Length-Dependent Electromechanical Coupling in Single Muscle Fibers

A. M. GORDON and E. B. RIDGWAY

From the Friday Harbor Laboratories, Friday Harbor, Washington 98250. Dr. Gordon's present address is the Department of Physiology and Biophysics, University of Washington, School of Medicine, Seattle, Washington 98195, and Dr. Ridgway's is the Department of Physiology, Medical College of Virginia, Richmond, Virginia 23298.

ABSTRACT In single muscle fibers from the giant barnacle, a small decrease in muscle length decreases both the calcium activation and the peak isometric tension produced by a constant current stimulus. The effect is most pronounced if the length change immediately precedes the stimulation. In some cases, the decrease in tension with shortening can be accounted for almost entirely by a decrease in calcium release rather than changes in mechanical factors such as filament geometry. During the constant current stimulation the muscle membrane becomes more depolarized at longer muscle lengths than at the shorter muscle lengths. Under voltage clamp conditions, when the membrane potential is kept constant during stimulation, there is little length dependence of calcium release. Thus, the effect of length on calcium release is mediated through a change in membrane properties, rather than an effect on a subsequent step in excitation-contraction coupling. Stretch causes the unstimulated fiber membrane to depolarize by about 1 mV while release causes the fiber membrane to hyperpolarize by about the same amount. The process causing this change in potential has an equilibrium potential nearly 10 mV hyperpolarized from the resting level. This change in resting membrane potential with length may account for the length dependence of calcium release.

INTRODUCTION

The active force that a muscle can develop depends strongly on muscle length. For most muscles the active force reaches a maximum at a length near the in situ length and declines at longer and shorter lengths. Since the work of Gordon et al. (1966) who measured the maximum tetanic tension in frog skeletal muscle as a function of sarcomere length, the explanation for this relationship has focused on mechanical factors such as filament geometry. This focus on mechanical factors occurred because of the striking correlation between the maximum tetanic tension and the extent of the overlap of thin filaments with “bridges” in the thick filaments at sarcomere lengths at and above that for which tension is a maximum. The decline in tension at shorter sarcomere lengths did not have as convincing an explanation in terms of mechanical factors although a number were possible. Activation was assumed to be maximal, so that all variations in force were attributed to variations in mechanical factors such as filament overlap, restoring forces, etc. More recent evidence (Fuchs, 1974) indicates that at
short sarcomere lengths, tetanic stimulation may not produce maximal activation (Rüdel and Taylor, 1971; Schoenberg and Podolsky, 1972; Taylor et al., 1975). Also the sarcomere length-peak-tension relationship for twitch contractions differs from that for tetanic stimulation and is very labile, again suggesting a variation in contractile activation with length (Close, 1972). Thus, factors other than mechanical ones may sometimes determine the relationship between muscle length and tension, particularly at short muscle lengths.

This paper demonstrates that in single muscle fibers from the barnacle Balanus nubilus, which have been microinjected with the luminescing calcium indicator aequorin, there is a length dependence of calcium release under conditions of constant current stimulation. Less calcium is released at shorter muscle lengths than at longer muscle lengths. Over some ranges of muscle length the length dependence of peak isometric tension can be accounted for almost entirely by the length dependence of Ca release rather than by mechanical factors including filament geometry. The second part of the paper deals with the response of the fiber under voltage clamp conditions. Here some of the eventual effects of length changes on force are shown to be mediated through an intermediate effect of length on the electrical properties of the muscle membrane. A preliminary account of this work has appeared (Ridgway and Gordon, 1975).

METHODS

Materials

Large single muscle fibers having lengths of 20-30 mm and weights of 30-60 mg were obtained from the barnacle Balanus nubilus.

