Relation between Membrane Potential Changes and Tension in Barnacle Muscle Fibers

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ABSTRACT Constant current pulses have been applied to single muscle fibers of the barnacle, *Balanus nubilus* Darwin, with an axial metal electrode. The membrane potential change, which took place over a large part of the muscle fiber, was measured with a similar electrode. Depolarizing pulses, if the voltage was greater than threshold, produced tension. The size of the tension was a function of the magnitude and the duration of the depolarizing pulses. The latency between the onset of depolarization and tension can be only in part attributable to mechanical factors. AC stimulation produced tension, but 5 to 10 seconds were required for the steady-state level of the tension to be reached. Muscles were depolarized in elevated K and studied after the contracture had terminated. If not too depolarized, further depolarization produced tension. Termination of hyperpolarizing pulses also produced tension, which decayed quite slowly. Hyperpolarizing pulses reduced, or abolished, any preexisting tension. Thus, it appears that at certain values of the membrane potential tension is set up, but there is also a slow process of accommodation present.

The relation between muscle tension and membrane potential in solutions with various potassium concentrations has been measured by Hodgkin and Horowicz (1960). In single muscle fibers of the frog, the tension was found to be related by an S-shaped curve to the external potassium concentration or the membrane potential. No tension was developed until the potential reached about −50 mv.

A similar relation between tension and potential has recently been described for single muscle fibers of the barnacle, *Balanus nubilus* Darwin (Hoyle and Smyth, 1963). These muscle fibers are large enough to permit the insertion of wire electrodes for passing current and recording changes in membrane po-

tential. We have used these fibers to study the changes in muscle tension set up by constant current pulses under various conditions.

METHODS

Single muscle fibers were dissected from the depressor muscles of large specimens of *Balanus nubilus* Darwin (see Hoyle and Smyth, 1963, for a complete anatomical description). A thread was tied to the tendon end of the muscle fiber and the fiber was cut as close as possible to the other end. The fiber was placed on a glass slide with vaseline covering about 1 cm of the fiber at the cut end (details in Hagiwara and Naka, 1964). The isolation of the cut end eliminated shortening due to spread of the injury potential. The rest of the fiber, about 1.5 cm, was covered with barnacle saline. Two metal electrodes, attached together, were inserted axially from the cut end. One electrode, of silver and 100 μ in diameter, was uninsulated for about 1 cm and was used for passing current. The voltage-monitoring electrode was nichrome and was 35 μ in diameter. It had a 2 mm uninsulated region close to, but not in contact with, the center of the uninsulated region of the first electrode. After insertion of the electrodes, the cut end was tied to the electrodes by a thread.

The tendon end of the muscle fiber was connected by thread to a condenser type strain gage, kindly loaned by Dr. Alan Brady, Department of Physiology, University of California at Los Angeles.

The muscles were not rugged, and the experiments were usually terminated within an hour by rupture of the fiber or of the membrane. Doubling the osmolarity of the bath solution with sucrose was tried to reduce the mechanical response and possibly prolong the experiment, but this was unsuccessful.

The usual bathing solution was made up after the formula of Hoyle and Smyth (1963), and had the following composition: NaCl, 466 mM; KCl, 8 mM; $CaCl_2$, 20 mM; $MgCl_2$, 12 mM; and $NaHCO_3$, 10 mM. For elevated potassium solutions, the NaCl and KCl were replaced with equimolar K methanesulfonate, and this was mixed with the usual solution in the appropriate ratio.

In most preparations there was little or no active membrane response following current pulses, though delayed rectification was sometimes present. The factors controlling the appearance of active responses have been reported in detail elsewhere (Hagiwara, Chichibu, and Naka, 1964; Hagiwara and Naka, 1964).

RESULTS

Tension Changes Set Up by Square Current Pulses The time courses of the tension and voltage changes set up by a series of square current pulses of different magnitudes are shown in Fig. 1. Depolarizing voltages of sufficient magnitude were followed, after a latency inversely related to the magnitude of the depolarization, by a transient increase in tension. The tension increased almost linearly after a slow start. After the termination of the current pulse, the rise of tension was soon slowed, and the tension eventually returned to the resting level.

