Effect of Calcium on Force-Velocity-Length Relations of Heart Muscle of the Cat

By Dirk L. Brutsaert, Victor A. Claes, and Mark A. Goethals

ABSTRACT

The influence of calcium concentration in the bathing solution on the force-velocity relations of the isolated, electrically excited cat papillary muscle was examined. Shortening velocity was directly measured at minimum loads by unloading or load clamping the muscle from the preload (at the length, Lmax, where maximum active tension was developed) to 0 (zero-load clamp), 2, 5, 10, and 15% of the total tension, P0. All measurements were made in the physiological length range between Lmax and 12.5% below Lmax. Increasing the calcium concentration augmented the measured maximum unloaded shortening velocity and shifted the normalized force-velocity relation when force was expressed as a fraction of P0. These observations suggest that the activating calcium has an action more complex than the binding to troponin alone.

KEY WORDS load clamping maximum unloaded shortening velocity normalized force-velocity relations papillary muscle

The activation role of calcium (Ca) in the excitation-contraction coupling of muscle is now well established (1, 2). The influence of Ca on the mechanical performance of isolated skinned and glycerinated fibers of skeletal muscle has been studied recently (3-9). Potentiating effects of Ca on the contractile properties of isolated intact cardiac muscle have been observed (10, 11); some investigators suggest that Ca augments force development (P0) and maximum shortening velocity (Vmax) extrapolated from force-peak velocity relations of afterloaded contractions (10, 12), but such derivations of Vmax are subject to methodological criticism (13, 14). More recently Ca has been shown to influence force development in glycerinated cardiac muscle fibers (15), but the important question of how Ca influences the whole force-velocity relation in heart muscle remains a controversial issue. From a biochemical standpoint, Katz (16) has challenged the view that in cardiac muscle Ca really affects Vmax or the maximum rate of turnover at a single myosin crossbridge, maintaining that the influence of Ca is wholly explicable in terms of the number of crossbridges it activates and that previous observations of changes in Vmax (10, 12) are unreliable or attributable to an internal load.

In the present study the influence of the Ca concentration in the bathing solution on the shortening velocity of heart muscle at minimum loads was examined by unloading the shortening muscle to loads approximating zero in the physiological length range between the length, Lmax, corresponding to maximum active tension development and 12.5% below Lmax (17).

Methods

Ten papillary muscles of the right ventricle of cats were studied. Table 1 summarizes the basic characteristics of the nine muscles in which the influence of Ca was studied. The tenth muscle was used to test the performance of the load-clamping apparatus and to analyze the theoretical basis of the load-clamping procedure (Figs. 1 and 2). The muscles were suspended vertically in a bath containing modified Krebs-Ringer's solution (mM): NaCl 118, KCl 4.7, MgSO4·7H2O 1.2, KH2PO4 1.1, NaHCO3 24, CaCl2·6H2O 2.5 (or 7.5), and glucose 4.5. The solution was bubbled with 95% O2-5% CO2. The temperature was maintained at 29°C and the pH at 7.4. The muscles were stimulated at 12/min with rectangular pulses 5 msec in duration and about 10% above threshold. The stimulus was provided through two platinum electrodes arranged longitudinally, one on each side of the muscle. The lower nontendinous end of the muscle was held by a light phosphor bronze clip soldered to the middle of the spring of the force transducer. The tendinous end of the muscle was tied with a short silk thread extending upwards to the electromagnetic lever system, which was mounted on a Palmer stand immediately above the bath. In all experiments the preload on the muscle was adjusted so that the initial muscle length corresponded to Lmax. The Lmax value was rechecked in the high Ca concentration and again at the end of the experiment. The mean ratio of the resting-to-total force at Lmax was 11.7 ± 0.8% (S.E.) in 2.5 mM Ca and 8.4 ± 0.5% in 7.5 mM Ca (Table 1).
Basic Characteristics of Papillary Muscles and the Influence of Calcium