Procedure

The fiber was dissected, cannulated, and then microinjected with 0.4 μl of the aequorin solution by methods similar to those described by Ashley and Ridgway (1970). The concentration of aequorin in the injection solution was about 5 × 10⁻⁴ M (estimated from solubility or from photon yield—this estimate is probably accurate to within a factor of 5). This implies that the average final concentration of aequorin in the fiber is of the order of 5 × 10⁻⁴ M, depending on the size of the fiber. Next, the aequorin-injected fiber was placed in the muscle fiber chamber illustrated in Fig. 1. The fibers were allowed to stand for a period of ~0.5 h to allow the aequorin to diffuse throughout the sarcoplasm. During this time the amplitude of the light response gradually increased to a plateau. For current clamp experiments, a single 100-μm tungsten wire which served as the internal electrode was inserted through the cannula to within 1-2 mm of the tendon end of the fiber. For current and voltage clamp experiments, a double spiral electrode (Hodgkin et al., 1952), having the voltage measuring wire (Ag-AgCl) exposed for 0.5 cm and the current injecting wire exposed for 2.5 cm, was used. The recording chamber was fitted with several transducers. First, a photomultiplier tube (PMT) was used to record the light produced in the reaction between intracellular calcium and the injected aequorin. Second, a force transducer was connected to the fiber by a chain and lever system. Third, a length transducer recorded muscle length. For some experiments a thermistor was incorporated to record temperature.

The entire muscle chamber was light tight and a second light-tight box surrounded both the transducers and the recording chamber. During the experiments the output
signals of the transducers and the electrodes (i.e. length, tension, calcium-mediated light, current, and voltage) were displayed on a cathode ray oscilloscope. Because the oscilloscope had only four independent channels, only four of the five measurements could be displayed simultaneously.

Sarcoplasmic Calcium Detection

Sarcoplasmic calcium concentration was detected by using the calcium luminescing protein, aequorin (Shimomura et al., 1962). The aequorin injection solution was prepared by desalting purified aequorin (Johnson and Shimomura, 1972) on a Sephadex G-25 column which had been equilibrated with 250 mM potassium chloride, 4 mM TES [N-Tris (hydroxymethyl) methyl-2-amino ethanesulfonic acid] pH 7.0. It is important to note that the desalted aequorin prepared in this way had no toxic effects on the single muscle fibers, and both normal membrane responses and normal force responses were recorded up to 8 h after injection.

The photomultiplier tube (PMT) used to record the calcium-mediated light output (calcium transient response) was an EMI number 9635A. The circuit for the PMT was similar to that given by Baker et al. (1971) with a time constant of 10 ms. The response of
the PMT was linear over the range of light intensities encountered in these experiments. The PMT output is expressed in terms of the anode current which is proportional to the incident light. A factor for converting the anode current to the approximate number of aequorin molecules reacting per second is: $1 \mu A = 1.6 \times 10^6$ molecules reacting per second. This assumes a quantum efficiency for aequorin of 0.25 (Shimomura and Johnson, 1969). Light was normally collected from the whole length of the fiber. However, to be certain that light from the ends of the fiber (either at the cannula or at the tendon near the tip of the electrode) was not giving spurious results, we used a mask in a number of experiments to exclude light from the ends. The results were the same both with and without the mask.

**Force and Length Measurements**

Fig. 1 shows how the barnacle muscle fiber was arranged in the chamber through which cold solutions could be passed. In this chamber, light and force output were measured at various lengths under conditions of constant current or constant voltage stimulation with the internal axial electrode. Force was measured with a transducer similar to that used by Hellam and Podolsky (1969) modified to accept the high force values of single barnacle muscle fibers. The response of the force transducer was linear to about 20 g. Muscle length could be changed by using a Shaker pot (LTV model 203) which was driven from pulse generators through a HP 6824B power amplifier. Muscle length was measured by a United Detector Technology PIN-SC/10 position transducer with a light emitting diode (LED) (United Detector Technology, Inc., Santa Monica, Calif.) mounted on the moving element of the Shaker pot. Mechanical connection to this fiber was achieved with a light gold chain and lever connections as indicated in Fig. 1. This was done to enable us to insert the electrodes vertically into the muscle fiber. Changes of muscle length and muscle force had to be corrected for the lever arm ratio. Because of the slow rate of development of force in these fibers, force was measured with adequate time resolution with this arrangement. It was determined by visual observations and markers that length changes were distributed fairly uniformly over the entire length of the fiber. The length change occurred rapidly and was not followed by subsequent creep.

