Effects of Calcium on Shortening Velocity in Frog Chemically Skinned Atrial Myocytes and in Mechanically Disrupted Ventricular Myocardium From Rat

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Effects of [Ca\textsuperscript{2+}] on isometric tension and unloaded shortening velocity were characterized in single chemically skinned myocytes from frog atrium and in mechanically disrupted myocardium from rat ventricle. The preparations were attached to a force transducer and piezoelectric translator and were viewed with an inverted microscope to allow continuous monitoring of sarcomere length during mechanical measurements. Unloaded shortening velocity was determined by measuring the time required to take up various amounts of slack imposed at one end of each preparation. Ca\textsuperscript{2+} sensitivity of isometric tension was assessed as pCa\textsubscript{50}, i.e., the Ca\textsuperscript{2+} concentration at which tension was 50% maximal, and was greater for frog atrial myocytes (pCa\textsubscript{50} 6.17) than for rat ventricular myocytes (pCa\textsubscript{50} 6.06). This difference in Ca\textsuperscript{2+} sensitivity may be due to variations in myofibrillar protein isoform composition in the two preparations. Inclusion of caffeine in the activating solutions substantially increased the Ca\textsuperscript{2+} sensitivity of tension, which may be a manifestation of a direct effect of caffeine on the myofibrillar proteins. Unloaded shortening velocity during maximal activation averaged 4.32 muscle lengths per second in frog atrial myocytes and 4.46 muscle lengths per second in rat ventricular myocytes. When [Ca\textsuperscript{2+}] was reduced, unloaded shortening velocity decreased substantially in both preparations. Possible mechanisms for the effect of Ca\textsuperscript{2+} on shortening velocity in myocardium include Ca\textsuperscript{2+} dependence of the rate of ADP dissociation from actomyosin complexes or a shortening-dependent internal load involving structures such as C protein or long-lived myosin cross-bridges. (Circulation Research 1992;70:885–892)

**KEY WORDS** • cardiac myocyte • shortening velocity • calcium • caffeine
while in both preparations, decreasing the concentration of Ca\(^{2+}\) reduces the velocity of unloaded shortening.

**Materials and Methods**

Single atrial cells from male *Xenopus laevis* were prepared using an enzymatic digestion technique. Minced atria were gently agitated for 1 hour at 32°C in Ca\(^{2+}\)-free Ringer's solution containing 200 units of Sigma collagenase 1 and 2,000 units trypsin II per milliliter. Atrial pieces were then repeatedly aspirated with a Pasteur pipette to dissociate the cells. To remove the extracellular membrane, frog atrial myocytes were placed for 30 minutes in relaxing solution containing 250 µg saponin per milliliter. Individual cells were attached to a force transducer (model 406, Cambridge Technology, Inc.) and piezoelectric translator (Physik Instrumente, Waldbronn, FRG) by using methods modified from Warshaw and Fay\(^{16}\): 1) the tip (5 µm o.d.) of a glass micropipette was dipped in silicone adhesive; 2) adhesive on the micropipette tip was brought into contact with a 20-µm-diameter anion exchange resin bead and the adhesive was allowed to cure; 3) by using de Fonbrune micromanipulators (Technical Products International, St. Louis, Mo.), the micropipettes and beads were brought into contact with each end of a single myocyte; 4) the ends of the cell were manipulated to introduce a double Blackwall's hitch at each end (see Figure 3 of Reference 16); 5) after the knots were loosely tied, the cell was stretched taut to a sarcomere length of approximately 2.4 µm; and 6) after the cell was initially activated in solution of pCa 4.5, sarcomere length was determined from a photomicrograph (Polaroid 107 film, ×640 at the film plane) of the cell while in relaxing solution. Microscopy, solution changing procedures, and recording instrumentation were described previously.\(^{17}\)

Female Sprague-Dawley rats (250–300 g body weight) were anesthetized by intraperitoneal injection of sodium pentobarbitol (40–50 mg/kg). The rat ventricular myocardial preparation, attachment procedure, and experimental apparatus were identical to those described by Hofmann et al.\(^{17}\) Freshly isolated ventricles from rat hearts were placed in relaxing solution and then homogenized in a blender, resulting in a suspension of small clumps of ventricular myocytes and cell fragments. Disrupted ventricular myocardium was attached to the experimental apparatus by touching the ends of the preparation with micropipettes coated with uncured silicone adhesive (Dow Corning Corp., Midland, Mich.). After the adhesive was allowed to cure, the preparation was lifted to the center of a drop of relaxing solution. Before any activations, the preparation was immersed in a 3% Triton X-100 solution for 10 seconds. Photomicrographs of rat ventricular myocardium were taken with a 35-mm camera system (Olympus model PM-10, ×540 at the film plane).