<table>
<thead>
<tr>
<th>Muscle</th>
<th>L&lt;sub&gt;max&lt;/sub&gt; (mm)</th>
<th>Cross-sectional area (mm&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>Resting tension (g/mm&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>Total tension, P&lt;sub&gt;L&lt;/sub&gt; (g/mm&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>Velocity at P/P&lt;sub&gt;0&lt;/sub&gt; = 0 (L&lt;sub&gt;max&lt;/sub&gt;/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>0.85</td>
<td>0.94</td>
<td>5.58 ± 0.1</td>
<td>3.16 ± 0.1</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>0.54</td>
<td>1.11</td>
<td>9.35 ± 0.2</td>
<td>2.71 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0.81</td>
<td>1.2</td>
<td>9.10 ± 0.3</td>
<td>2.78 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.90</td>
<td>1.11</td>
<td>9.89 ± 0.4</td>
<td>2.84 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.45</td>
<td>1.11</td>
<td>10.7 ± 0.5</td>
<td>2.75 ± 0.2</td>
</tr>
<tr>
<td>6</td>
<td>6.25</td>
<td>0.98</td>
<td>0.92</td>
<td>8.67 ± 0.2</td>
<td>2.66 ± 0.2</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>0.72</td>
<td>1.25</td>
<td>8.54 ± 0.3</td>
<td>2.87 ± 0.3</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>0.90</td>
<td>1.0</td>
<td>10.5 ± 0.4</td>
<td>2.82 ± 0.3</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>0.50</td>
<td>1.2</td>
<td>15.2 ± 0.5</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>7.80 ± 0.4</td>
<td>0.74 ± 0.07</td>
<td>1.09 ± 0.04</td>
<td>9.75 ± 0.8</td>
<td>13.5 ± 0.9</td>
</tr>
</tbody>
</table>

All measurements were made with the preload required to set initial muscle length at L<sub>max</sub>.

ELECTROMAGNETIC LEVER SYSTEM

The lever system was similar to that described previously (18), but with a smaller equivalent moving mass. The aluminum lever (1 mm wide and 40 mm long) had a tapered end to decrease the moving mass and was cemented with epoxy resin to a coil that was suspended in a strong field of a permanent magnet. The equivalent moving mass of the whole system was measured by applying a sinusoidal current of known amplitude and frequency to the coil and recording the amplitude of the displacement at the end of the lever. The amplitude of the current determines the amplitude of the force (F), and the amplitude of acceleration (A) can be calculated from the frequency (f) and the amplitude of displacement (S) by \( A = 4\pi S/F. \) The equivalent moving mass then equals \( F/A \) or 155 mg for the present system. The compliance of the system measured by imposing a known force on the lever fixed against a stop was 2 \( \mu \)g. The current through the coil was controlled by an operational amplifier current source calibrated for step changes in force of 0.1, 1, or 10 g to a total of 29.9 g. Displacement of the lever was measured 13 mm from its fulcrum with a photoelectric transducer which had a linear range up to 2.2 mm of muscle shortening. The force calibration changed less than 0.3% with the position of the lever over this entire range. An active differentiator was used to derive the velocity of displacement of the lever and thus of muscle shortening. The relation between the highest frequency component of the input signal of the differentiator and its critical frequency was smaller than 0.02.

FORCE TRANSDUCER

The force transducer was similar to the type described in another publication (19), but its total compliance was reduced to 3 \( \mu \)g.

RECORDING SYSTEM

Force and shortening velocity were recorded as functions of length during shortening on a storage oscilloscope (Tektronix 5031) and photographed with a Polaroid camera. All variables were also displayed as functions of time on a second storage oscilloscope (Tektronix 5031).

