing</td>
<td>6.63</td>
<td>5.98</td>
<td>20</td>
<td></td>
<td></td>
<td>41.2</td>
</tr>
<tr>
<td>Preactivating</td>
<td>6.31</td>
<td>5.98</td>
<td>0.5</td>
<td>19.5</td>
<td></td>
<td>41.8</td>
</tr>
<tr>
<td>Activating</td>
<td>6.20</td>
<td>6.11</td>
<td></td>
<td>20</td>
<td>41.5</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed in mM. Ca-EGTA was made by mixing equimolar amounts of CaCl$_2$ and EGTA. In addition, all solutions contained the following (in mM): 10 phosphocreatine, 100 N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid, 0.1 leupeptin, 0.1 phenazine-methosulfate, 1 dithiothreitol, and 4 U/ml creatine phosphokinase. The free Mg$^{2+}$ and Mg-ATP concentrations were calculated at 1 and 5 mM, respectively (9). Relaxing and activating solutions were appropriately mixed to obtain a range of free [Ca$^{2+}$] by using an apparent Ca$^{2+}$ binding stability constant of $10^{6.39}$ at 15°C. The preactivating solution with low Ca$^{2+}$ buffering capacity contained 1,6-diaminohexane-N,N,N’,N’-tetraacetic acid (HDTA). The ionic strength of the solutions was kept at 200 mM by adding an appropriate amount of potassium propionate (KProp). The pH was adjusted to 7.0 at 15°C with KOH.

**SL measurement.** Each skinned trabecula was monitored by video microscopy with an inverted microscope using a ×40 long working distance objective (Olympus), a ×10 video adapter tube, and a gray-scale CCD-based video camera (10). The image was displayed on a video monitor (video field of view, 100 μm × 100 μm) and sampled by computer for on-line analysis of SL using custom-designed software (LabView) as follows. A region of the image encompassing a large portion of the trabecula was selected, and each horizontal pixel line was transformed by fast Fourier transformation (FFT) into a spatial frequency domain. The power spectra were averaged, and the spatial frequency at peak power of the first-order harmonic in the spatial frequency domain was determined (Fig. 1). This spatial frequency was then converted into...
median SL across the region. The system was calibrated with glass gratings of known spacing: resolution was \( \sim 0.01 \) \( \mu m \) with an acquisition speed of 0.5 s.

**Experimental protocol.** The \( \text{Ca}^{2+} \) sensitivity of force was determined as previously described (20) with slight modifications. A \( \text{Ca}^{2+} \)-force relationship was determined in each skinned trabecula at each of the five SLs that were selected in random order as follows. Before activation, the relaxing solution in the muscle bath was exchanged with the preactivating solution (see Solutions). Use of an exchange volume that was three to four times the trough volume ensured complete exchange. The solution exchange was accomplished such that the muscle was never exposed to air. Each incubation period was 3–4 min, allowing full equilibration of the skinned trabecula with the solution. \( \text{Ca}^{2+} \)-dependent force was determined by activating the muscle during a series of preactivating-activating-relaxation cycles using a range of free \([\text{Ca}^{2+}]\) in the activating solutions selected in random order. During each activation, measurements were made at each of the five SLs, again selected in random order, that was achieved by changing muscle length during the contraction. SL was kept constant at the selected SL by appropriately adjusting muscle length via a micromanipulator throughout the experimental protocol, that is, both during maximal and submaximal activations. Force was allowed to reach a new steady state when the next random SL was selected during activation before measurements were recorded; steady-state force was reached within \( \sim 10 \) s. This protocol was adopted to minimize both the number and duration of contractions that were imposed upon the muscle preparation. Preliminary experiments were performed using the alternative approach in which a full \( \text{Ca}^{2+} \)-force relationship was determined at each individual SL using multiple contraction-relaxation cycles. Of the few \((n = 3)\) muscle preparations that exhibited close to acceptable rundown using this alternative protocol revealed, in general, similar results as those presented here. However, because the success rate of this approach was unacceptably low, this method was not further pursued in the present study. The amount of stretch required to maintain SL amounted to \( \sim 10\% \) of overall muscle length, consistent with our previous observations (7). In addition, during each activation, the muscle was examined laterally by means of the movable stage so as to assess homogeneity of the fiber and uniformity of SL along the muscle, except for the regions close to the T-clips \((\sim 50–100 \) \( \mu m \)). If SL varied by \( >0.05 \) \( \mu m \) along the length of the muscle preparation during activation, it was discarded. Active force at each \([\text{Ca}^{2+}]\) was calculated as the difference between total force and relaxed passive force at that SL as assessed in relaxing solution. To determine any decline in tension-generating capacity (preparation rundown), the muscle was maximally activated at the beginning and at the end of the protocol at a SL of 2.05 \( \mu m \). Trabeculae that did not maintain 90\% of initial maximal force or that lost a visible striation pattern at any point during the experimental protocol were discarded. On average, based on these criteria, 80–90\% of the examined muscle preparations were discarded. It should be noted that the preparations that were excluded displayed, in general, similar steep force \( \text{Ca}^{2+} \)-force relationships at all SL. Furthermore, detailed analysis of the data from the excluded muscle preparations was not attempted.