The maximum tension reached was a function of both the pulse duration and magnitude (Fig. 2). For a given pulse width, tension was developed only

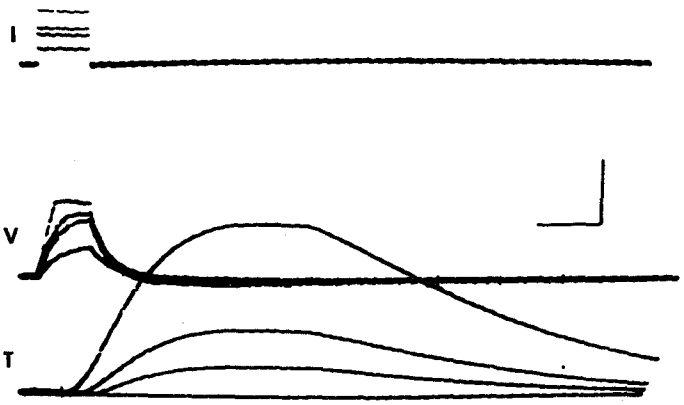

FIGURE 1. Tension and voltage changes produced by square current pulses of various sizes. Calibration, horizontal, 0.1 sec.; vertical, current (I), 2×10^{-5} amps, voltage (V), 20 mv, tension (T), 1.3 gm.

after a certain potential change had been exceeded; the plot of tension as a function of voltage first rises gradually and eventually becomes approximately linear, as might be expected from the S-shaped relation reported by Hoyle and

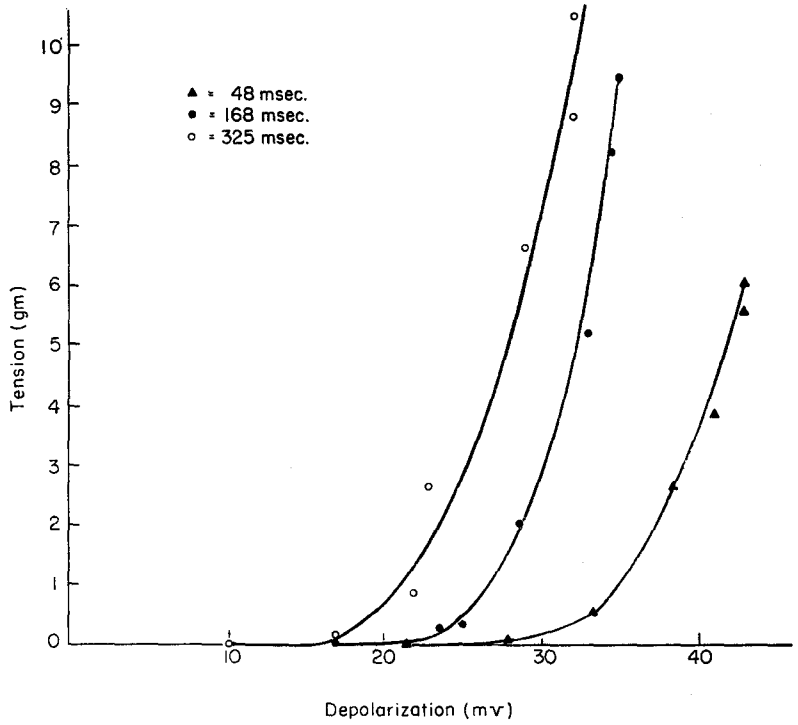

FIGURE 2. Maximum tension as a function of membrane potential change during square current pulses of different durations.

Smyth (1963). A similar relation has been reported in crayfish muscle fibers by Orkand (1962). The saturation part of the S-shaped curve was not found in these experiments probably because the currents were kept low to minimize mechanical trauma to the fiber. The extrapolated intercept of the linear region is presumably some measure of the threshold, and appears for the shorter pulses to be inversely related to the pulse width, so that the threshold follows the classical strength-duration relation.

