ENERGETICS OF ACTIVATION IN FROG SKELETAL MUSCLE AT SARCOMERE LENGTHS BEYOND MYOFILAMENT OVERLAP

DANIEL M. BURCHFIELD AND JACK A. RALL
Department of Physiology, The Ohio State University, Columbus, Ohio 43210

ABSTRACT Experiments were designed to gain information about the effects of extremely long sarcomere lengths (>3.8 μm) on muscle activation. The amount of energy liberated in an isometric twitch by muscles stretched to sarcomere lengths where myofilament overlap is vanishingly small (>3.6 μm) is thought to be an indirect measure of the Ca^{2+} cycled during contraction. The effects of altering sarcomere length from 3.8 to 4.3 μm on the amount of Ca^{2+} cycled was measured using twitch energy liberation as an indicator of the Ca^{2+} cycled. Twitch energy liberation decreased by ~20% over this sarcomere length region, suggesting that the amount of Ca^{2+} released by a single action potential is not altered dramatically when a muscle is stretched to extreme lengths.

INTRODUCTION

Activation processes in vertebrate striated muscle are often investigated under conditions where acto-myosin or cross-bridge interaction is greatly depressed. This depression is usually achieved by stretching muscles to sarcomere lengths where myofilament overlap is vanishingly small. For example, structural studies of activation employing x-ray diffraction techniques have been conducted on isolated muscles stretched to sarcomere lengths from 3.6 to 4.5 μm (1) and from 3.8 to 4.3 μm (2). Optical measurements of the transient increase in intracellular free Ca^{2+} concentration after stimulation have been made in cells stretched to sarcomere lengths of 3.6 to 4.3 μm (3, 4). In energetic studies of activation, muscles are usually stretched to a resting sarcomere length of ~3.8 μm (5). The interpretation of some of the results from these investigations depends on the supposition that the activation processes are not altered in a major way when the muscle is stretched. Nonetheless, the amplitude of the Ca^{2+} transient, as measured with the photoprotein aequorin during a twitch in frog skeletal muscle, diminishes by 25–70% as a cell is stretched to a sarcomere length of 3.6 μm (6). With aequorin it is difficult to quantitate the decrease in amount of Ca^{2+} released from the sarcoplasmic reticulum (SR) because: (*a*) the signal is sensitive to free Ca^{2+} to the 2.5 power, (*b*) the distribution of Ca^{2+} is longitudinally non-uniform during transient activation, and (*c*) the majority of Ca^{2+} released binds to myoplasmic proteins. Recent optical observations of free Ca^{2+} transients in the range of sarcomere lengths from 3.0 to 4.1 μm and subsequent calculations have led to the conclusion that it is unlikely that stretch exerts any great effect on the amount of Ca^{2+} that is released after a single action potential (4). To gain further information about the effects of long sarcomere lengths on muscle activation, twitch energy liberation has been measured in the sarcomere length range of 3.8–4.4 μm and beyond. The energy liberated in an isometric twitch at long sarcomere lengths has been attributed to the cyclic movements of Ca^{2+} released with muscle activation (7–9). The amplitude of the energy liberated is thought to be an indirect measure of the amount of Ca^{2+} released with activation (9). It was reasoned that if stretch caused a major decrease in Ca^{2+} release from the SR, then further stretch to extreme sarcomere lengths might greatly diminish twitch energy liberation. The main result from this investigation is that the energy liberated during an isometric twitch decreases by ~20% in the sarcomere length range of 3.8–4.3 μm. This result leads to the suggestion that the amount of Ca^{2+} released in a twitch is modestly decreased in this sarcomere length range.

METHODS

Experiments were carried out on isolated semitendinosus muscles from frogs (*Rana pipiens*). Methods for measurement of force and energy liberation, for stimulation, for control of bath temperature, as well as the composition of the Ringer's solution, have been described (10). Resting sarcomere length at which twitch force production was maximum averaged 2.43 ± 0.07 μm (mean ± standard error of the mean) (n = 5). Sarcomere length was determined by laser diffraction after the energy liberation measurements were made. At each muscle length, sarcomere length measurements were made at 4–5 positions evenly distributed along the length of the muscle and results were averaged.

Address correspondence to Dr. Rall.

The chamber containing the thermopile and muscle was immersed in a thermostatic bath at 0°C. After a 30–45-min equilibration period, the Ringer's solution was drained from the chamber to permit determination of the stimulus parameters and muscle length required to elicit maximal twitch force and energy liberation. During experimental runs of 20–35-min duration, reproducible mechanical and energetic records were obtained at an average sarcomere length of 2.43 μm and then, in most cases, the muscle was stretched to an average sarcomere length of 3.93 ± 0.06 μm, where virtually all twitch force was eliminated. From this length the muscle was stretched in 0.5-mm steps and stimulated at each length until the energy liberation records exhibited a large decrease, at which point the muscle was returned to 2.4 μm and force and energy liberation were measured again. Between runs the muscle was immersed in Ringer's solution and rested for 30 min.