CONTROL UNITS FOR THE LOAD-CLAMPING PROCEDURE AND ANALYSIS OF DATA

Both sudden and slow load alterations were imposed on the shortening muscle. For the sudden load alteration, the control voltage of the current source was switched electronically at the end of the latent period either from one level to another (load clamping) or to zero (zero-load clamping). During these abrupt load alterations, the electromagnetic lever system was slightly damped by changing the output voltage of a second active differentiator, which delivered a feedback signal to the coil. This damping hardly affected the timing of the force step which occurred within 3–5 msec.

Figure 1 illustrates a typical complete unloading experiment in a representative cat papillary muscle. Curve 1 represents an abrupt zero-load clamp from a load of 0.8 g (or 10% of \( P_0 \)). The calibrations of lever movement, the velocity of lever movement, and the time scale were adjusted so that the major portion of active muscle shortening was visible on the oscilloscope. On the phase plane, the zero-load clamp velocity first passed through a high velocity transition phase and then followed a plateau (within 5% of the peak value) extending to a length somewhat less than 15% below \( L_{max} \).

The slow load alterations were performed according to the resting length-tension relation, using the same technique described previously (17). A representative example is illustrated by curve 2 of Figure 1. On the phase plane, the unloading velocity rose to a common plateau with the zero-load clamp velocity (curve 1) over the same length range. The application of this unloading technique according to the resting length-tension relation requires some analysis in terms of the most appropriate analogue model for heart muscle. In the Maxwell model in which resting force is supported by an elastic element in parallel with both the contractile element and the series elastic element, subtraction of the resting force would prevent the transfer of load from the parallel elastic element to the...
series elastic element during shortening (13). Thus, the contractile element would be clamped at zero load during the entire course of shortening, and the maximum shortening velocity would be measured directly over a wide range of contractile element lengths. In terms of the Voigt model, in which the series elastic element and the parallel element, or a two component analogue, or any related model, the interpretation is more complex. Shortening velocity of the muscle after removal of the external load according to the resting length-tension relation would not necessarily correspond to the maximum unloaded shortening velocity of the contractile element at any instant. Because no analogue model can explain all experimental data in heart muscle (13, 14, 20), the unloading according to the resting length-tension relation can also be considered as a means of slowly unloading the muscle at physiological lengths rather than in terms of any of the proposed models. Moreover in Figure 2, when arbitrary unloadings (curves 7 and 8) were imposed at unloading rates which were intermediate between the zero-load clamp (curve 1) and the resting length-tension curve (curve 5), it appeared that the maximum shortening velocity of the muscle at zero load was the same regardless of the preceding mode of unloading. Hence, maximum shortening velocity is determined only by the total load, albeit zero load, on the muscle and not by the sequence of length changes or the mode of unloading the muscle to zero load, at least within this range of lengths and times.

These results further support previous findings that heart muscle always senses total load and that at any given length the shortening velocity always adjusts to this instantaneous total load, independent of the sequence of length and tension changes through which the muscle arrived at that length and load (18). Any consideration of analogue models, especially regarding the role and the origin of the resting tension, will have to be reconsidered for heart muscle relative to these findings.

INERTIAL AND DAMPING FORCES

It is important to question how closely zero load was approached. Inertial force reached peak levels of 2% of $P_0$ in low Ca during the initial velocity transients associated with muscle and equipment compliance during abrupt zero-load clamps. During slow unloading this force was only 0.2% of $P_0$. In the higher Ca concentration, the force was 1.48 and 0.15% of $P_0$, respectively. However, consideration of these forces was not relevant to measurements of velocity after the initial transition phase when shortening velocity was almost constant and, hence, the inertial force negligible.