**Voltage and Current Clamp Experiments**

The voltage clamp circuit used was similar to that described by Hagiwara et al. (1968). Currents were measured at virtual ground by a current-to-voltage converter. To assure relatively constant currents, a resistance of 47 kΩ was placed in series with the current electrode. For the temperature and solutions used, these fibers do not generate action potentials during the constant current stimulation but instead give graded responses.

**Saline**

The saline used was prepared according to the formula of Hoyle and Smythe (1963) except that 4 mM TES, pH 7.5, replaced the bicarbonate. The temperature of the saline in contact with the single fiber was held at about 11°C.

**RESULTS**

**Basic Effect of a Length Change**

Under constant current stimulating conditions, the muscle length at the time of stimulation markedly affects both the height of the “calcium transient” (the light signal) and the peak isometric force as illustrated in Fig. 2. Records 2 A and 2 C were taken at the control (in situ) length. When the muscle is prereleased
(allowed to shorten) by about 1% of the initial muscle length (2B), both the height of the calcium transient and the isometric force are substantially decreased. Prestretch (lengthening, 2D) has the opposite effect: both the height of the calcium transient and force are substantially increased. Thus a small length change has a large effect: the decrease in light and force is about 80% in Fig. 2B for a 1% shortening while the increase is about 30% for a 1% lengthening (2D). The sensitivity to length change was somewhat variable from fiber to fiber but the length dependence of the Ca transient and the force was seen in all fibers.

![Figure 2](image)

**Figure 2.** Effect of 1% prerelease and 1% prestretch under constant current conditions. A, B, C, and D are control, release (shortening), control, and stretch (lengthening), respectively. Trace 1 is fiber length recorded at 1.05 mm/cm, increasing length is in the upwards direction. Trace 2 is the calcium transient recorded at 200 nA cm⁻¹. Trace 3 is isometric force recorded at 0.55 g cm⁻¹. Trace 4 is stimulus current recorded at 50 μA cm⁻¹. Horizontal sweep is 200 ms cm⁻¹. Calibration bar 1 cm. Temperature 11° C. Initial fiber length 22 mm, fiber weight 60 mg.

**Timing of the Length Change**

Part of the effect of a length change on the calcium transient and force persists long after the length change is accomplished. Fig. 3 is a plot of the peak height of the calcium transient, the peak isometric force, and the passive force in a muscle fiber as a function of muscle length. In these experiments, the muscle was allowed to equilibrate for 15 s at each new length before stimulation. This time was long enough for a steady state to be reached. As shown in the figure, for length changes which are about 16% of the initial muscle rest length, there is a substantial variation in both the peak force and peak light output with length. Fibers were usually not extended to a length at which the maximum active force began to decrease as the length was increased. In several fibers where this range was investigated, it appears as if the decrease in peak active force was not
accompanied by a substantial decrease in the height of the calcium transient. In Fig. 3, the active force shows a small decrease only at the longest length.

As the delay between the length change and the stimulus pulse is reduced, the effect of the length change on both the calcium transient and the force becomes more pronounced. Both Figs. 4 and 5 illustrate this observation. In Fig. 4 A, B,

![Graph](image_url)

**Figure 3.** Relationship between passive force (□), peak active force (△), and peak of the Ca transient (○) as a function of muscle length. The muscle was held at this length for 15 s before stimulation. Force is plotted on the scale on the left, the Ca transient on the scale on the right. The points were taken as muscle length was changed from longest to shortest. Fiber length change measured from an initial length of 24.5 mm. Fiber weight 59.6 mg.

