MAGNESIUM ION-DEPENDENT CONTRACTION OF SKINNED FROG MUSCLE FIBERS IN CALCIUM-FREE SOLUTION

JAGDISH GULATI
Departments of Medicine and Physiology and Biophysics, Cardiovascular Research Center, Albert Einstein College of Medicine, Bronx, New York 10461

ABSTRACT Skinned frog fibers were reversibly activated in Ca-free solutions containing 0 mM KCl, 23 μM free Mg, and having an ionic strength of ~50 mM. Contractile force was nearly maximal at 22°–25°C and decreased at lower temperatures. Maximal force in Ca-free solution at 50 mM ionic strength was close to twice the calcium-activated force with pCa 5 and 190 mM ionic strength. The force in Ca-free solution could be reduced to zero by raising the concentration of free Mg from 23 μM to 1.0 mM at the same ionic strength (50 mM). On stretching the fiber from 2.0 to 3.2 μm the force decreased; this effect was similar to that seen with Ca-activated fiber and the data support the idea that Ca-free tension is made at the cross-bridge level. Isotonic contraction during Ca-free activation showed a velocity transient as in Ca-activated fiber at 190 mM ionic strength, but the transient in the present case was very much prolonged. This finding suggests that contraction mechanisms for force generation and for shortening are essentially the same in the two conditions, but that certain rate constants of cross-bridge turnover are slower for the Ca-free contraction. Also, the results indicate that, in low ionic strength, Ca binding to thin filaments is not essential for unmasking the cross-bridge attachment sites, which suggests that the steric blocking mechanism is modified under these conditions.

INTRODUCTION

The presence of calcium ions at a concentration above 10^{-8} M is normally required for the contraction of skinned skeletal muscle fibers (Hellam and Podolsky, 1969). These ions bind troponin, initiating a series of events in the thin filaments that lead to a release in the block of cross-bridge attachment (Ebashi et al., 1969). A few years ago, Gordon et al. (1973) observed that at very low ionic strength substantial force is developed at room temperature even in the absence of calcium (free Ca $< 10^{-8}$ M), and suggested that cross-bridges were involved. This force was reversed on raising the ionic strength to ~190 mM. The mechanism underlying this Ca-free activation and the cross-bridge properties in this contraction are not known. Because the shortening behavior of activated fibers gives information on the cross-bridge properties, the present study was undertaken to measure properties of the skinned fibers under both isometric and isotonic contractions in Ca-free solutions (Gulati, 1981).

I find that the contractile force developed by frog skinned fibers in Ca-free solutions is reversed on lowering the temperature to 0°C as well as by raising the free magnesium concentration to 1 mM. Studies of the shortening response to a quick release in the Ca-free condition at low ionic strength showed a velocity transient that is qualitatively similiar to the response during normal contraction with pCa 5 at 190 mM ionic strength (Podolsky et al., 1974).

The results indicate that, in solutions of low ionic strength, binding of Ca to the thin filaments is not essential for unmasking the cross-bridge attachment sites, suggesting complexity in the steric blocking mechanism. On the other hand, the cross-bridge mechanisms for force generation and for shortening are essentially the same for contractions with and without calcium. The rate constants for turnover in this mechanism appear slower in the present study than the rate constants in Ca-activated fibers, however.

MATERIALS AND METHODS

Methods

The mechanically skinned fiber preparation from the semitendinosus muscle frogs (*Rana pipiens pipiens*) was the same as that described earlier (Gulati and Podolsky, 1978, 1981). Fiber selection and attachment and the recoding procedures were also similar to the procedures described there. In addition, the fiber was examined before the experiment for sarcomere uniformity with a Zeiss microscope (model ACM) at 430x (Carl Zeiss, Inc., Thornwood, NY). The attachment of the fiber to each transducer was made with a piece of 8-0 nylon monofilament (Ethicon, Inc., Somerville, NJ). Sarcomere length was adjusted to 2.2–2.3 μm) with laser diffraction (CW Rad model 5R He-Ne laser; beam size, 1 mm).

Solutions

The Ca-free solution contained 5 mM Na_2-ATP, 1 mM $MgCl_2$, 10 mM imidazole, and 5 mM EGTA; its ionic strength was ~50 mM. The

standard relaxing solution contained 130–140 mM KCl in addition to the above, and its ionic strength was 190 mM. The activating solution with calcium contained 4.9 mM CaEGTA plus 0.1 mM EGTA (pCa = 5) (apparent stability constant of CaEGTA = 2.51×10^6). In some experiments in solutions with low and high ionic strengths, 1–5 mM creatine phosphate (CP) and creatine phosphokinase (CPK 100 IU/ml) were added, and ionic strength was maintained with KCl. Free Mg in these solutions was 23 μM, as calculated with selected association constants for the various ligands (Fabiato and Fabiato, 1979). In a series of experiments, the magnesium concentration was raised by making the needed adjustments in ATP and $MgCl_2$. Calculations for these adjustments were made with a microcomputer (Hewlett Packard Co., Palo Alto, CA, model HP-85). The pH was adjusted to 7.00 ± 0.01 at room temperature. Detailed compositions of the various solutions are given in Table I.