RESULTS

Fig. 1 shows one experiment in detail. In Fig. 1 *A* the amount of energy liberated in an isometric twitch is plotted against peak force development at resting sarcomere lengths ranging from 2.36 to 4.43 μm. The straight line is the result of linear regression of the data in the sarcomere length range of 2.36–3.78 μm (solid squares). The intercept, 1.96 mJ/g, is 27% of the energy liberated when maximum twitch force was developed, and is thought to reflect the amount of Ca^{2+} cycled during contraction. Fig. 1 *B* displays twitch energy liberation plotted vs. resting sarcomere length. Energy liberation decreases dramatically with increasing sarcomere length until a sarcomere length of 3.78 μm is reached. Beyond this point energy liberation (open squares) decreases slightly (by 14%), with further increase in sarcomere length until an inflection point is reached. The inflection point occurs at a resting sarcomere length of 4.29 μm. Beyond the inflection point, energy liberation decreases abruptly. The average value of the energy liberation in the sarcomere length range of 3.89–4.29 μm is 2.02 mJ/g, which compares to the extrapolated value of 1.96 mJ/g.

Using the data from Fig. 1 *B*, linear regression analysis was performed to determine the relationship between energy liberation and sarcomere length in the regions 2.36–3.78 μm (*a*) and 3.89–4.29 μm (*b*). The slopes of the regression lines are significantly different with a ratio (*a*/*b*) of 4.8, and the lines intersect at a sarcomere length of 3.68 μm. This sarcomere length corresponds to the length where active force production is predicted to be zero according to the sliding filament theory of contraction. Thus energy liberation varies linearly with sarcomere length, but the relationship changes dramatically at a sarcomere length of 3.6–3.7 μm.

Fig. 2 is a plot of twitch energy liberation vs. resting sarcomere length in the region beyond 3.8 μm. This figure displays the results of all experiments. Energy liberation decreases modestly as the muscle is stretched until an inflection point is reached between 4.18 and 4.67 μm. For each of the experiments a linear regression relationship was established between energy liberation and sarcomere length in the region up to and including the inflection point. The mean value of the ratio of predicted twitch energy liberation at 4.3 μm to that at 3.8 μm is 0.81 ± 0.04 (range of 0.91–0.68).

DISCUSSION

The present results indicate that in the resting sarcomere length range of 3.8–4.3 μm, twitch energy liberation decreases in amplitude and this decrease is ~20%. These results suggest that the amount of Ca^{2+} released with stimulation decreases in this sarcomere length region by ~20%. These results do not address the sarcomere length region <3.8 μm because significant energy liberation due to cross-bridge cycling complicates the analysis. Nonetheless, Baylor et al. (4) have observed an ~20% decrease in the amplitude of the Ca^{2+} transient measured with the dye arsenazo III in an isometric twitch in the sarcomere length range of 3.0–4.1 μm. Since their theoretical analysis indicated that a twofold change in the Ca^{2+} transient would result from a 21% change in the amount of Ca^{2+}

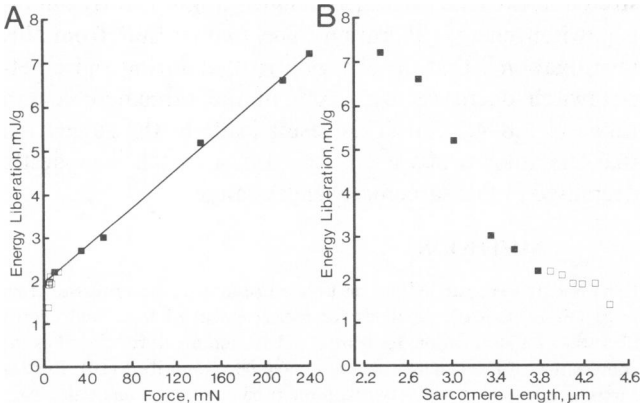

FIGURE 1 Plots of energy liberation in an isometric twitch vs. peak force development (*A*) and resting sarcomere length (*B*). Filled squares: data recorded in the sarcomere length region of 2.36–3.78 μm. Open squares: data recorded when the muscle was stretched beyond 3.78 μm.