**Data analysis.** Each force-\( \text{Ca}^{2+} \) relationship as determined in each individual trabecula was fit by nonlinear regression analysis to a modified Hill equation

\[
F = F_{\text{max}} \cdot \left[ \frac{[\text{Ca}^{2+}]}{[\text{Ca}^{2+}]_{\text{EC}50} + [\text{Ca}^{2+}]} \right] \tag{1}
\]

where \( F \) is steady-state force, \( F_{\text{max}} \) is maximum \( \text{Ca}^{2+} \)-saturated force, \( \text{EC}_{50} \) is \([\text{Ca}^{2+}]_{\text{EC}50} \) at which force is half-maximal, and \( n \) is slope of the force-\( \text{Ca}^{2+} \) relationship (Hill coefficient).

Force in submaximally activating solutions was expressed as a fraction of the maximum force \( F_{\text{max}} \) at every SL. Next, force was normalized to maximal force at a SL of 2.05 \( \mu m \). To correct for preparation rundown, the \( F_{\text{max}} \) value used for this normalization procedure was estimated for each contraction by linear interpolation of the \( F_{\text{max}} \) obtained during the test contractions at a SL of 2.05 \( \mu m \) as performed at the beginning and end of the experimental protocol; that is, we assumed that each contraction equally contributed to preparation rundown, as described previously (6). Note, however, that the magnitude of this correction was small, since only trabeculae with minimal rundown (<10\%) were included in the present study (see also below).

To assess the asymmetry in the force-\( \text{Ca}^{2+} \) relationship, linear regression analysis was performed using a modified, linearized form of the Hill equation (28, 33, 34)

\[
\log \left[ \frac{F}{F_{r}} \right] = n \cdot (\log ([\text{Ca}^{2+}]) - \log (\text{EC}_{50})) \tag{2}
\]

in which \( F_{r} \) represents force development as a fraction of \( F_{\text{max}} \). The slope of this relationship is governed by the Hill coefficient \((n)\), while the zero crossing represents the \( \text{EC}_{50} \) parameter. Two separate linear segments, using data above or below the \( \text{EC}_{50} \), were fit by linear regression analysis to the data, thus yielding two separate estimates of the Hill coefficient: \( n_1 \) and \( n_2 \) (see Table 2).

**Statistical analysis.** Differences among the Hill-fit parameters in each group at each SL were analyzed by one-way ANOVA, followed by a Bonferroni-corrected Student’s \( t \)-test to assess differences among mean values. Fit parameters obtained from the nonlinear fit to the Hill equation in each individual muscle preparation were averaged to obtain the mean and SE. Furthermore, direct analysis of the SL dependence of the Hill-fit parameters was accomplished by multiple linear regression.