The solutions were contained in temperature-controlled experimental chambers (Gulati and Podolsky, 1978). The temperature (0°–25°C ± 1°C) was adjusted with a bipolar controller (Cambion, Campbell, CA).

Sarcomere uniformity was checked in eight fibers by an earlier procedure (Gulati and Podolsky, 1981), both by obtaining the laser diffraction pattern and by photographic observation with a light microscope (250×) during contractions in separate experiments. The fibers selected were determined to contract uniformly along their entire length. Sarcomere uniformity was stable for several minutes of continuous contraction in the test solution at low ionic strength and 13°–16°C, when the force was between 50 and 70% of force in pCa 5 solution.

Experimental Protocol

At a given temperature the fiber was transferred from the standard relaxing solution (Solution I in Table I) to an appropriate solution for activation, and then it was relaxed in the standard solution. The total number of contraction cycles in Ca-free solution (40–50 mM ionic strength) at 13°C and above on each fiber ranged from one to three. In experiments in which isometric force levels in Ca-free solution at low ionic strength were compared with force at 4°–6°C in pCa 5 at 190 mM ionic strength, two successive contractions were used for each data point. The first contraction cycle was made in low ionic strength solution followed by a second cycle at standard ionic strength and pCa 5. This protocol was used because of the tendency of the force level to drop 5–20% in each subsequent contraction in the Ca-free solution at 22°–25°C. The response was somewhat more stable at 16°C than at higher temperatures. Resting tension was measured in the standard relaxing solution after each contraction by manually shortening the fiber by ~5% of its resting length. The experiment was stopped if there was any measurable increase in resting tension.

For isotonic contractions at 16°C the number of releases for a fiber was limited to three. The displacement traces were analyzed by fitting the function $A_1 e^{A_2 t} + A_3$. An algorithm for a least-squares fit of nonlinear parameters was used on the microcomputer. This function gave a good fit for the entire trace in pCa 5 at 16°C and for the transient phase in the Ca-free solution (inset in Fig. 4). Initial velocity v_0 was taken as $A_1 A_2$ in each case. The speed v_t was obtained by fitting a hairline over a part of the trace after the transient, during which the motion was steady, and calculating its slope.

RESULTS

Force Development in Ca-free Solution at 50 mM Ionic Strength

Effect of Temperature. When the skinned fibers were transferred from the standard relaxing solution (190 mM ionic strength) to Ca-free low ionic strength solution, containing ~20 μM free Mg, force was registered in a temperature-dependent manner (Fig. 1). In determining this force-temperature relation, the fiber was equilibrated at a given temperature in the standard relaxing solution for at least 5 min and then transferred to the low ionic strength solution (50 mM) at the same temperature. The force response in this solution was recorded and the fiber was returned to the standard relaxing solution. Next, the temperature was raised to 25°C and the above cycle (standard solution—low ionic strength solution—standard solution) was repeated at this temperature. Fiber developed high force at 25°C, which confirms the finding of Gordon et al. (1973). The force in the first cycle at a given temperature (<25°C) was normalized to the force in the second cycle at 25°C. This combination of the two cycles, the first at a temperature below 25°C and the second at 25°C, are referred to as one round. The above procedure

TABLE I
SOLUTIONS

Materials	Standard ionic strength (190 mM)		Low ionic strength (40 to 50 mM)			
			Fixed Mg ATP (0.8 mM)		Fixed ATP (0.5 mm)	
	I	II	Low Mg III	High Mg IV	Low Mg V	High Mg VI
Na_2ATP (mM)	5	5	4	0.9	0.63	6.2
$MgCl_2$ (mM)	1 (23μM)*	1 (23μM)	0.8 (23μM)	2 (1 mM)	0.12 (23μM)	6.7 (1 mM)
K_2 EGTA (mM)	5	0.1	2	2	2	2
CaK_2 EGTA (mM)	0	4.9	—	—	—	—
Imidazole (mM)	10	10	10	10	10	10
KCl (mM)	130	130	0	23	25	0
Creatine phosphate (mM)	5	5	4	4	1	1
Creatine phosphokinase (units/ml)	100	100	100	100	100	100
Estimated ionic strength (mM)	190	190	52	52	42	42
pCa (estimated)	<8	5	<8	<8	<8	<8
pH (at 22°–25°C)	7	7	7	7	7	7

*In the case of $MgCl_2$, the values within parentheses indicate estimated free Mg.