![Graph](image_url)

**Figure 4.** Effect of timing of length change on calcium transient and force. Each record shows the responses to two identical stimuli with a 4% length change (shortening) imposed before the second (test) stimulus. Recovery of the calcium transient to control levels is greater in A than in B. Trace 1, stimulus current, recorded at 4.2 μA cm⁻¹. Trace 2, fiber length recorded at 0.42 mm cm⁻¹. Trace 3, isometric force recorded at 0.7 g cm⁻¹. Trace 4 is the aequorin light signal recorded at 40 nA cm⁻¹. Horizontal sweep 1 s cm⁻¹. Calibration bar 1 cm. Temperature 11°C. Initial fiber length 21 mm, fiber weight 25 mg.

the fiber length was reduced by about 4% at two different times between two constant current stimuli which otherwise would have given equal "calcium transients" and peak isometric force responses. It can be seen in Fig. 4, where the length change immediately precedes the second stimulus, that the effect of the length change is greater than for a length change which is delivered earlier (Fig. 4 A). In Fig. 5, these data are plotted for several release times. In this figure
the time of release is measured from the beginning of the second stimulus. The peak light output for the second stimulus is plotted as a fraction of the value it would have had there been no release (the value at the initial length, $L_0$). As is shown in this figure, the decrease in the peak light output is maximized if the length change is made immediately before the constant current stimulus pulse. If the change is made a few seconds before the stimulus pulse, the effect of the length change has decreased substantially by the time of the occurrence of the stimulus pulse. The half-time of this adaptation process was on the order of a few seconds. In most fibers the effect of a length change did not adapt completely so that fibers showed an effect of steady-state length on peak light output and peak twitch force.

*Relationship between Light and Force after a Length Change*

From the data presented in Figs. 2-4 it is clear that under constant current stimulating conditions, both the calcium transient and the force decrease with decreasing muscle length. The question naturally arises as to how much of this decrease in peak isometric force is due to a decreased calcium release and how much is due to mechanical factors such as filament geometry. To answer this question we varied the force and light output in two separate ways. First, force and light output were varied by changing the intensity of the stimulus. This

---

**Figure 5.** Relationship between time of release and the effect of the release on the height of the calcium transient. The protocol is as illustrated above and in Fig. 4. The fiber was allowed to shorten at a time between two constant current stimuli which gave identical responses if there was no length change. The time of release (shortening) is measured from the beginning of the second stimulus, negative meaning before, positive after. The calcium transient observed when the length change occurred at that time is plotted as a fraction of the value it would have had there been no length change (the value at the initial length). Initial fiber length 21 mm, fiber weight 25 mg. Temperature 11°C.
procedure changes force by varying Ca release. Then by use of the same fiber, force and light output were varied by changing muscle length. This second experiment changes force by varying mechanical factors and/or Ca release. If variations in force with muscle length are caused entirely by variations in the calcium release (reflected in the peak light output), then the relationship between peak force and peak light output should be similar whether force is varied by changing length or by changing stimulus intensity.

In Fig. 6, it can be seen that over a range of length changes and stimulation conditions, the relationship between peak light and peak isometric force is the same whether these parameters are varied by changing stimulus intensity or changing length. Thus, over this range the length dependence of peak isometric force is being determined by the length dependence of the Ca release and not by mechanical factors.

When mechanical factors become important (for example, when the length change is large), one would expect a deviation between the two curves. This sort of behavior is shown in Fig. 3 where the force begins to fall off at longer lengths.
even though the Ca transient is still increasing. For the fiber shown in Fig. 3 and others there was a significant deviation of the two curves plotting peak force against peak light at the longer lengths consistent with some influence of mechanical factors decreasing force at a given Ca level.

The overall relationship between the peak of the Ca transient and peak isometric force was usually linear but sometimes curved. Because of the problems in quantifying the aequorin light signal in terms of Ca concentration (Baker et al., 1971), we have not attempted to attach any special significance to shape of the curve.

Another phenomenon demonstrated by the fiber shown in Fig. 6 is the nonzero intercept along the light axis, i.e. there is additional light (Ca) on stimulation but no detectable force. Apparently for barnacle fibers, the aequorin is more sensitive to Ca than the myofilaments, at least to the extent that myofilaments can turn bound Ca into detectable force. This intercept was observed previously (Ashley and Ridgway, 1970).