<table>
<thead>
<tr>
<th>SL, ( \mu m )</th>
<th>( F_{\text{max}}, \text{mN/mm}^2 )</th>
<th>( \text{EC}_{50}, \mu M )</th>
<th>Hill Coefficient</th>
<th>( n_2 )</th>
<th>( n_1 )</th>
<th>Transition ( F_{\text{rel}}, % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.85</td>
<td>49.1 ± 4.0</td>
<td>4.43 ± 0.08</td>
<td>7.4 ± 0.5</td>
<td>8.6 ± 0.6</td>
<td>4.8 ± 0.4</td>
<td>61.6 ± 7.1</td>
</tr>
<tr>
<td>1.95</td>
<td>56.8 ± 4.8</td>
<td>4.03 ± 0.05</td>
<td>7.2 ± 0.3</td>
<td>10.6 ± 1.0</td>
<td>4.7 ± 0.3</td>
<td>59.2 ± 6.1</td>
</tr>
<tr>
<td>2.05</td>
<td>60.6 ± 4.8</td>
<td>3.74 ± 0.06</td>
<td>7.3 ± 0.3</td>
<td>9.9 ± 0.3</td>
<td>5.4 ± 0.3</td>
<td>56.6 ± 5.0</td>
</tr>
<tr>
<td>2.15</td>
<td>65.3 ± 5.1</td>
<td>3.51 ± 0.06</td>
<td>7.0 ± 0.4</td>
<td>8.8 ± 0.7</td>
<td>5.6 ± 0.3</td>
<td>61.8 ± 7.0</td>
</tr>
<tr>
<td>2.25</td>
<td>72.0 ± 6.0</td>
<td>3.24 ± 0.07</td>
<td>6.9 ± 0.7</td>
<td>7.3 ± 0.7</td>
<td>4.7 ± 0.5</td>
<td>59.4 ± 11.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Average parameter values of the fit to the single Hill relationship (Eq. 1; first 3 columns) and a biphasic linearized form of the Hill relationship (Eq. 2; last 3 columns). See text for details. A total of 10 trabeculae were studied at the indicated sarcomere length (SL). Data were analyzed for each individual muscle. Fit parameters were analyzed by multiple linear regression analysis; SL significantly only affected maximum \( \text{Ca}^{2+} \)-saturated force \((F_{\text{max}}, P < 0.001)\) and \( \text{Ca}^{2+} \)-sensitivity \((\text{EC}_{50}, P < 0.001)\).

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RESULTS

Ca\(^{2+}\)-dependent force development was determined in 10 skinned rat cardiac trabeculae that qualified the inclusion criteria (see METHODS). On average, rundown of maximum Ca\(^{2+}\)-saturated force development in these trabeculae was 7.2 ± 1.7\% over the course of the entire experimental protocol. Because of damage inflicted on the ends of the trabeculae during sample preparation, a compliance is introduced into the preparation that causes uncontrolled sarcomere shortening upon contractile activation (20, 24, 41). To correct for this, SL was kept constant by appropriately adjusting muscle length both during maximal and submaximal activations. Hence, myofilament force development was measured under conditions of strict SL control along the length of the skinned muscle preparation during the contraction. This is illustrated by the CCD video image in Fig. 1A, which shows a clearly resolved striation pattern during maximal activation in a typical skinned rat cardiac trabecula. FFT analysis of the CCD images allowed SL to be determined within 0.01 \(\mu\)m. FFT analysis of the image in Fig. 1A is shown in Fig. 1B, which revealed median SL of 2.25 \(\mu\)m as well as minimal SL dispersion (width at half-maximum power = 0.03 \(\mu\)m). Previous studies on skinned rat cardiac trabeculae have employed laser diffraction techniques to assess SL, however (20, 24, 41). Accordingly, we directly compared the FFT method (10) with laser diffraction analysis (20, 24, 41) in a preliminary series of experiments under conditions of varied levels of contractile activation and SL. We found that the difference between these two methods of SL measurement was, on average, 9.2 ± 0.1 nm, a value similar to the nominal resolution of either system (0.01 \(\mu\)m).