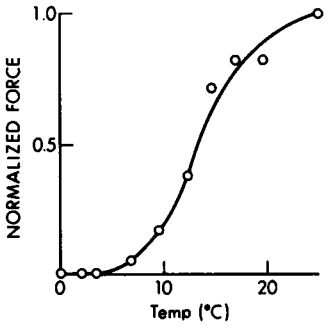

FIGURE 1 Force development in Ca-free solution of calcium low ionic strength (50 mM) as a function of temperature. The results shown are for four fibers; the force in each case was normalized to the measurement at 25°C.

was repeated for the same fiber for a second round, except that the temperature during the first cycle in this round was adjusted to a new (<25°C) value. However, the result of the second round was recorded as a data point only if the force in the second cycle at 25°C, during this round, was within 20% of the force in the second cycle of the first round. Following this criteria, many frog fibers used in this study yielded only a single data point.[1] The results are given in Fig. 1. They show that the force in a Ca-free low ionic strength solution is maximal at 25°C and is practically zero at 0°C. The increase in force is relatively modest from 0° to 10°C, but it increases markedly between 10° and 16°C.

Effect of Triton X-100, Caffeine, and EGTA. Experiments were made to examine the possibility that the force at the low ionic strength in Ca-free solution is a result of Ca release from sites in the sarcoplasmic reticulum. In these experiments, 0.1 or 0.2% Triton X-100 detergent was included in both the standard relaxing solution and in the Ca-free activating solution. Presence of the detergent did not block the tension development during 10 min of incubation at 22°C. Similarly, varying the EGTA concentration between 1 and 5 mM with the ionic strength fixed at 50 mM did not significantly alter the tension in Ca-free solution (Gordon et al., 1973). Furthermore, adding 5 or 10 mM of caffeine also had no measurable effect on force during 30 min of incubation. A 6- to 7-min incubation in 0.05% Triton X-100 empties the sarcoplasmic reticulum of Ca (Stephenson and Podolsky, 1977). Also caffeine drastically reduces the ability of the sarcoplasmic reticulum to accumulate Ca (Moisescu and Thieleczek, 1978). Because these agents did not block the ability of the fibers to develop force in Ca-free solution, I conclude that the release of Ca is not a factor in the development of force in Ca-free solution under the present experimental conditions.

Effect of Sarcomere Length. To ascertain the influence of filament overlap on force development in Ca-free solution, experiments were made at stretched length (SL = 3.0 to 3.3 μm) and compared with those at rest length (SL = 2.0 to 2.2 μm) on fibers from five frogs. Because the force level in low ionic strength is not constant in repeated cycles, these experiments at stretched and rest lengths were carried out in pairs, on separate segments from the same muscle. The results of a typical experiment, on two fiber segments from the same muscle, are shown in Fig. 2. The first segment was activated at rest length (upper panel, Fig. 2) to determine the relation between force in the presence of Ca (pCa = 5, temperature = 5°C, ionic strength = 190 mM) and in the absence of Ca (solution III, temperature = 20°C). Force was 44 mg in the pCa 5 solution. Force in the Ca-free, low ionic solution was 64 mg, which gives a factor of 1.5 for the relation between force level in this solution to the level in pCa 5 solution.

The lower panel in Fig. 2 gives the force responses on the second fiber segment at the sarcomere length of 3.2 μm (contractions 2 at 20°C and 3 at 5°C in Fig. 2). The first contraction (1) in this case was made at the sarcomere length of 2.2 μm with pCa = 5 at 5°C. Two observations may be made from this result. First, the calcium-activated force in the stretched fiber is lower than the force at rest length, by a factor of 0.46 (compare contractions 3 and 1). The mean value of this factor, from results on five fibers, was 0.35 ± 0.14. This is in agreement with previous results

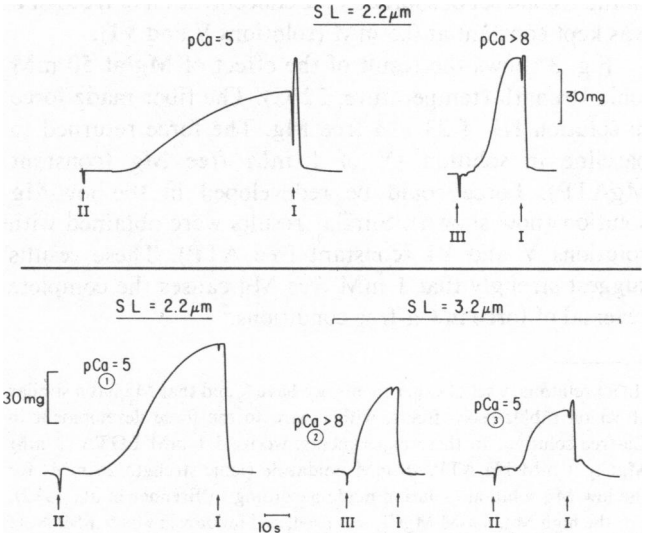

FIGURE 2 Effect of fiber stretching on tension in Ca-free solution. The sarcomere length is 2.2 μm in the *upper* panel and 3.2 μm in the *lower* panel at left. In each case the first contraction relaxation cycle was made in pCa 5 (solution II) at SL = 2.2 μm. This was followed by the cycle in the Ca-free solution (ionic strength, 50 mM) at the appropriate length. The temperature was 20°C.