All data were obtained at 22°C. Relaxing and activating solutions contained 4 mM MgATP, 1 mM free Mg\(^{2+}\), 20 mM imidazole, 10 mM caffeine, 7 mM EGTA, 14.5 mM creatine phosphate, pH 7.0, and sufficient KCl to adjust ionic strength to 180 mM. Free Ca\(^{2+}\) concentration was varied between 10\(^{-9}\) M (relaxing solution) and 10\(^{-4.5}\) M (maximally activating solution) and is expressed as pCa (−log[Ca\(^{2+}\)]. The apparent stability constant for CaEGTA was corrected to 22°C and an ionic strength of 180 mM.\(^{18}\) The computer program of Fabiato\(^{18}\) was used to calculate concentrations of each metal, ligand, and metal-ligand complex. For V\(_{\text{max}}\), measurements on rat ventricular myocytes, solutions were identical to those above except that caffeine was excluded.

Figure 1 presents photomicrographs of a frog chemically skinned atrial myocyte (panel A) and a preparation of rat mechanically disrupted ventricular myocardium (panel B) in relaxing solution and of the same rat ventricular preparation during maximal activation (panel C). After a photograph of a frog myocyte in relaxing solution was obtained, additional photomicrographs of the activated myocyte were not possible since the motion associated with removing the Polaroid camera from the microscope to withdraw the next exposed film broke the cell attachment to the apparatus. Instead, sarcomere length was monitored during activation by using a filar micrometer ×10 eyepiece. Frog myocytes that shortened during maximal activation by more than 0.30 µm per sarcomere were discarded. For measurements on rat myocardium, a 35-mm camera back was installed on the inverted microscope and photomicrographs were taken during steady activations. From the photomicrographs, it was evident that mean sarcomere length in every preparation varied by less than 0.20 µm between rest and maximal activation (compare panels B and C in Figure 1). Within individual preparations of rat ventricle, the standard deviation of sarcomere length measurements was approximately 0.10 µm in relaxing solution and 0.11 µm during maximal activation. These measurements indicate that the compliance of the preparations at the points of attachment was relatively small, suggesting that attachment with silicone adhesive caused minimal damage to the preparation.

Typically, a tension–pCa relation was obtained by initially measuring force during maximal activation (pCa 4.5), followed by contractions at randomly chosen submaximal pCa values and again at pCa 4.5 to assess any decline in the performance of the cell. Tensions at submaximal Ca\(^{2+}\) concentrations were expressed as fractions of the maximum tension developed by the same myocyte at pCa 4.5. Thus, tension–pCa data are presented as normalized, relative tension–pCa relations. Hill plots of the data were fit by least-squares regression with the Hill equation

\[
\log[P_i/(1-P_i)]=n(\log(Ca^{2+}))+\log k
\]

where \(P_i\) is tension as a fraction of \(P_o\) (or \(P/P_o\)), \(n\) is the Hill coefficient, and \(k\) is the intercept of the fitted line with the x axis. By using the constants derived from the Hill equation, tension–pCa curves were fit by computer with the equation

\[
P/P_o=([Ca^{2+}]^n)/(k^n+[Ca^{2+}]^n)
\]

Hill plot transformation of the data allowed direct comparisons of the forms and midpoints of the tension–pCa relations in the two myocardial preparations. V\(_{\text{max}}\) was measured with the slack test method.\(^{19,20}\) Myocytes and disrupted ventricular myocardium were first activated with Ca\(^{2+}\). When steady tension was reached, the preparation was rapidly (within 5 msec) slackened; the preparation was then returned to relax-
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A.

B.

C.

Figure 1. Light photomicrographs of a frog skinned atrial myocyte while relaxed (panel A) and rat disrupted ventricular myocardium while relaxed (panel B) and during maximal activation (panel C). pCa was 9.0 in panels A and B and 4.5 in panel C. For the frog atrial myocyte (panel A) sarcomere length was 2.49±0.14 μm. For the rat ventricular myocardium, the sarcomere length was 2.27±0.04 μm while relaxed (panel B) and 2.17±0.07 μm during maximal activation (panel C). Bars, 25 μm.