Ca\(^{2+}\)-force relationships were determined at five SLs (1.85, 1.95, 2.05, 2.15, and 2.25 \(\mu\)m) in each trabecula. The average pooled data are shown in Fig. 2A, where force is normalized to the maximum Ca\(^{2+}\)-saturated force measured at a SL of 2.05 \(\mu\)m. Increasing SL from short (SL = 1.85 \(\mu\)m) to long (SL = 2.25 \(\mu\)m) induced a leftward shift of the Ca\(^{2+}\)-force relationship, as well as an increase in maximum, Ca\(^{2+}\)-saturated force development. The data were fit to the modified Hill relationship (Eq. 1) for each trabeculae at each SL; the average fit parameters are summarized in Table 2. Ca\(^{2+}\) sensitivity, as indexed by the EC\(_{50}\) parameter, was an inverse and approximately linear function of SL as illustrated by the average pooled data in Fig. 2B (\(P < 0.001\)). On average, maximum force development increased with an increase in SL, on average, 9% for each 0.1- \(\mu\)m increase in SL (\(P < 0.001\)). Thus these data indicate that myofilament Ca\(^{2+}\) sensitivity and maximum Ca\(^{2+}\)-saturated force development are a sensitive function of SL, consistent with previous findings (1, 17, 22). In contrast, the level of cooperativity, as indexed by the Hill coefficient, was not affected by SL (\(P = 0.2\); Table 2), a finding that differs from previous findings (22). In addition, the level of cooperative activation was much greater than has previously been reported for skinned rat cardiac trabeculae at physiological \([\text{Mg}^{2+}]\) (13).

The generalization of the Hill equation is an equilibrium formulation (37, 38). Because muscle contracts under nonequilibrium conditions, however, the Hill equation may not provide an accurate description of cooperative activation in myocardium. Inspection of the Ca\(^{2+}\)-force relationships shown in Fig. 2A indeed suggests that this may be the case, since there was a consistent overestimation of predicted myofilament force development at the higher levels of activation at all SL. This asymmetry in the force–Ca\(^{2+}\) relationship was quantified by regression analysis of the linearized form of the Hill relationship (Eq. 2) (28, 33, 34). The average Hill parameters that were obtained via this analysis are summarized in Table 2 (parameters \(n_1\) and \(n_2\)). Figure 3A shows examples of this analysis for the data that were obtained at SL values of 2.15 and 1.85 \(\mu\)m (to reduce clutter, only 2 SLs are displayed). At either SL, there were two clear phases of the Hill relationship, indicative of reduced cooperativity of
respectively; Table 2). Relative force development (Frel) given SL. Data are presented as means calculated as a percentage of the maximum developed force at a

at the apparent phase transition between

n1

relationship (solid lines; see METHODS). Data for only two SLs are displayed for clarity; full analysis results are reported in Table 2. The

calculation of length dependency has a direct impact on the shape

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of which depends on inotropic state (4).

In striated muscle, the actin-activated ATPase activity of myosin depends on the presence of Ca$^{2+}$ in the solution (42). Despite similar steps leading to myofilament activation in all striated muscles, the regulation of Ca$^{2+}$-activated myosin ATPase differs among striated muscle types: fast skeletal troponin C (TnC) contains two regulatory Ca$^{2+}$-binding sites (35), whereas cardiac TnC contains only one binding site that confers Ca$^{2+}$ regulation (18). Therefore, in both cardiac and skeletal muscle, cooperative and allosteric interactions among thin and thick filament components during activation are required for full activation (40). Strongly bound cross bridges enhance the binding of additional cross bridges in a cooperative manner, presumably by initiating the movement of tropomyosin to a more favorable position (25, 39). Myofilament length-dependent activation has been proposed to be due to a direct influence of interfilament separation distance on the probability of cooperative cross-bridge formation (11, 12, 15, 28, 36). Kinetic alterations in this critical reaction step of the cross-bridge cycle would be expected to affect the level of myofilament cooperativity. Indeed, early results in skinned rat cardiac trabeculae with SL control suggested an increase in the Hill parameter at longer SL (22). However, these results were obtained at unphysiologically high (3 mM) concentrations of Mg$^{2+}$ in the skinned fiber solutions (16, 21). In addition, insufficient data were collected in that study in the low SL range to allow for accurate determination of the level of cooperativity. More recently, SL independence of the Hill coefficient has been illustrated in studies employing single skinned cardiac myocytes at physiological [Mg$^{2+}$] (10, 28). However, those studies were performed under conditions in which internal shortening was minimized, but not altogether eliminated. Consequently, although the Hill parameter was not SL dependent in those studies, the estimation of myofilament cooperativity was likely underestimated (22). To our knowledge, this is the first detailed study of cardiac myofilament cooperativity at physiological [Mg$^{2+}$] under conditions where 1) SL was strictly controlled, 2) sufficient data were collected at all SL, and 3) analysis was restricted to muscle preparations that completed the entire protocol with minimal rundown. Our results clearly show SL independence of cooperativity (Fig. 2; Table 2), consistent with data obtained in skinned skeletal muscle. (29). Hence, those and our results suggest that this behavior is a general property of all striated muscle. Even though the mechanism that underlies length-dependent activation cannot be determined from our study, these data indicate that any model of myofilament activation must include SL independence of myofilament cooperativity.