[1]In preliminary experiments, I have found that the skinned fiber preparations (Gulati, 1975) from rabbit and hamster psoas muscles are much more stable in a series of contractions at low ionic strength, than was the case for the frog preparation. The reason for this difference in the stability of force response with the preparations from different species (i.e., mammalian or amphibian) was not further investigated.

on tetanically stimulated intact fibers (Gordon et al., 1966) and on skinned fibers (Schoenberg and Podolsky, 1972) which have been taken to indicate that the level of force development is related to the extent of filament overlap and the number of cross-bridge attachments.

The second observation to be made from the results of Fig. 2 is that the contractions in Ca-free and pCa 5 solutions at the stretched length give a value of 1.5 for the ratio of force levels in the two solutions at this length (compare contractions 2 and 3 in the lower panel of Fig. 2). The factor of 1.5 is the same as that found above at rest length (upper panel in Fig. 2), indicating that the influence of Ca-free solution on the force-generating mechanism is similar at the two lengths, and that the force in Ca-free solution is affected by the change in filament overlap in the same proportion as the Ca-activated force.

Effect of Magnesium. Magnesium ions can compete with part of Ca on troponsin (Potter and Gergely, 1975) and influence the interaction of tropomyosin with actin in solution (Eaton et al., 1975). Because troponin and tropomyosin constitute the major regulatory system of thin filaments in vertebrate fibers, I examined the effect of magnesium on tension development in Ca-free conditions.

The fiber was allowed to develop tension in Ca-free solution at 40–50 mM ionic strength and 23 μM Mg, and then was transferred to a solution having the same low ionic strength but containing 1 mM free Mg. Force of the fiber was recorded continuously. Such experiments of raising the free Mg concentration in the absence of Ca were made in two sets of solutions. In the first set, the concentration of MgATP was maintained at 0.8 mM, and free Mg was either 23 μm or 1 mM (solutions III and IV). In the second set of solutions the concentration of free ATP was kept constant at 0.5 mM (solutions V and VI).

Fig. 3 shows the result of the effect of Mg at 50 mM ionic strength (temperature, 22°C). The fiber made force in solution III of 23 μM free Mg. The force returned to baseline in solution IV of 1 mM free Mg (constant MgATP). Force could be redeveloped in the low-Mg solution (now shown). Similar results were obtained with solutions V and VI (constant free ATP). These results suggest strongly that 1 mM free Mg causes the complete reversal of force in Ca-free conditions.[2]

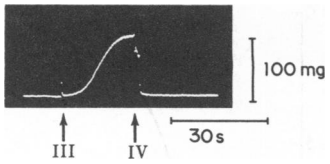

FIGURE 3 Effect of high Mg (1 mM) on the contractile force in Ca-free solution of low ionic strength and 23 μm free Mg. The fiber resting in the standard relaxing solution (solution I) was transferred to the chamber containing low Mg, low ionic strength solution (solution III; Mg, 23 μm) at the arrow marked III. After the development of force was complete, at arrow IV, the fiber was transferred to the solution with high free Mg (solution IV; Mg, 1 mM; ionic strength, 50 mM). Note that the force trace returns to the original baseline, showing that force is completely abolished in the presence of 1 mM Mg. Similar results were obtained when solutions III and IV were replaced with solutions V and IV, respectively. Temperature was 25°C.

It is possible that the CP-CPK "regenerating" system, routinely included in solutions, might be more effective at high Mg than at low free Mg, implying that if a MgATP gradient existed along the fiber radius, it would then be more effectively reduced at high Mg than at low Mg. To check the effect of this gradient on the present results, new sets of solutions III and IV were made in which CPK was not included, so that the regenerating system was not effective in either solution. The force responses in these solutions were the same as above, being reversed in the presence of high Mg. This does not support the explanation requiring decrease in MgATP gradient for the observed reversal in force in high Mg, but favors our idea that the force development in Ca-free solution is directly influenced by free Mg. For, if the former explanation were correct, the force level would have remained unaffected in the high Mg solution without CPK, since total MgATP is kept the same in both solutions III and IV.[3]

Effects of High (1 mM) Mg on Ca-activated Force. As an additional control for the above experiments, the effect of magnesium was also studied on the isometric force response in the presence of calcium (pCa 5), at fixed concentrations of MgATP (1 mM) and ionic strength of 50 and 190 mM. The results are shown in Table II for 11 fibers. In each case the force measurement is normalized to the force in solution II.