**Length-Dependent Effects at Higher Force Levels**

In the results previously described, the peak isometric force developed was a few grams. This amounted to force levels per unit cross-sectional area of the order of 100–400 g/cm². At higher force levels it appears that for the small length changes investigated, there is less dependency (measured as a fractional change) of calcium release on length than at the lower force levels. For example, in one experiment when the muscle was released or restretched by 0.4 mm (about 2% of the muscle length) before the stimulation, the ratio between the peak light output at the longer lengths to that at the shorter lengths was 1.83 for a peak isometric force of about 0.7 g and was 1.02 for a peak isometric force of 4.7 g.

In another experiment for a length change of 1%, the ratio between the peak light output at the longer length to that at the shorter length varied from 1.9 at a peak force of 0.7 g compared to 1.5 at a peak force of 7.8 g. However, even though the fractional change in the peak of the Ca transient is greater at the lower force levels, the absolute change might be equal or greater at the higher force levels. This absolute difference is just a smaller fraction of the total height when the force and Ca transient are high. Maximum force levels were not attainable in these cannulated preparations.

**Is Calcium Release Length Dependent?**

We considered the question of whether the length dependence of the Ca transient is due to a length dependence of calcium release or to a length dependence of some other process having to do with calcium binding or uptake.

It is conceivable that length dependence of Ca uptake by the sarcoplasmic reticulum or a length dependence of calcium binding to proteins other than those activating contraction might produce this effect. Length dependence of calcium binding is ruled out as a reasonable hypothesis by data presented in the next section where it will be shown that under voltage clamp conditions the length dependence of the Ca transient is minimized or eliminated. If the binding were length dependent it would be hard to understand how voltage clamp conditions would eliminate the length dependence.
Length dependence of calcium uptake is shown to be of little importance by the following two observations. The declining phase of the calcium transient occurs after the stimulus current has been decreased to zero. This declining phase follows a single exponential and presumably reflects the disappearance of free Ca from the sarcoplasm, either uptake-binding to the sarcoplasmic reticulum or binding by other proteins in the sarcoplasm. In either case, the time constant of this process does not appear to be length dependent, implying that changes in uptake with length are not responsible for the decline in the height of the calcium transient with muscle length. The evidence then suggests that these two alternatives are not tenable and that it is Ca release which is affected by muscle length.

**Length Effect on the Membrane Potential**

Because the length dependence of the calcium transient, and hence force, could be produced in a number of possible ways, we began by investigating the effects of length on the membrane potential.

In Fig. 7 are shown the results of an experiment where the membrane potential was recorded during constant current stimulation at two different muscle lengths. Here the magnitude of the voltage response seen at the shorter muscle length is smaller than that seen at the longer length. The smaller depolarization for the same constant current at shorter length could well be the direct explanation of the reduced calcium release and reduced peak isometric force. This explanation of the effects of length puts the site of action at the first step in excitation-contraction (E-C) coupling. It also makes the results shown in Fig. 3 more understandable since both the stimuli and the length are operating through a common element, namely the membrane potential. In addition, this explanation makes the testable prediction that controlling the membrane potential should eliminate the length effects.

Fig. 8 shows records from a voltage clamp experiment designed to test the effects of length. The effect of a length change on the calcium transient is virtually eliminated by the voltage clamp, yet there is still a small length dependence of force. Although Figs. 7 and 8 are not taken from the same fiber in the same experimental session, we did perform both kinds of experiments a number of times in the same fiber. In all cases, the voltage clamp reduced the length dependence of the calcium transient to a negligible percentage of its value under constant current stimulating conditions. These results strongly imply that the length dependence of the calcium transient is mediated by a process which ultimately affects the membrane potential and not by some other process such as, for example, a change in the relationship between membrane potential and calcium release. The voltage clamp experiments have a second implication which is not as obvious as the first. If the voltage clamp had not eliminated the length effects, one possible explanation would have been that the clamp was inadequate. However, one voltage clamp did eliminate the length dependence of the calcium transient and therefore, whatever the voltage our electrode is recording, it is proportional to the appropriate “coupling” voltage.