**DISCUSSION**

Length-dependent activation of cardiac muscle is believed to underlie the ability of the intact ventricle to generate greater end-systolic pressures at increased end-diastolic filling volumes; this is the Frank-Starling law of the heart (1, 22, 41). In cardiac muscle, length-dependent activation is more pronounced than in skeletal muscle and, consequently, the length-tension relationship is steeper in cardiac muscle (8). The extent of length dependency has a direct impact on the shape of the length-isometric tension relationship in skinned cardiac fibers and the shape of the end-systolic sarcomere length-tension relationship in intact cardiac fibers (22). These results suggest that this phenomenon also underlies the shape of the end-systolic pressure-volume relationship, which is the ventricular counterpart to the end-systolic length-tension relationship, the shape of which depends on inotropic state (4).

**Fig. 3.** A: asymmetry in the Ca$^{2+}$-force relationship. Data were analyzed as two linear segments of the linearized version of the Hill relationship (solid lines; see METHODS). Data for only two SLs are displayed for clarity; full analysis results are reported in Table 2. The level of cooperative activation (slope of fitted line) was reduced at activation levels above the EC$_{50}$ (indicated by the dotted line). B: force level at the transition between high and low cooperativity was not affected by SL. The transition between the two phases of the Ca$^{2+}$-force relationship was estimated from the intersection of the two fitted line segments above and below the EC$_{50}$ (see A). Force was calculated as a percentage of the maximum developed force at a given SL. Data are presented as means ± SE.

myofilament activation at levels of activation that were above the EC$_{50}$ (dotted line). As was the case for the single Hill relationship analysis, neither $n_1$ nor $n_2$ parameter was affected by SL ($P = 0.6$ and $P = 0.2$, respectively; Table 2). Relative force development ($F_{rel}$) at the apparent phase transition between $n_1$ and $n_2$ was estimated from the intersection of the two linear line segments (Fig. 3A; Table 2). This force was consistently above half-maximal force and, also, not affected by SL ($P = 0.6$; Fig. 3B). On average, at all SL, $F_{rel}$ at the transition was 59.7 ± 1.0% $F_{max}$. 

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Detailed analysis of the Ca\(^{2+}\)-force relationships in this study revealed a marked asymmetry, such that cooperative myofilament activation was significantly reduced at higher levels of activation (Fig. 3; Table 2). These results are consistent with previous studies that showed a transition from a steep, high cooperative phase to a shallow, low cooperative phase at a [Ca\(^{2+}\)] close to that at which tension development is half maximal (that is, at the EC\(_{50}\)) (28, 33, 34). It should be noted that in this study, the phase transition occurred consistently above the EC\(_{50}\) at all SL (Fig. 3B), whereas in the other studies this point was closer to the EC\(_{50}\). It has been suggested that a greater number of force-bearing cross bridges induce a slower rate of cross-bridge cycling (2, 42) and that this phenomenon results in a reduction of cooperative cross-bridge formation (5). The decreased cooperativity at the higher [Ca\(^{2+}\)] observed in this study might be explained by such a mechanism. The overall shape of the Ca\(^{2+}\)-force relationship was maintained at all SL, being shifted to the left and increased in magnitude at longer SL. Again, although the underlying mechanism for this behavior cannot be determined from this study, it is tempting to conclude that the length-sensing mechanism involves the recruitment of additional cross bridges at higher SL that are functionally identical, despite the increased sensitivity to activator Ca\(^{2+}\).

Clearly, future modeling efforts aimed to describe cardiac myofilament activation processes will also have to incorporate this phenomenon.