The first two columns in Table II summarize the results in the Ca-free condition. As described above, the tension at 22°C is completely reversed by raising the concentration of

[2]In a preliminary set of experiments, we have found that Mg has a similar effect on rabbit psoas fibers, with regard to the force development in Ca-free solution. In these experiments, we used 1 mM EGTA, 3 mM $MgCl_2$, 1 mM Na_2ATP, 10 mM imidazole (ionic strength-20 mM) for the low-Mg solution (solution made according to Brenner et al., 1982). For the high Mg, 5 mM $MgCl_2$ was used, and imidazole was 5 mM. Note that in these experiments, MgATP and free ATP were practically constant when free Mg was raised from 2 to 4 mM. Force was developed in the Ca-free solution (25°C) at 2 mM free Mg, and was completely reversed at 4 mM Mg. Note that the Mg effect requires higher free Mg in rabbit than in frog fibers, but this could be due to the fact that ionic strength is 20 mM in the experiments with rabbit fibers and 50 mM with frog fibers (Gulati, unpublished).

[3]The concentration of creatine phosphate in the present solutions was at most 5 mM, which amount is much lower than that estimated for intact cells (20–30 mM; Dawson et al., 1977). The question arises, therefore, is 5 mM creatine phosphate sufficient for a completely effective regenerating system at any Mg? This point was not possible to check for low ionic strengths in the present study, because of the relatively high contribution of CP (due to its having the valence of 2) to the total ionic strength. But it is worth emphasizing that the conclusion of the Mg-effect is still correct, because, as pointed out in the text, this effect occurs whether or not the regenerative system was included.

TABLE II
RELATIVE FORCE LEVELS* IN THE PRESENCE AND ABSENCE OF CALCIUM

Ca-free solution		pCa = 5, 190 mM ionic strength		pCa = 5, 50 mM ionic strength	
Low Mg‡ (20–22°C)	High Mg	Low Mg	High Mg (0°C)	Low Mg	High Mg (0°C)
1.85 ± 0.1(11)§	0(10)	1.0(21)	1.05 ± .03(8)	2.37 ± 0.2(12)	2.14 ± .3(5)

*Each force level was normalized to that in pCa = 5, 190 mM ionic strength at 0°C and low Mg concentration.
‡Low Mg = 23 μM, high Mg = 1 mM.
§The values are means ± SEM. Numerics with parenthesis indicate the number of value determinations.

free Mg from 22 μM to 1 mM. In contrast, there is relatively little change in the Ca-activated tension with Mg, either at 190 or 50 mM ionic strength at 0°C. This lack of effect of Mg at pCa 5 at 0°C is in agreement with the finding of Donaldson and Kerrick (1975), who found that Mg (ionic strength, 150 mM) has relatively little effect on maximal Ca-activated tension at room temperature.

Isotonic Contraction During Ca-free Activation at 16°C

Because the isotonic contraction properties of muscle fibers in quick-release experiments give information on the cross-bridge turnover (Civan and Podolsky, 1966; Sugi and Tsuchiya, 1981), such experiments were performed in Ca-free solution of low ionic strength. Fig. 4 shows the displacement response of a fiber to a force step P_{rel} (= P_L/P_o, where P_o is isometric force before the release and P_L is external load during shortening) of 0.43 in the Ca-free solution (ionic strength, 50 mM, right panel). Also shown (left panel) is the control response to a similar force step (0.48) at physiological ionic strength (pCa = 5, ionic strength = 190), both at 16°C. The displacement trace for the response with calcium is practically linear. In the Ca-free solution the trace is greatly curved and the speed of shortening decreases continuously over the recorded duration (~350 ms). The initial speed of shortening (indicated in each case by the sloping dashed lines) in the Ca-free solution (right panel) is 0.7 times that of the Ca-activated fiber in the left panel.

The presence of an enhanced curvature during the contraction in Ca-free solution suggests that the mechanism underlying the isotonic shortening in this solution may have time- and/or distance-dependent factors that are different from those operative in contraction with calcium at standard ionic strength. To study these differences in more detail, the isotonic response in the Ca-free solution was recorded on a slower time scale. Fig. 5 shows the typical records obtained at two different force steps. After the initial curvature (Fig. 5, right panel), the speed increases to nearly a steady value (dashed line in Fig. 5,