Results

Average values and ranges of preparation length, width, sarcomere length, maximum tension, and passive tension are presented in Table 1 for frog chemically skinned atrial myocytes and mechanically disrupted ventricular myocardium from rat. Because we were unable to measure cell depth, force could not be normalized to cross-sectional area. One of the noteworthy differences between the two cell types is the large difference in width, which presumably accounts for the smaller maximum tension generated by frog atrial myocytes and the significantly greater passive tensions in our preparation of rat ventricular myocardium.

Comparison of cumulative tension-pCa relations from frog atrial myocytes and rat ventricular myocardium reveals a difference in Ca\textsuperscript{2+} sensitivity of isometric tension (Figure 2). The [Ca\textsuperscript{2+}] for half-maximal tension

...ing solution and reextended to its original length. This procedure was repeated several times on each preparation, except that the amount of slack introduced was varied. The time required to take up the imposed slack was measured as the interval between the beginning of the length step and the onset of tension redevelopment. During submaximal activations of frog atrial cells, the cell was repeatedly slackened by varying degrees and then reextended during continuous activation\textsuperscript{5} to reduce the decline in tension that was otherwise observed when the cell was subjected to numerous solution changes. Plots of change in length versus duration of unloaded shortening were fit with straight lines using a least-squares method. V\textsubscript{max} in muscle lengths per second was calculated from the slope of each line. A fit was calculated only if a line contained four or more points, and data were discarded for r<0.95 for the fitted lines.
was less for frog myocytes (pCa<sub>50</sub> 6.17) than for rat ventricular myocardium (pCa<sub>50</sub> 6.06). The slopes of the two tension–pCa relations were similar as quantified by Hill plot analysis. In our previous studies with an identical preparation of rat skinned ventricle, but in which the activating and relaxing solutions had no caffeine, a pCa<sub>50</sub> of 5.77 was observed at 22°C. Work by others suggests that caffeine may have a direct effect on myofibrillar proteins to increase Ca<sup>2+</sup> sensitivity. For this reason, we measured the effects of 10 mM caffeine on pCa<sub>50</sub> in nine additional ventricular preparations and found that pCa<sub>50</sub> increased by an average of 0.32 pCa units (Figure 3). Addition of 10 mM caffeine also altered the form of the tension–pCa relation in that the relation became biphasic and was less steep for pCa <6.0.

Fast time-base recordings of tension obtained during slack test measurements on a frog chemically skinned atrial myocyte during maximal and submaximal activation are shown in Figure 4. When steady active tension had developed, the myocyte was slackened by various amounts, in these examples by 10% of maximum length (left) and by 17% of maximum length (right). By visual comparison, it is apparent that the time required to take up a given amount of slack increased as [Ca<sup>2+</sup>] was reduced, indicating a decrease in V<sub>max</sub>. Data from the recordings in Figure 4, together with additional data from the same myocyte, were plotted as change in length versus duration of unloaded shortening (Figure 5), and V<sub>max</sub> was calculated as the slope of a straight line fit to the data. At pCa 4.5, V<sub>max</sub> was 3.43 muscle lengths per second, whereas V<sub>max</sub> during submaximal activation (pCa 6.5) was 1.26 muscle lengths per second. Table 2 presents mean V<sub>max</sub> data from 18 frog atrial myocytes during maximal and submaximal activations. We consistently observed that as [Ca<sup>2+</sup>] was reduced, velocity of shortening also decreased.

For preparations of disrupted ventricular myocardium, fast time-base slack test recordings were obtained during steady activation with Ca<sup>2+</sup> and also under relaxing conditions to obtain a force baseline and a measure of passive tension. These recordings were then superimposed to allow clear delineation of the onset of active tension redevelopment (Figure 6). Change in length was subsequently plotted versus duration of unloaded shortening to obtain V<sub>max</sub>. For the preparation in Figure 7, V<sub>max</sub> decreased substantially when [Ca<sup>2+</sup>] was reduced. Averaging the data from the rat ventricular preparations (n=8) yielded a mean V<sub>max</sub> of 4.46 muscle lengths per second during maximal activation and a mean V<sub>max</sub> of 2.16 muscle lengths per second during submaximal activation (Table 3).