Some damping force had to be present to reduce the initial peak velocity transiently so that active velocity of muscle shortening could be measured. The use of a light system with small equivalent moving mass reduced the amount of damping force needed, but some damping was still necessary even with the mechanical friction forces that were present in the system associated with muscle movement and friction at the bearings. As
Influence of damping the load-clamping apparatus (top, curves 1–4) and of the speed of switching the load control units from the preload to zero load at constant damping (bottom, curves 7 and 8) on subsequent active muscle shortening at zero load. All tracings are displayed as functions of time on the left and as phase plane velocity-length relations on the right. In the bottom two sections, curves 7 and 8 have been simply superimposed on the tracings of the top two sections. Curve 5 represents a slow unloading according to the resting length-tension relations but could equally well be obtained by appropriately adjusting the speed of switching the load control units. At slower speeds of unloading (not illustrated) subsequent shortening velocity of the muscle was always reduced at any instantaneous length. The circled crosses indicate the velocity which in the present study was taken as the maximum shortening velocity of the muscle. Same muscle as in Figure 1.

illustrated in Figure 2, this additional damping was critically adjusted for each muscle to give optimal conditions for measuring maximum velocity of active shortening. Damping was necessary to reduce initial peak velocity so that active muscle shortening and shortening velocity could be measured at longer muscle lengths. The total force then present was 1 mg/mm sec⁻¹, amounting to 21 mg (0.3% of P₀) at a velocity of 21 mm/sec, the mean value for maximum velocity with the low Ca concentration (Table 1), or to 28 mg (0.3% of P₀) at 28 mm/sec, the mean value for maximum velocity with the high Ca concentration. The markedly higher velocity values in the high Ca concentration despite the higher damping force can only emphasize the validity of the present results.

Although it was not possible to measure the true Vmax with this technique, a good approximation could be obtained, resulting in a much more accurate point on the force-velocity curve than that obtained with Vmax calculated from long mathematical extrapolations of force-peak velocity curves from afterloaded contractions at relatively high preloads, e.g., at Lmax.

Results

Figure 3 illustrates an experiment performed in muscle 6 at Lmax with Ca concentrations of 2.5 mM (A) and 7.5 mM (B); curves 1 and 2 represent the phase plane velocity-length relations at zero load. These curves were obtained by progressive unloading of the shortening muscle according to the length-tension relation (curve 1) and by imposing an abrupt zero-load clamp immediately following the latent period (curve 2). The other four curves represent load clamps from the preload to 2%
Technique employed for measuring force-velocity values at minimum loads and over the physiological length range (muscle 6). A: Calcium concentration of 2.5 mM. B: Calcium concentration of 7.5 mM. In each Ca concentration six contractions are superimposed as phase plane velocity-length relations. All velocity-length relations were obtained starting from the preload required to set the initial muscle length at Lmax. The mean value of this preload was 11.7% of P₀ in the low and 8.4% of P₀ in the high Ca concentration (Table 1). The circled crosses indicate the velocity values used for plotting force-velocity relations in Figures 4 and 5.

The computed mean normalized force-velocity curves in Figures 4 and 5 have been replotted in Figure 6 for direct comparison. In skeletal muscle fibers, P/P₀ has been used to correct for the total number of myosin crossbridges activated (5, 6). If Ca affected only the number of crossbridges activated, one would expect both curves to superimpose. The distinct separation between the curves in the initial portion between 0 and 15% of P₀ could thus indicate that the observed difference in the maximum shortening velocity in the two Ca concentrations persisted regardless of the difference in the number of crossbridges activated in the two states.

However, in the present study P₀ was also greatly enhanced in the high Ca concentration. A small unrecognized internal load would therefore have a relatively smaller effect on the normalized force-velocity relations in the high Ca concentrations with the high P₀ than it would in the low Ca concentration with the low P₀, and a small shift in the normalized force-velocity relation with changes in the Ca concentration would not necessarily exclude the presence of such an unrecognized

(curve 3) and to 5% (curve 4) of P₀ imposed immediately following the latent period, a control curve of the muscle carrying the preload throughout shortening (curve 5), and an afterloaded contraction at 15% of P₀ (curve 6). In the high Ca concentration, an afterloaded contraction normalized at a value corresponding to the normalized preload in the low Ca concentration was also recorded (not illustrated in Fig. 3). Velocity was measured at minimum loads and at lengths between Lmax and 12.5% below Lmax despite the relatively high preload.