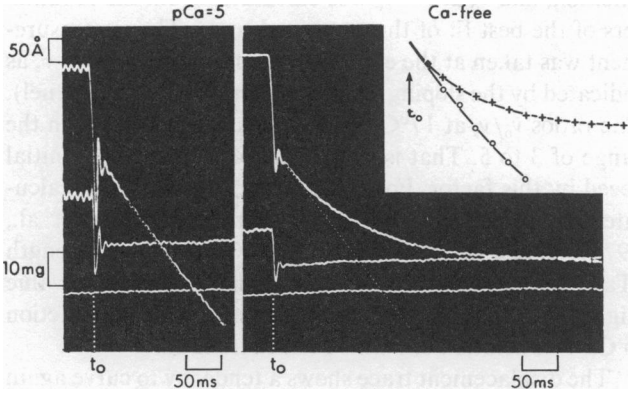

FIGURE 4 Isotonic contraction at 16°C. *Left* panel: contraction in pCa 5, 190 mM ionic strength. *Right* panel: contraction in Ca-free solution, 50 mM ionic strength. *Top* trace in each panel shows displacement; *middle* trace, force; and *bottom* trace, zero of force. Fiber length = 1.9 mm. Isometric force in pCa 5 = 29 mg, P_{rel} = 0.48. Isometric force in Ca-free solution = 19 mg, P_{rel} = 0.43 in the *right* panel. The displacement marker indicates a length of 50 Å per half sarcomere. The vertical dashed lines indicate the start of isotonic motion at $t = t_o$. Initial speed in each case is shown by a sloping dashed line. The parameters for these lines were estimated from the computer best nonlinear least-squares fit to the actual displacement trace. *Inset*: goodness of the computer exponential fit. x, data points from the *right* panel; o, data points from the *left* panel. The lines joining data points are the computer fits.

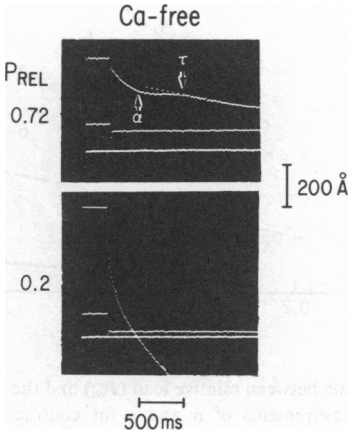

FIGURE 5 Isotonic velocity transient in low ionic strength Ca-free solution, at a slow time base. Temperature, 17°C. The different traces in each of the two panels are as identified in Fig. 2. P_{rel} values are as indicated; fiber length = 1.5 mm; isometric force levels are 30 and 26 mg in the top and bottom panels, respectively. The dashed line shows the manner in which the speed v_s was measured at the end of the transient. The end of the fast phase is marked by α and the null time of the transient is marked by τ.

upper panel). In the example shown, this feature is prominent for where P_{rel} = 0.72. This type of response is qualitatively the same as that seen by Podolsky et al. (1974) in Ca-activated fibers at 190 mM ionic strength and 0°–4°C.

For the quantitative analysis of this response, the time τ (which indicates the null time of the transient and is found by extrapolating the steady speed to its intersection with the instantaneous motion) and the fast phase α of the transient (during which the speed is higher than at steady state) were marked off (Podolsky et al., 1974). Fig. 6 shows these measurements as a function of relative load on 12 fibers. The lowest P_{rel} was around 0.25 where τ = 270 ms and α = 85 ms; at the highest value of P_{rel} (0.78) in this study, τ = 950 ms and α = 220 ms. Such measurements of the transient were difficult at P_{rel} below 0.25. Least-squares fitting of the data points in Fig. 6 indicates a tendency for a and t to increase with relative load. The ratio α/τ is calculated for each set of measurements (inset to Fig. 6, and Table III A) and is constant with a mean value of 0.33 at 16°–17°C. For comparison, Table III A also lists the mean value of these measurements on transients in Ca-activated skinned fibers at 0°–5°C. The mean values for α/τ are almost the same in both Ca and Ca-free activation, although the null times differ appreciably in the two cases (Fig. 6). This suggests that the factors underlying the various phases of the transient are changed in Ca-free activation, from those with Ca, but that the effects are uniform throughout the course of the transient.

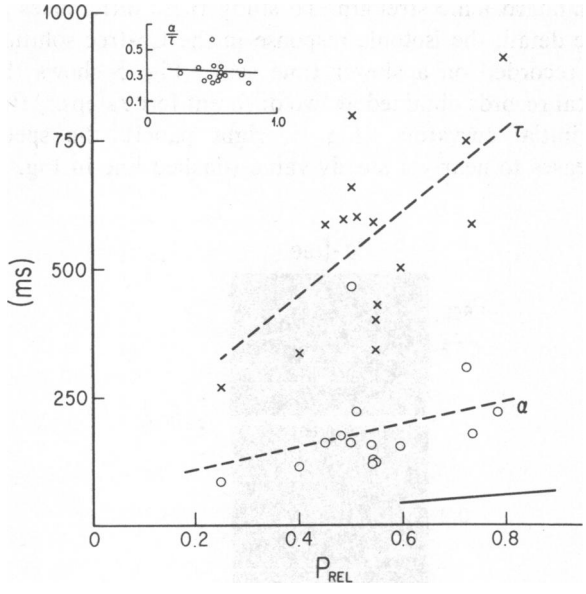

FIGURE 6 Relation between relative load (P_{rel}) and the isotonic velocity transient. The measurements of α and τ for contractions in Ca-free solution (13°–17°C) of 12 fibers from different frogs are shown by the dashed lines. These lines are least-squares fits to the corresponding set of data points. The solid line is the fit for measurements of τ in Ca-activated skinned fibers (these data taken from Fig. 2 in Podolsky et al., 1974). The τ values obtained in the present study for contractions in Ca-free, low ionic strength solution are ~10 times higher than those for Ca-activated fibers at 190 mM ionic strength. *Inset*: α/τ as a function of relative load and the least-squares fit of the data. The ratio α/τ is practically unchanged with relative load.