Because sarcomere length during isometric tension development was measured directly, it was possible to estimate the sarcomere length range of shortening during the slack test by assuming proportionality between changes in overall length and sarcomere length. In most cases, the estimated sarcomere length range of shortening was between 2.2 and 1.8 µm, although some preparations shortened to lengths below these (e.g., recordings

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**TABLE 1. Characteristics of Frog Chemically Skinned Atrial Myocytes and Rat Mechanically Disrupted Ventricular Myocardium**

<table>
<thead>
<tr>
<th></th>
<th>Frog (n=18)</th>
<th>Rat (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (µm)</td>
<td>98.7±5.9</td>
<td>108.7±13.6</td>
</tr>
<tr>
<td>Range</td>
<td>63.0–144.5</td>
<td>69.6–171.8</td>
</tr>
<tr>
<td>Width (µm)</td>
<td>3.46±0.30</td>
<td>28.8±2.44</td>
</tr>
<tr>
<td>Range</td>
<td>1.54–5.87</td>
<td>19.7–39.0</td>
</tr>
<tr>
<td>Sarcomere length (µm)</td>
<td>2.36±0.05</td>
<td>2.22±0.02</td>
</tr>
<tr>
<td>Range</td>
<td>1.97–2.57</td>
<td>2.11–2.37</td>
</tr>
<tr>
<td>Maximum tension (mg)</td>
<td>0.09±0.02</td>
<td>1.41±0.13</td>
</tr>
<tr>
<td>Range</td>
<td>0.04–0.23</td>
<td>1.21–1.91</td>
</tr>
<tr>
<td>Passive tension (%P&lt;sub&gt;a&lt;/sub&gt;)</td>
<td>5.1±2.1</td>
<td>9.9±2.0</td>
</tr>
<tr>
<td>Range</td>
<td>1.5–8.1</td>
<td>6.0–20.0</td>
</tr>
</tbody>
</table>

Values are mean±SEM. P<sub>a</sub>, maximum isometric tension.

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**FIGURE 2. Cumulative plot of mean relative tension (P/P<sub>0</sub>) (±SEM) as a function of pCa. For frog atrial myocytes (n=7), pCa<sub>50</sub> was 6.17 and the Hill coefficient was 2.20. For rat ventricular myocardium (n=9), pCa<sub>50</sub> was 6.06 and the Hill coefficient was 2.62. Caffeine (10 mM) was present in all solutions used for these measurements.**

**FIGURE 3. Cumulative plot of mean relative tension (P/P<sub>0</sub>) (±SEM) as a function of pCa obtained from rat ventricular myocardium (n=9) in the presence and absence of caffeine. Mechanically disrupted myocardium was chemically skinned by bathing the preparations for 10 seconds in solution of pCa 9.0 containing 3% Triton X-100. In the absence of caffeine pCa<sub>50</sub> was 5.74, whereas in the presence of 10 mM caffeine pCa<sub>50</sub> was 6.06. The Hill plot (not shown) of data obtained in the absence of caffeine was best fit by a single straight line with a slope (Hill coefficient) of 2.33. In the presence of 10 mM caffeine, the Hill plot was biphasic, with Hill coefficients of 2.32 for pCa >6 and 1.05 for pCa <6.**
in Figure 6). Additional experiments were done to directly assess the sarcomere length range of shortening during the velocity measurements on rat ventricular myocardium. In video recordings made through the microscope, sarcomere length never shortened to less than 1.60 μm, even when extreme shortening steps of 45% maximum length were applied during maximal activation. The video recordings of these preparations showed that the amount of sarcomere shortening in response to a given length change applied during maximal and submaximal activations agreed within 0.10 μm.

**Discussion**

In the present study, static and dynamic mechanical properties of chemically skinned single myocytes from frog atria and mechanically disrupted myocardium from rat ventricles were assessed as functions of [Ca$^{2+}$]. Our results show that the Ca$^{2+}$ sensitivity of isometric tension is greater in frog atrial myocytes than in rat ventricular myocardium. In both types of myocardium, $V_{\text{max}}$ decreased as [Ca$^{2+}$] was reduced. For a given preparation, when identical slack steps were introduced during maximal and submaximal activations, similar changes in sarcomere length were observed, indicating that the reductions in $V_{\text{max}}$ as [Ca$^{2+}$] was lowered were not due to different length ranges of sarcomere shortening.