The fact that the voltage clamp eliminated the length dependence of the
calcium transient while decreasing substantially but not eliminating the length dependence of force implies again that mechanical factors can also affect the length dependence of force.

The Mechanism of Length Effect

Having concluded from the voltage clamp experiments described here that the primary effect of length occurs at the level of the membrane potential, we next

---

**FIGURE 7.** Responses of membrane potential to prestretch and prerelease with constant current stimulus. The records are superimposed. For each trace, the upper one was recorded at the longer muscle length. Trace 1, membrane potential recorded at 10 mV cm\(^{-1}\). Trace 2, calcium transient recorded at 100 nA cm\(^{-1}\). Trace 3, isometric force recorded at 0.55 g cm\(^{-1}\). Trace 4, stimulus current recorded at 100 \(\mu\)A cm\(^{-1}\). Horizontal sweep 100 ms cm\(^{-1}\). Calibration bar 1 cm. Temperature 11°C. Initial fiber length 22 mm, fiber weight 60 mg.

**FIGURE 8.** Response of fiber to length changes under voltage clamp conditions. A, prerelease (0.3 mm). B, prestretch (0.3 mm). Trace 1, membrane potential recorded at 10 mV cm\(^{-1}\). Trace 2, calcium transient recorded at 200 nA cm\(^{-1}\). Trace 3, isometric force recorded at 0.5 g cm\(^{-1}\). Trace 4, clamp current recorded at 50 \(\mu\)A cm\(^{-1}\). Horizontal sweep, 100 ms cm\(^{-1}\). Calibration bar 1 cm. Temperature ~11°C. Initial fiber length 25 mm, fiber weight 80 mg.

considered several mechanisms which might underlie the length effect seen with constant current stimulation. One possibility, suggested by the work of Keynes et al. (1973) is that there is a length-dependent ion accumulation which tends to accentuate the depolarization produced by the current pulse. However, we could find no evidence for ion accumulation in our preparations which are not internally perfused as was the case in Keynes et al. (1973). A second possible mechanism is that there is a basic change in the membrane properties with length. This could be a change in effective membrane resistance with muscle
length so that particular channels or current paths into the sarcoplasm are closed at longer muscle lengths, producing a higher membrane resistance and thus a larger depolarization for a constant current. Another membrane property change with length which could produce this effect would be one which would cause the fiber to hyperpolarize with releases (decreases in length) and depolarize with stretches (increases in length).

In principle, a change in the "effective" membrane resistance with length should be detected by I-V curves taken under voltage clamp. However, in a number of experiments where we looked specifically for a change in "effective" membrane resistance with length, we observed the change less than half the time even though the voltage change with length seen in the constant current condition (Fig. 7) was always visible. This does not eliminate the possibility of length affecting membrane channels (like Ca or Na) which may contribute little to membrane resistance, but would tend to cause substantial depolarization under the less controlled constant current conditions.