TABLE IIIA
DURATION OF THE FAST PHASE (α) RELATIVE TO THE NULL TIME (τ) OF THE VELOCITY TRANSIENT

P_{REL}	α/τ	
	Ca-free, 0 KCl (16–17°C)	pCa = 5, 140 KCl*
0.25–0.78	0.33 ± 0.02 (15)	0.39 ± 0.01

TABLE IIIB
RELATION BETWEEN INITIAL SPEED (v_o) AND STEADY SPEED (v_s)

P_{REL}	(v_o/v_s) in Ca-free, 0 KCl (16–17°C)	(v_o/v_s) in pCa = 5, 140 KCl* (0 – 5°C)
0.2–0.49	4.8 ± 0.52 (6)	
0.50–0.78	4.5 ± 0.39 (13)	6.2 ± 2.5 (3)
0.2–0.78	4.6 ± 0.31 (19)	6.2 ± 2.5 (3)

*The measurements for α/τ in pCa 5 were taken from Table 1 in Podolsky et al. (1974). The values for v_o/v_s in pCa 5 were estimated from Figs. 1 and 3 in Podolsky et al. (1974). P_{REL} range in these cases was 0.75–0.80, and the temperature range was between 0° and 5°C.

Table III B shows the ratio (v_o/v_s) of initial speed at $t = t_o$ to steady speed after τ. To estimate this ratio, the transient phase of the isotonic response to approximately two-thirds the duration of τ was fitted with an exponential function, and v_o at $t = t_o$ was calculated from the parameters of the best fit of the computed trace. The v_s measurement was taken at the end of the transient at $t = t_o + \tau$, as indicated by the sloping dashed line in Fig. 5 (upper panel). The ratios v_o/v_s at 17°C for Ca-free contraction are in the range of 3 to 5. That is, steady speed is lower than initial speed by this factor. For comparison, v_o/v_s was also calculated on previously published records (Podolsky et al., 1974) for Ca-activated fibers at 190 mM ionic strength (Table III B). The mean of v_o/v_s in this case is 6.2, a value slightly larger than that observed presently for contraction in Ca-free solution of 50 mM ionic strength.

The displacement trace shows a tendency to curve again after the steady phase (Fig. 5, top panel). This part of the motion was not analyzed.

DISCUSSION

The present study shows that contractile force in low Mg, Ca-free solution at 50 mM ionic strength and 20° to 25°C is nearly the same as the force with pCa 5 at the same ionic strength and 0°C (Table II). The force development in Ca-free solution decreased when sarcomere length was increased, and this effect is also practically the same as that seen in Ca-activated fibers, which indicates that

cross-bridges are involved in the development of force in the absence of Ca, as in the presence of Ca. However, the force in Ca-free solution was decreased at lower temperatures and was practically abolished at 0°C. Furthermore, the force in Ca-free solution at high temperature was completely reversed at high (1 mM) free Mg, but similarly raising the free Mg concentration had no effect on maximal force with pCa 5. These findings suggest that mechanisms for transitions in the cross-bridge between the nonforce and force-generating states and/or the mechanisms of control are different in the two types of activation.

Another finding is that isotonic displacement response of the fiber following a quick release during contraction in Ca-free medium (ionic strength, 50 mM) contains a velocity transient similar to the response during activation with Ca (ionic strength, 190 mM). The null time and duration of the fast phase of the transients with both types of activations decrease with decreasing relative load, and the ratio of fast phase duration to null time is constant (Fig. 6). A major difference in the transient response under Ca-free activation from that of fibers activated in pCa 5 solution at 190 mM ionic strength and 0° to 5°C is that the null time at a given load step in the present case is over 10 times longer than in the Ca-activated fiber (Podolsky et al., 1974). A common explanation for the various types of mechanical transients suggests the existence of detailed relations between the rate constants of different steps in the cross-bridge turnover and suggests that there is an influence of these rate constants on the properties of the transients (Hill, 1974). On the basis of this explanation, the present results suggest that the rate constants may be slower for contraction in the absence of Ca (ionic strength, 50 mM) than for contraction in the presence of Ca (ionic strength, 190 mM).

Before the transients were measured for contraction without Ca, the curved trace for the isotonic response, such as in Fig. 4, had suggested that the cross-bridges might be deactivated during shortening (Gulati and Podolsky, 1981). However, the fact that the curved trace in the present case is the initial part of the prolonged velocity transient, is a strong indication that a simple deactivation is not a major mechanism for the overall shortening behavior in the Ca-free (0 KCl) solution.

Mechanism of Regulation in the Absence of Calcium

The most widely held view of the regulation process, with calcium, in the case of vertebrate skeletal muscle, is the steric blocking mechanism. According to this theory, a sequence of events is initiated by calcium binding to troponin, which leads to an appropriate shift in the position of tropomyosin, thereby opening the active sites on the thin filament for cross-bridge attachment.