The level of myofilament cooperativity determined in this study was similar to values reported for fast skeletal muscle (31, 32). Because cardiac TnC has a single Ca\(^{2+}\) regulatory site compared with the two sites in skeletal TnC, there must be a fundamental difference between cooperative regulation of thin filament activation in these different muscle types. It has been shown that the rate of force redevelopment after a release-restretch protocol (\(k_r\)) in fast skeletal muscle varies more than in cardiac muscle over a similar range of activator [Ca\(^{2+}\)]. This difference in Ca\(^{2+}\) dependence of \(k_r\) between cardiac and fast-twitch skeletal muscle has been attributed to a greater level of cooperativity in fast skeletal muscle, especially at low levels of activation (30, 43). Modeling efforts of cross-bridge kinetics aimed to account for these experimental observations indicate a requirement for a higher level of cooperativity (Hill parameter) in fast skeletal muscle than in cardiac muscle (5). Our data, however, are not consistent with that notion (Figs. 2 and 3; Table 2). This discrepancy could perhaps be explained by a difference in the absolute number of cross bridges that are required for full activation in each muscle type; that is, if activation in cardiac muscle were to require the attachment of fewer strong cross bridges, \(k_r\) may be less sensitive to Ca\(^{2+}\), despite a similar level of cooperativity as that in fast skeletal muscle. Alternatively, the differences in \(k_r\) and its regulation between the muscles types (30) may simply involve the difference in the number of Ca\(^{2+}\) regulatory sites on TnC. Detailed comparative studies of the different muscle types employing strict length control under well-controlled conditions may be required to resolve this issue.

Our results were obtained in skinned muscle preparations in which all membranous structures were removed by detergent treatment. Recently, it has been suggested by Gao et al. (13) that the level of cooperative activation in skinned myocardium is greatly reduced compared with intact, that is, nonskinned, myocardium (13). However, the data in that study were obtained without strict SL control throughout the contraction. It is well known that isolated cardiac muscle exhibits damage end-compliance that allows for substantial SL shortening during the contraction (20, 24, 41). Uncontrolled SL shortening leads to a significant underestimation of the level of myofilament cooperativity (22). The data obtained in this study employing strict SL control revealed levels of myofilament cooperative activation that are similar to those obtained in intact isolated myocardium (13, 23, 44). Hence, our data do not support the notion of loss of some critical factor or moiety from the cytosol that modulates myofilament function upon removal of all membranous structures upon “skinning,” as has been proposed by Gao et al. (13).

Several limitations of our study need to be considered. First, SL was kept constant by adjusting overall muscle length during the contraction. The amount of stretch of the muscle was <10% of overall muscle length. Nevertheless, it is possible that eccentric contractions caused by this stretch during the activation affected measured force in a way other than through changes in SL. This could have affected the steepness of the Ca\(^{2+}\)-force relationships in our study and thus led to overestimation of the Hill parameters. Second, chemical permeabilization (skinning) of a muscle fiber causes swelling of the myofilament lattice, as we have confirmed recently in rat myocardium (19). Interfilament spacing is a sensitive function of SL (11, 12, 19). Furthermore, significant changes in interfilament spacing have been reported to occur during activation in skeletal muscle (3, 27). There may be a direct effect of interfilament spacing on myofilament Ca\(^{2+}\) responsiveness (11, 12, 15, 28, 36). However, we did not measure interfilament spacing during contraction in our study, nor did we attempt to adjust interfilament spacing by addition of high molecular compounds, such as dextran, to the solution (11, 12, 15, 19, 28). Hence, whether the steepness, asymmetry, or SL dependence of the Ca\(^{2+}\)-force relationship is affected by changes in interfilament spacing during the contraction can neither be determined nor excluded based on the data collected in this study. Clearly, this issue awaits further experimentation.

In summary, the present study was undertaken to determine the impact of SL on the level of cooperative activation of the cardiac myofilament at physiological [Mg\(^{2+}\)]. We found that 1) the level of cooperative myofilament activation did not vary with SL (Fig. 2A), 2) Ca\(^{2+}\) sensitivity was inversely proportional to SL (Fig. 2B), 3) the force-Ca\(^{2+}\) relationship was asymmetric (Fig. 3), and 4) the level of cooperativity was compara-
ble to that reported for intact, nonskinned myocardium. Our results suggest that an increase in SL induces an increase in the Ca$^{2+}$ responsiveness of the cardiac sarcomere without affecting the level of cooperativity.

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