Kinetics of Muscle Tension Change If a second depolarizing pulse were given during the rising phase of the tension, there followed an increase in the rate of rise of tension (Figure 3 A). The latency between the start of the second pulse and the increase in the rate of rise was shorter than that between the

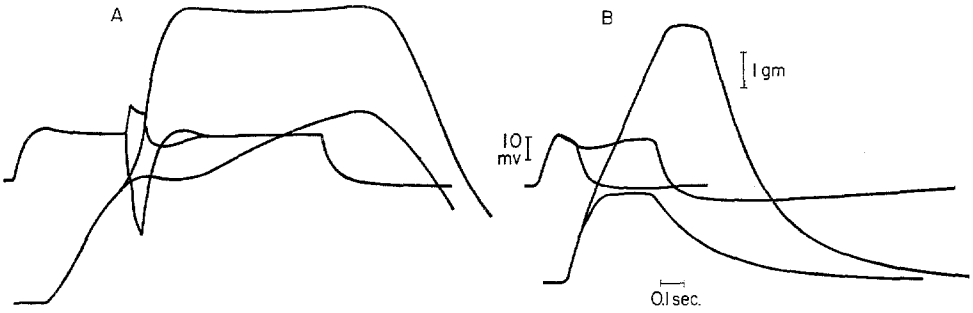

FIGURE 3. A, time course of tension and membrane potential when brief hyperpolarizing and depolarizing pulses were added during a long depolarizing pulse. B, time course of tension and membrane potential for two square current pulses giving equal membrane potential changes, but of different durations.

first pulse and the start of the initial tension change. Further, if the tensions set up by two pulses of approximately constant height, but different widths, be compared (Fig. 3 B), the latency between the termination of the shorter pulse and the change in slope of the rising phase of the tension was also shorter than the latency between the start of the pulse and the start of the initial tension change.

From these results the latency between the onset of potential change and tension might be thought to be purely mechanical in origin; *e.g.* as if some series elastic element must be stretched before there were an appreciable increase in tension following the initial stimulus. This delay would not be present with a second depolarizing pulse applied to a contracting muscle. This cannot be the entire explanation, however, as indicated by the following results.

Three tension records of approximately the same maximum tension are shown in Fig. 4, along with the voltage changes. While the rates of rise of tension are not too dissimilar, the latencies are strongly dependent on the magnitude of the voltage change. The curves show further that a reduction in the

depolarizing voltage can be compensated for by an increase in duration of the depolarization as far as peak tension alone is concerned. However, the voltage-duration relation does not appear to be a simple one.

The entire time course of the tension change seems to be determined by the time course and magnitude of the depolarizing pulse. Fig. 5 shows that the tension set up by a depolarizing current pulse (lowest trace of Fig. 5 B) was unaltered by a hyperpolarizing current pulse applied during the time at which the tension was at its maximum value (Fig. 5 A).

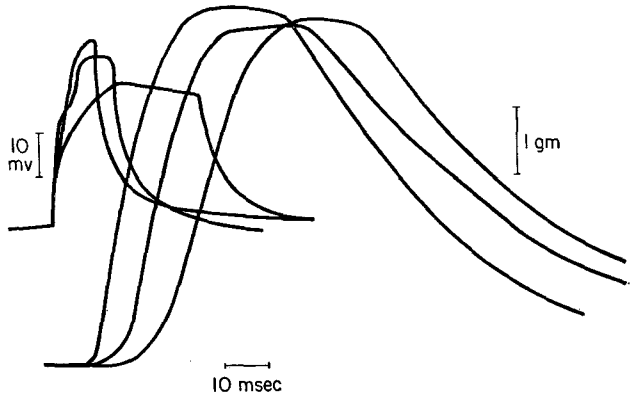

FIGURE 4. Time course of tension and membrane potential for three current pulses producing approximately equal maximum tensions. The shortest, largest depolarization produced the earliest tension maximum, and the smallest, longest depolarization gave the latest maximum.

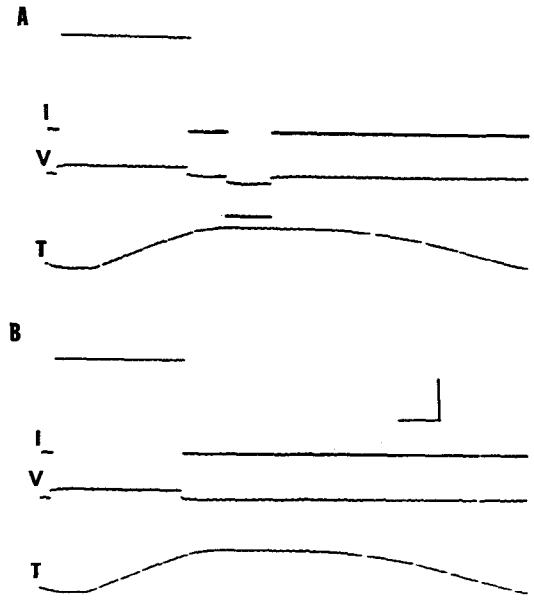

FIGURE 5. Absence of effect of hyperpolarizing square current pulse on tension produced by depolarizing pulse. A, depolarizing current pulse followed by hyperpolarizing current pulse. B, depolarizing current pulse only. Calibration, horizontal, 0.1 sec.; vertical, current (I) 2×10^{-5} amps, voltage (V), 20 mv, tension (T), 1.3 gm.