This type of steric mechanism gives the simplest explanation for activation in the absence of Ca also, provided the initial steps in the above sequence of events are modified in solutions of low ionic strength. One possible modification is suggested by the observed difference in the effects of Mg in the presence and absence of calcium. High Mg reversed the tension produced in the absence of Ca, but had no measurable effect on the Ca-activated force at pCa 5. It is interesting that magnesium ions also increase the binding of tropomyosin to actin in solution at low (~25–45 mM) ionic strength (Eaton et al., 1975). Raising the ionic strength to 115 mM had a similar effect to high Mg, i.e., ionic strength increased the binding of tropomyosin to actin in low Mg. With ionic strength, as with high Mg, the binding of tropomyosin is correlated with increasing inhibition of the acto-HMM ATPase in the absence of Ca, suggesting that the binding of regulatory proteins to actin in solution may be equivalent to tropomyosin shift in the fiber. According to this view, therefore, the tropomyosin shift for cross-bridge attachment can be produced also as a direct effect of agents other than Ca (e.g., ionic strength, Mg), under appropriate experimental conditions.[4]

The presence of the Mg-effect suggests that the light-chain moieties of myosin may be involved also in the mechanism of activation under the present conditions. There is evidence that the association of at least one of the light chains (LC2) is affected by EDTA (presumably in part due to chelation of Mg) (Chantler and Szent-Gyorgyi, 1980) and the removal of these light chains has physiological effects on the speed of shortening in the skinned skeletal muscle fibers (Moss et al., 1982). However, since the Mg effect in the present study is fully reversible, the influence of low ionic strength, involving the light chains, is probably more subtle than the EDTA treatment.

Effect of Temperature. The effect of temperature on force (Fig. 1) could be explained if the tropomyosin shift on actin filaments in low ionic strength were modulated by temperature in some way (e.g., as in the presumed switchlike action of calcium), so that an increasing number of cross-bridges were attached and producing force at temperatures above 0°C. This would imply that the activation is graded with temperature. Another possible mechanism for the effect of temperature is that the number of attached bridges is constant at different temperatures (at a given low ionic strength), but that force per bridge is increased as the temperature is raised. This latter possibility is supported by the recent studies of Brenner et al.

[4]On the other hand, on the basis of recent biochemical studies, also in low ionic strength, Eisenberg and his coworkers have proposed an alternative to the steric blocking mechanism (Chalovich et al, 1981). They suggest that tropomyosin acts not by blocking the cross-bridge binding sites on actin filaments but by changing the rate constant of a specific kinetic step (the Pi release step) in the cross-bridge turnover. However, the influence of free Mg on the above step is not known, which makes it difficult presently to relate these new ideas of regulation to the fiber results in Ca-free solutions.

(1982) on rabbit psoas fibers, who confirmed the finding that force in Ca-free solution of low ionic strength at 25°C is reversed at 0°C (Gulati, 1981), and showed in addition that the stiffness (measured by the application of a fast, 0.1 ms, length step; ionic strength, 20 mM; 5°C) remained high. These results suggested that the bridges were attached in a zero-force conformation at 0°C, and shift to a high-force conformation with temperature.[5] In this light it is interesting that recent ^{31}P NMR studies of myosin-nucleotide complexes in solution (Shriver and Sykes, 1981 *a*, *b*) and quantitative studies relating nucleotide binding to mechanical strain in rigor fibers (Marston et al., 1979), have also indicated two distinct cross-bridge conformations. Furthermore, the relative distribution of these conformations is altered with temperature (Shriver and Sykes, 1981 *a*, *b*), which suggests in addition that there may be a relation between these various NMR conformations of the myosin in the solution experiments and the various possible force conformations of the cross-bridges in an active muscle at low ionic strength.

Relation to Studies on Intact Cells

The resting tension in whole frog muscle (Okada and Gordon, 1972) and in single fibers (Gulati and Gross, unpublished) is constant on transfer of the tissue from normal solution to solution with 0.3 times the physiological tonicity at 25°C. This result may be similar to that seen in the skinned fibers in Ca-free solution high in Mg and provides additional support for the idea that free Mg in the intact cell is in the millimolar range (Gupta and Moore, 1981; Baylor et al., 1982).

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[5]The explanation that the force development in low ionic strength solution is possibly due to a shift in equilibrium amongst the two force conformations, would also require a comment on the effect of Ca in low ionic strength. The results in Table II show that the force in pCa 5 at 50 mM ionic strength and 0°C is very similar to the force in Ca-free solution at 50 mM ionic strength but at 22°C. This suggests that in a low ionic strength solution, Ca is able to produce the same effect on cross-bridge force conformation as temperature. Therefore, the results can be taken as additional evidence that the Ca-effect in low ionic strength may be different from, or in addition to, its switchlike action at physiological ionic strength (Gulati and Podolsky, 1981).

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