Some muscle fibers showed a depolarization with stretch and a hyperpolarization with release. Fig. 9 illustrates one record of stretch and release of an unstimulated muscle fiber. The record shows ~1 mV of depolarization on stretch of greater than 1 mm and up to 0.5 mV of hyperpolarization with releases of up to 2 mm. These are among the largest seen. This depolarization on stretch, hyperpolarization on release, did not involve just a simple nonselective permeability increase. Under voltage clamp conditions, it could be shown that the process producing the voltage change had an equilibrium potential in the order of 10 mV hyperpolarized from the resting potential (actually the zero current point). This was ascertained from records such as those in Fig. 10. Stretches or releases produced changes in holding current under voltage clamp conditions. When the membrane potential was of the order of 10 mV hyperpolarized from the resting level, the current necessary to keep the potential constant during a stretch (or release) changed sign. This implies that channels permeable to K⁺ or Cl⁻ closed or were made ineffective (for example, in a cleft which closes off) on stretch or appeared on release. This also implies that length changes are not acting by damaging the membrane and producing a nonselective shunt. For, if this were the case, the equilibrium potential should be depolarized from the resting level and near a membrane potential of zero.
The question is, does this length-sensitive membrane potential account entirely for the length-dependent Ca release? Certainly, the extra depolarization seen during the constant current pulse at the longer length is greater than the depolarization seen in the unstimulated muscle with the same length change. Moreover, with nonlinear current voltage relations and time-dependent conductances a small initial depolarization on stretch might be amplified (i.e. if it allowed more Ca channels to open) during the current pulse. An additional factor to be considered is the steepness of the relationship between membrane depolarization and the peak height of the Ca transient. The peak height increases e-fold for an additional 2–4 mV depolarization. At present we can say only that this mechanism of the effect of length on the resting membrane potential may be able to account for most of the length-dependent Ca release.

DISCUSSION

The results presented in this paper support the conclusion that changes in muscle length can directly affect the calcium activation process. Of course length is also known to affect force through mechanical factors, and thus the question arises of the importance of each factor at a given length. With constant current stimulation, the influence of mechanical factors becomes increasingly observable as the length is varied greatly from the resting value (see Fig. 3), while calcium activation factors are clearly important at or near the rest length (see Fig. 4).
to whether activation factors are also important at lengths far from rest length, we have no systematic evidence at present. This limitation is due to the constraints imposed by the internal electrode which would obviously (see Fig. 1) damage the tendon end of the fiber with extreme shortening or which would lose the space clamp ability with extreme lengthening. Nevertheless, over the range of lengths that we were able to investigate the calcium activation factors remained important.

On the basis of data such as those shown in Figs. 7-10, we concluded that length changes affect calcium release by directly affecting membrane properties. A question which is not answered by these experiments concerns a site of the length-induced change in membrane properties. The geometry of barnacle muscle (see Hoyle et al., 1973) is such that the stimulus current can flow outward via the membrane of the transverse tubular system and via the membrane of the extensive “cleft system,” as well as via the surface (sarcolemmal) membrane. Because these separate membranes are in parallel, the length-induced change could, in principle, occur in one or perhaps several of these possible pathways. We are unable to measure these currents separately and therefore cannot distinguish which of the pathways is length sensitive. Whatever membrane might be involved, our data (see Figs. 9 and 10) imply that the $P_{Cl}$ or $P_{K}$ of that membrane must be elevated by release and decreased by stretch. It seems less likely that the permeability of a peripheral membrane would be increased more by release than by stretch, but this cannot be ruled out. Another alternative is that access to some membrane with significant $P_{Cl}$ or $P_{K}$ is opened up by release or closed off by stretch. This latter might be the case if the $P_{K}$ or $P_{Cl}$ were in the transverse tubules or clefts and access to these was impeded (resistance in series increased) by stretch and was enhanced (resistance in series decreased) by release. If the access resistance to these membranes increased substantially and the area of the membranes closed off as a current path by stretch was large enough, one might see a decreased effective membrane capacity. Several attempts to observe changes in the time constant of the voltage response to a step of constant current applied via an axial electrode gave negative results, but the precision of the measurements was such that a 5% change in capacity would not have been seen. However, a change in membrane permeability or effective membrane area could produce a change in membrane resistance. One would predict that the fall in $P_{Cr}$ or $P_{K}$ or the rise in access resistance to membranes with high $P_{Cl}$ or $P_{K}$ would cause an increase in effective membrane resistance with stretch. As was noted earlier, this was observed occasionally, but not in a majority of the cases in which it was investigated. Nevertheless, if the resting membrane has a significant Na or Ca permeability, it may not take a large decrease in $P_{Cl}$ or $P_{K}$ (thus a large increase in effective membrane resistance) to cause this depolarization on stretch. This cannot be argued in a more quantitative manner as the appropriate data are not available.