All individual peak velocity data obtained with the technique illustrated in Figure 3 were then plotted as functions of normalized force, P/P₀, for the low Ca concentration of 2.5 mM (Fig. 4) and for the high Ca concentration of 7.5 mM (Fig. 5). The force-peak velocity values of any given muscle were always consistently and significantly enhanced in the high Ca concentration, despite some minor overlap of the low Ca velocity values in some muscles and the high Ca velocity values in a few other muscles. Values for maximum unloaded shortening velocity at zero P/P₀ are shown in Table 1.
Individual force-velocity data obtained in all muscles at a Ca concentration of 2.5 mM. The symbols X, +, *, o, □, △, and ▽ correspond respectively to muscles 1, 2, 3, 4, 5, 6, 8, and 9 of Table 1. The data for muscle 7 are not illustrated, since they were not obtained at all percents of P0. Force is normalized as P/P0. With the method of the least square, a hyperbolic relation (solid line) was calculated to fit the experimental data on an IBM 1130 computer.

Discussion

The fact that maximum unloaded shortening velocity of isolated, electrically excited heart muscle is enhanced by Ca agrees with the results obtained in skinned fibers (4) and in glycerinated single fibers of skeletal muscle (6, 7). However, another study on skinned fibers of skeletal muscle (5) reported that Ca did not increase maximum unloaded shortening velocity, and controversy about the exact role of Ca in such preparations still remains. Although the present findings on electrical-
load, \( V_{\text{max}} \) would fall with a decreasing degree of overlap of the filaments due to a reduction in the number of crossbridges. However, the experiments of Gordon et al. (21) demonstrated that, instead of an increase in speed as the degree of overlap increased with fiber shortening, there was actually a slight decrease.

The isolated papillary muscle represents a more complex experimental preparation than the glycerinated skeletal muscle fiber, and additional factors such as heterogeneity and high series compliance can make the analyses of papillary muscle rather difficult and must therefore be considered. Inhomogeneity of the activation process was minimized by applying mass stimulation along the entire length of the muscle. In heart muscle, morphological inhomogeneity of the sarcomere lengths has been demonstrated only at rather short lengths below 85\% of \( L_{\text{max}} \) (22, 23). In the present experiments variation in sarcomere length was therefore reduced by using a high resting length (\( L_{\text{max}} \)) and by measuring all velocities within 12.5\% below \( L_{\text{max}} \). Furthermore, this length range corresponds to the physiological lengths of heart muscle. In this muscle, \( L_{\text{max}} \) is associated with a sarcomere length of approximately 2.2 \( \mu \text{m} \) (22, 24). Since in the intact ventricle sarcomere length decreases from diastolic values of 2.05-2.15 \( \mu \text{m} \) in the midwall to a length of 1.85-1.95 \( \mu \text{m} \) with systole (25), which explains an ejection fraction of 60\%, the physiological length range of cardiac muscle in the ejecting intact heart would encompass lengths between \( L_{\text{max}} \) and 10-15\% shorter than \( L_{\text{max}} \). Moreover, in a previous study (17) it was shown that maximum unloaded shortening velocity is maintained at a constant level, within 5\% of the peak value, over a corresponding length range between \( L_{\text{max}} \) and 12.5\% below \( L_{\text{max}} \); all velocity measurements in the present study were obtained in this physiological length range (Figs. 1-3). Velocity obtained with unloadings at zero-load clamps initiated from shorter initial lengths, e.g., from preloads smaller than 4-5\% of \( P_{\text{o}} \), would be measured below this length range. At these shorter nonphysiological lengths maximum unloaded velocity falls with decreasing length for reasons which are still largely unknown (9, 17, 26-29).

The present challenging observations on heart muscle, the data obtained in glycerinated skeletal muscle fibers (6, 7), and the findings that myosin bridges appear to move in the absence of overlap with actin (30) further emphasize the need for considering other possible and still lacking biochemical links in excitation-contraction coupling.

References


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