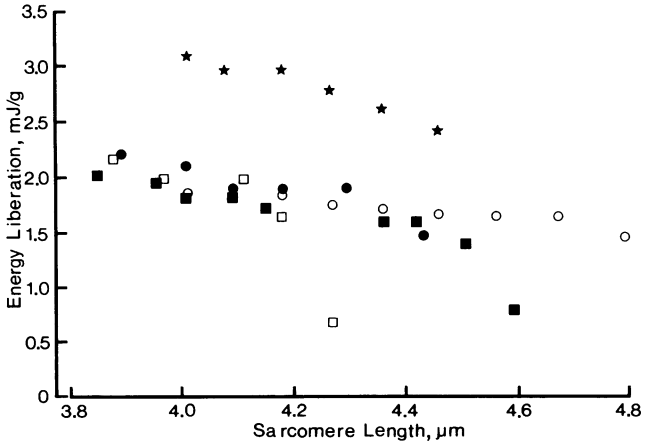

FIGURE 2 Plot of energy liberation in an isometric twitch versus sarcomere length in the region beyond 3.8 μm. Five separate experiments. Twitch energy liberation at 3.93 μm averaged 31 ± 3% of the value at 2.43 μm.

released, they concluded that it seemed unlikely that stretch exerts any great effect on the amount of Ca^{2+} that is released after a single action potential. Further detailed analysis is speculative because of the likelihood of unmeasured longitudinal nonuniformities of Ca^{2+} distribution that could considerably complicate the analysis. Results of Blinks et al. (6) indicate that the amplitude of the Ca^{2+} transient measured with the photoprotein aequorin diminishes by 25–70% as a cell is stretched from a sarcomere length of 2.3–3.6 μm. Because of the 2.5 power dependence of luminescence on free Ca^{2+} concentration, the aequorin signal gives an exaggerated impression of the actual decrease in free Ca^{2+} concentration, which may actually be ~10–40%. This result is similar to the data of Baylor et al. (4), and thus would lead to a similar conclusion. Consistent with these results, neither the properties of voltage-dependent charge movement nor of mechanical activation are significantly affected when frog muscle cells are stretched from a sarcomere length of 2.5–3.8 μm (11).

Taken together, these results indicate that the amount of Ca^{2+} released by a single action potential decreases modestly as a muscle is stretched from a sarcomere length of 2.3–4.3 μm. Ca^{2+} release may decrease by ~20% of the maximum value at sarcomere lengths approaching 4.3 μm. This is an important conclusion because it affects interpretation of the shape of the length-tension relation as muscles are stretched beyond optimal myofilament overlap. Further, the observation that myosin heads do not move on activation in vertebrate striated muscle stretched to long sarcomere lengths (2) is critically dependent on the extent to which activation is independent of muscle length. Thus the current results strengthen the conclusion that myosin heads are not directly sensitive to free Ca^{2+} concentration during activation of intact muscle (2). This conclusion has important implications for molecular modeling of muscle activation.

This research was supported, in part, by Public Health Service grant AM-20792 from the National Institutes of Health and a Research Career Development Award 1K04NS-00324 to J. A. Rall.

Received for publication 26 May 1985 and in final form 19 July 1985.

REFERENCES

1. Huxley, H. E. 1972. Structural changes in the actin- and myosin-containing filaments during contraction. *Cold Spring Harbor Symp. Quant. Biol.* 37:361–378.
2. Yagi, N., and I. Matsubara. 1980. Myosin heads do not move on activation in highly stretched vertebrate striated muscle. *Science (Wash. DC).* 207:307–308.
3. Baylor, S. M., W. K. Chandler, and M. W. Marshall. 1982. Use of metallochromic dyes to measure changes in myoplasmic calcium during activity in frog skeletal muscle fibres. *J. Physiol. (Lond.).* 331:139–177.
4. Baylor, S. M., W. K. Chandler, and M. W. Marshall. 1983. Sarcoplasmic reticulum calcium release in frog skeletal muscle fibres estimated from arsenazo III calcium transients. *J. Physiol. (Lond.).* 344:625–666.
5. Curtin, N. A., and R. C. Woledge. 1981. Effect of muscle length on energy balance in frog skeletal muscle. *J. Physiol. (Lond.).* 316:453–468.
6. Blinks, J. R., R. Rudel, and S. R. Taylor. 1978. Calcium transients in isolated amphibian skeletal muscle fibres: detection with aequorin. *J. Physiol. (Lond.).* 277:291-323.
7. Homsher, E., W. F. H. M. Mommaerts, N. V. Ricchiuti, and A. Wallner. 1972. Activation heat, activation metabolism and tension-related heat in frog semitendinosus muscles. *J. Physiol. (Lond.).* 220:601–625.
8. Smith, I. C. H. 1972. Energetics of activation in frog and toad muscle. *J. Physiol. (Lond.).* 220:583–599.
9. Rall, J. A. 1982. Energetics of Ca^{2+} cycling during skeletal muscle contraction. *Fed. Proc.* 41:155–160.
10. Rall, J. A. 1979. Effects of temperature on tension, tension-dependent heat, and activation heat in twitches of frog skeletal muscle. *J. Physiol. (Lond.).* 291:265–275.
11. Hui, C. S., and W. F. Gilly. 1979. Mechanical activation and voltage-dependent charge movement in stretched muscle fibres. *Nature (Lond.)* 281:223–225.