**TABLE 2. Velocity of Unloaded Shortening in Frog Chemically Skinned Atrial Myocytes**

<table>
<thead>
<tr>
<th>Cell No.</th>
<th>pCa 4.5</th>
<th>pCa 6.0</th>
<th>pCa 6.5</th>
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<tr>
<td>100288-2</td>
<td>3.31</td>
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<td>...</td>
</tr>
<tr>
<td>100488-2</td>
<td>4.23</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>100488-3</td>
<td>3.98</td>
<td>...</td>
<td>...</td>
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<tr>
<td>100588-4</td>
<td>4.08</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>101088-3</td>
<td>7.32</td>
<td>...</td>
<td>...</td>
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<tr>
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<td>...</td>
<td>...</td>
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<td>093088-1</td>
<td>...</td>
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<td>...</td>
</tr>
<tr>
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<td>...</td>
<td>2.31</td>
<td>...</td>
</tr>
<tr>
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<td>...</td>
<td>1.63</td>
<td>...</td>
</tr>
<tr>
<td>100588-2</td>
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<td>...</td>
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<td>...</td>
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<td>...</td>
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<td>...</td>
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<td>121588-2</td>
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<td>...</td>
<td>2.13</td>
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<td>121588-4</td>
<td>3.43</td>
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<td>1.26</td>
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</tr>
<tr>
<td>122288-1</td>
<td>4.24</td>
<td>...</td>
<td>1.96</td>
</tr>
</tbody>
</table>

**Mean** | 4.32 | 2.46 | 1.89 |
**SEM**  | 0.35 | 0.25 | 0.32 |
**n**    | 10   | 6    | 6    |

Values are in muscle lengths per second.
myocardium for mechanical studies, three main criteria were used to assess the quality of each preparation and its attachments to the apparatus. First, to be certain that the compliance of attachments was relatively low, preparations were discarded if sarcomere length varied by more than 0.30 μm in the transition from rest to maximal activation. Second, measurements on a particular preparation were discontinued if P\text{\textsubscript{0}} fell by more than 15%. Third, preparations were used only if a given amount of slack applied at various times during the measurement protocol resulted in similar durations of unloaded shortening. Application of these criteria in this study resulted in elimination of nearly 50% of preparations after initial attachment, but in those cases in which attachment was successful, the preparations typically yielded 20–30 usable contractions.

Maximum isometric tensions generated by the frog skinned atrial myocytes in this study (Table 1) were somewhat greater than the 0.02 mg peak tension reported by Tarr et al\textsuperscript{11} for frog living atrial myocytes during auxotonic contractions. Maximum isometric tensions developed by the rat skinned ventricular myocardium were similar to values found earlier in this laboratory\textsuperscript{17,22} and were also similar to values reported for single skinned ventricular myocytes.\textsuperscript{23} The latter result suggests that the cross section of our mechanically disrupted rat ventricle preparation is approximately equivalent to the cross section of a single ventricular

| Table 3. Mean Velocities of Unloaded Shortening in Rat Mechanically Disrupted Ventricular Myocardium |
|-------------------------------------------------|--------------|--------------|
| Maximal activation                              | 1.00          | 4.46±0.56    |
| (n=8)                                           |              |              |
| Submaximal activation                           | 0.52±0.04     | 2.16±0.27    |
| (n=4)                                           |              |              |

Values are mean±SEM. Velocity measurements are in muscle lengths per second. P/P\text{\textsubscript{0}}, mean relative tension.
myocyte. Both myocardial preparations used in the present study had sigmoidal tension-pCa relations (Figure 2), although the Ca\(^{2+}\) sensitivity of tension developed by the rat atrial myocytes was somewhat greater. This difference in Ca\(^{2+}\) sensitivity of tension developed between frog atrial and rat ventricular myocardium may be due to differences in thin filament protein isoform composition, which has previously been shown to be associated with altered affinity of troponin C for Ca\(^{2+}\).24

The increased Ca\(^{2+}\) sensitivity of tension observed in the presence of caffeine (Figure 3) is most likely due to a direct effect of caffeine on the myofibrillar proteins, possibly due to an alteration of the affinity of troponin C for Ca\(^{2+}\). Because our myocyte preparations were treated with Triton X-100 and the Ca\(^{2+}\) buffering capacity of our solutions was high, the altered sensitivity of tension cannot be explained as an effect of caffeine to mobilize Ca\(^{2+}\) from the sarcoplasmic reticulum. Caffeine also reduced the mean slope of the tension-pCa relation in the range of pCa >6, although the basis for this effect is unclear.