Under normal conditions, barnacle muscle fibers are activated via local, graded depolarizing currents produced by transmitter action at endplates scattered over much of the muscle fiber surface, thus normal activation is closer to being constant current than constant voltage. Therefore, the length dependence of calcium release seen here under constant current stimulation is potentially of
some physiological significance to the barnacle. It constitutes a negative feedback control acting at the cellular level.

These results show that muscle length can affect the Ca activation process. From work on other muscles (Gordon et al., 1966) it is clear that mechanical factors such as filament geometry are important at longer muscle lengths. Also the work of others (Fuchs, 1974) implies a decreased calcium release or decreased Ca sensitivity, particularly at shorter muscle lengths. The clear demonstration here by Taylor et al. (1975) of the length dependence of Ca release means that it may be important in other muscles. For example, in cardiac and skeletal muscle, how much of the fall-off in force at short muscle length is attributable to a decreased Ca release? Our results were shown for constant current stimulation. For excitable cells, even though the action potential is more like a constant voltage (wave form) rather than a constant current stimulus, the depolarization triggering Ca release spreads along the transverse tubules (T tubules) that penetrate deep into muscle fibers. Although there are Na channels in some T tubules (Costantin, 1970) the T tubular membranes may not support regenerative action potentials but instead attenuate the depolarization as it approaches the center of the fiber. If this were the case, length-induced changes in T tubules' membrane properties might influence depolarization and Ca release in the center of the muscle fiber. In this context, it is interesting to note that although stretch has been shown to increase the sub maximal potassium contractures in twitch fibers from the frog (Gonzalez-Serratos et al., 1973) and toad (Lännergren, 1975), stretch does not increase the sub maximal potassium contractures in slow muscle fibers from the toad (Lännergren, 1975). The T tubule system in slow fibers has a somewhat different, less regular morphology with fewer triadic junctions (Page, 1965) and less area than in twitch fibers (Adrian and Peachey, 1965). There is also evidence that extracellular Ca may play an activator role in slow fibers (Kirby, 1970). If stretch was affecting either T tubule membrane properties (see below) or Ca release caused by coupling at triadic junctions, slow fibers might be expected to have contractions affected less than for twitch fibers.

If all of the membrane property changes in barnacle muscle fibers with changes in length occur down in the clefts or tubules, could these mechanisms be applicable to muscle fibers that did not have this elaborate cleft system (see Hoyle et al., 1973)? This question cannot be answered unequivocally, but it is not inconceivable that T tubules could open or close or that access to these tubules could change with muscle length. For example, the extent of membrane folding and the opening of caveolae on a frog muscle membrane change with muscle length (Dulhunty and Franzini-Armstrong, 1975). It would be of interest to know how the contribution of transverse tubules to the electrical equivalent circuit changes with sarcomere length. Initial reports by Valdiosera et al. (1974) indicate that in frog muscle, the membrane capacity (both surface and T tubule) may depend on sarcomere length increasing at shorter sarcomere lengths.

There is clear evidence in many excitable and nonexcitable cells that stretch causes a depolarization of the cell membrane such as that seen here (Fig. 9). However, where this has been investigated, stretch tends to produce a nonselective permeability increases (i.e., crayfish stretch receptor [Terzuolo and Wash-
Although it is not inconceivable that stretch could close off K or Cl channels, it seems more likely that stretch might close off access to membranes with these channels. All of the other cells in which it has been reported that stretch depolarizes do not have elaborate T tubular systems. It would be of interest if there were other muscle cells with T tubes and no clefts that were depolarized by stretch. The depolarization described in these papers is small (Fig. 9) and might be difficult to detect in other muscles.

We thank the Director, A. O. D. Willows, and the staff at Friday Harbor Laboratories for their assistance.

This research was supported by National Institutes of Health grants NS08384 to A. M. Gordon and NS10919 to E. B. Ridgway.

Received for publication 1 June 1976.

REFERENCES