Absolute values of V\(_{\text{max}}\) during maximum activation at 22°C were 4.32 muscle lengths per second for frog atrial myocytes and 4.46 muscle lengths per second for rat ventricular myocardium (Tables 2 and 3). This V\(_{\text{max}}\) value for frog skinned atrial cells is in approximate agreement with results from Tarr et al.11 who found sarcomere shortening velocities of 6–12 \(\mu\)m/sec in frog living atrial cells stimulated with long-duration stimuli at 25°C. Assuming a sarcomere length of 2.3 \(\mu\)m, this corresponds to a V\(_{\text{max}}\) of 2.6–5.2 muscle lengths per second. In mammalian skinned cardiac muscle, a V\(_{\text{max}}\) of 4.2 muscle lengths per second was previously observed in rat trabeculae at 17.1°C, as determined by the slack test.15 In living preparations, V\(_{\text{max}}\) has been obtained by extrapolation from force-velocity relations measured during twitch contractions, yielding values of 6.0 muscle lengths per second in rat trabeculae (25°C)12 and 3.6 muscle lengths per second in cat papillary muscles (29°C).9 Thus, our V\(_{\text{max}}\) values from mechanically disrupted ventricular myocardium agree well with values previously published for both skinned and living mammalian myocardium.

During analysis of the velocity data from single atrial myocytes and disrupted ventricular myocardium, a concern arose about possible effects on the results of passive tension in these preparations. Recent work by Claffin et al.20 on living skeletal muscle fibers showed that when passive tension was increased by stretch, V\(_{\text{max}}\) obtained with the slack test did not vary as long as the slack steps were applied from the same initial sarcomere length or passive tension, although the intercepts of slack test plots increased. In the present study, the initial sarcomere length and passive tension were constant during velocity measurements on a given myocyte, making it likely that the passive tensions characteristic of our ventricular myocytes did not affect V\(_{\text{max}}\). Consistent with the high resting tensions expressed by our preparation of disrupted ventricular myocardium, the y intercepts of the slack test plots (Figure 7) were much higher for this preparation than was previously observed in skeletal muscle and were usually higher than observed in the single atrial myocytes from frog (Figure 5). The y intercept of slack test plots is thought to be a measure of extension of series elasticity,19 and increases in the y intercept in the presence of high passive tension have been associated with an initial rapid recoil of sarcomere length subsequent to the length step.19,25 Our findings are consistent with this idea in that the greater y intercepts in slack test plots from disrupted ventricular myocardium were generally associated with the greater passive tensions in this preparation. For example, the resting tension borne by the frog atrial myocyte in Figure 5 was 0.01 Pa, whereas resting tension for the rat ventricular myocardium preparation in Figure 7 was 0.08 Pa. In addition, the relatively slow response time of our force transducer (from 0% to 100% in 5 msec) would be expected to yield artifically high y intercepts in the slack test plots from both of our myocardial preparations.

Our results show that V\(_{\text{max}}\) decreased substantially when [Ca\(^{2+}\)] was reduced (Tables 2 and 3). Previous work on intact bundles of myocardium has shown that increased extracellular [Ca\(^{2+}\)] increases V\(_{\text{max}}\) as estimated from the intercept of the force-velocity relation.9–13 Using laser diffraction on rat ventricular myocytes floating free in Ringer’s solution, Krueger et al.14 reported that the velocity of sarcomere shortening was 9.07 \(\mu\)m/sec when [Ca\(^{2+}\)] was 1.0 mM and decreased to 3.67 \(\mu\)m/sec when [Ca\(^{2+}\)] was reduced to 0.5 mM. Their cells had an average sarcomere length of 1.8 \(\mu\)m, which yields maximum velocities of 5.03 and 2.03 muscle lengths per second, respectively. However, in similar experiments, Haworth et al.26 found no differences in shortening velocity when [Ca\(^{2+}\)] was increased from 1 to 2 mM. This discrepancy might be explained on the basis of the different ranges of [Ca\(^{2+}\)] used in the two studies, since Daniels et al.12 showed that V\(_{\text{max}}\) in living myocardium increased with [Ca\(^{2+}\)], only to concentrations that resulted in twitch tensions of less than 50% of maximum. The sensitivity of V\(_{\text{max}}\) to a restricted range of low [Ca\(^{2+}\)] might also explain why Herland et al.15 observed no effect of Ca\(^{2+}\) on V\(_{\text{max}}\) in skinned trabeculae, since steady tensions in their study were greater than 50% of maximum at every concentration of Ca\(^{2+}\) that was used.

In skinned skeletal muscle fibers, slack test plots become biphasic as Ca\(^{2+}\) concentration is reduced; i.e., there is an initial phase of high-velocity shortening and a subsequent phase of low-velocity shortening. V\(_{\text{max}}\) in both phases decreases when [Ca\(^{2+}\)] is reduced; however, the low-velocity phase has greater sensitivity to Ca\(^{2+}\).5–7 In the high-velocity phase, V\(_{\text{max}}\) remains relatively constant as activation is reduced to achieve isometric tensions as low as 0.3 Pa, but decreases when activation is reduced below this level. V\(_{\text{max}}\) in the low-velocity phase progressively decreases as [Ca\(^{2+}\)] is reduced. In the present study, we did not observe biphasic shortening in myocardial preparations at low levels of activation, although V\(_{\text{max}}\) was significantly reduced when [Ca\(^{2+}\)] was lowered. Although this difference between skinned skeletal and cardiac muscle preparations may be real, we cannot exclude the possibility that there is a fast phase of shortening in cardiac muscle that we are unable to detect because of the slow response time of our force transducer (i.e., from 0% to 100% response in 5 msec). The apparently greater intercept of slack test plots when activation was reduced (Figures 5 and 7) suggests the presence of an undetected high-velocity phase.5

At least two mechanisms could account for a decrease in V\(_{\text{max}}\) when activation is reduced by lowering the
concentration of Ca²⁺. One possibility dealt with by control experiments in the present study is that the preparations shortened to sarcomere lengths less than 1.65 μm, thereby encountering an internal load that would slow shortening.²⁷ In the presence of such a fixed load, the velocity of shortening would be expected to decrease at low levels of activation as a result of reduced tension-generating capability. This possibility was excluded in the present study by monitoring sarcomere length by direct observation and with a video recording system, which showed that sarcomere length was always between 2.2 and 1.6 μm. Another argument against possible contributions of a passive internal load is that the time course of shortening revealed in the slack test plots is linear. If a structural internal load were impeding shortening, it would be expected to have a greater effect as shortening proceeded; i.e., velocity would be expected to become progressively slower as shortening continued, which is not the case in the present study. The possibility remains that an internal load does slow velocity in heart muscle at low levels of activation,²⁸ although our results suggest that such an internal load does not accumulate as shortening continues, which generally eliminates simple compression of passive structural elements as a likely cause for such a load.

Another mechanism that could account for the decrease in Vₘₐₓ observed at low [Ca²⁺] is that, as the level of thin-filament activation decreases, cross-bridge detachment slows because of a Ca²⁺-dependent decrease in the rate of ADP release,³ which could give rise to long-lived cross-bridges that slow velocity.³ In addition, there is evidence from skeletal muscle that C protein, a thick-filament accessory protein,²⁹ is involved in the slowing of velocity at low levels of activation. Partial extraction of C protein has been shown to reversibly increase Vₘₐₓ in the low-velocity phase of shortening.³⁰ This result, together with evidence that C protein associates with the subfragment 2 portion of myosin,³¹ is compatible with the idea that at low levels of activation C protein places a structural constraint on the crossbridge, which in turn slows velocity.³² The presence of C protein in cardiac muscle³² suggests that cardiac and skeletal muscles may share at least one common mechanism for falloff of Vₘₐₓ at low levels of activation.

Whatever its specific mechanism, Ca²⁺ dependence of Vₘₐₓ in myocardium is likely to play an important role in cardiac function. Extrapolation to the whole heart of our results from isolated myocardial preparations would suggest that, under conditions that reduce myoplasmic [Ca²⁺], the rate of rise of ventricular pressure would be slowed, stroke volume reduced, and cardiac output diminished.

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