Tension Changes Produced by AC Stimulation The output of an audio generator was used to apply sinusoidal currents of various magnitudes and frequencies. The voltage change produced by a sinusoidal current of given magnitude was a function of the frequency, as expected from the capacitative properties of the muscle membrane. The time course of the tension and voltage (peak depolarization) during AC stimulation at 15 CPS, when the voltage was first increased and then decreased stepwise, is shown in Fig. 6. Above threshold, the tension appeared to reach a steady-state value some 5 to 10 seconds

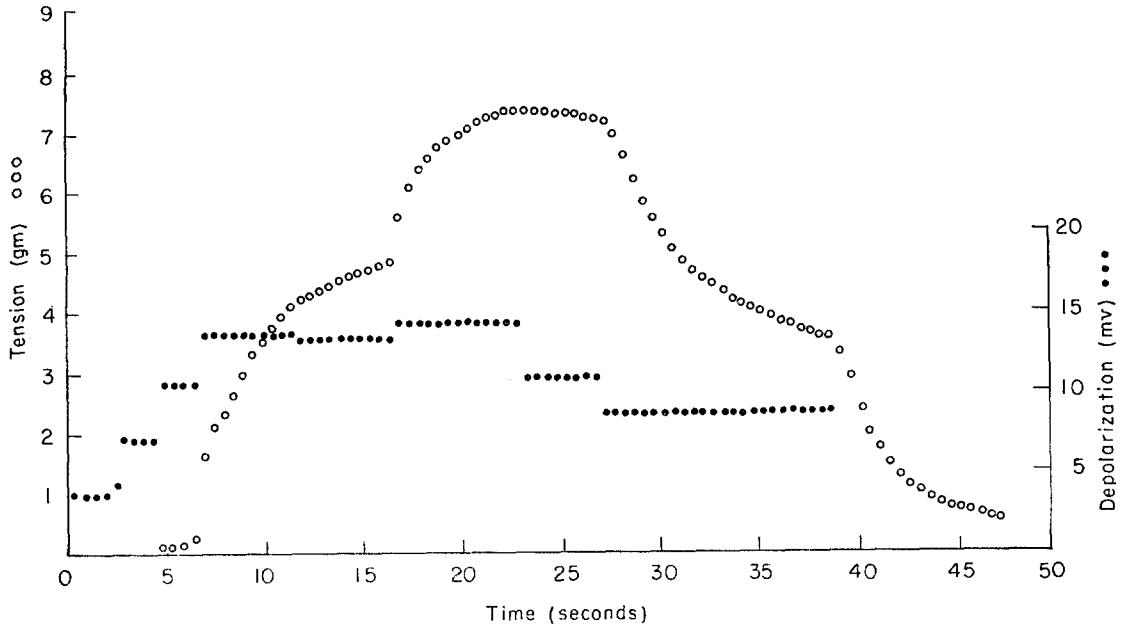

FIGURE 6. Time course of tension and peak membrane depolarization during AC stimulation at 15 CPS. Tension, open circles and ordinates on left. Peak value of membrane depolarization, filled circles and ordinates on right.

after a steady voltage level was reached. The steady-state tension was a function of the peak amplitude of the voltage change following increases in the voltage, and was independent of the frequency of the sine wave (Fig. 7). The steady-state tension during AC stimulation was greater than that found for stimulation by square current pulses with the same value of peak depolarization.

When the voltage was decreased during AC stimulation, there appeared to be some hysteresis; the first decrease in voltage in Fig. 6 (at 23 seconds) was accompanied by only a slight reduction in the tension. Further, following the second voltage decrease (at 26 seconds), considerable tension was maintained, though a slow decay was apparent.

Stimulation Following Elevation of Potassium Elevation of the external potassium causes single barnacle muscle fibers to shorten. However, after a few minutes the fibers relaxed and electrodes were then mounted as previously described.

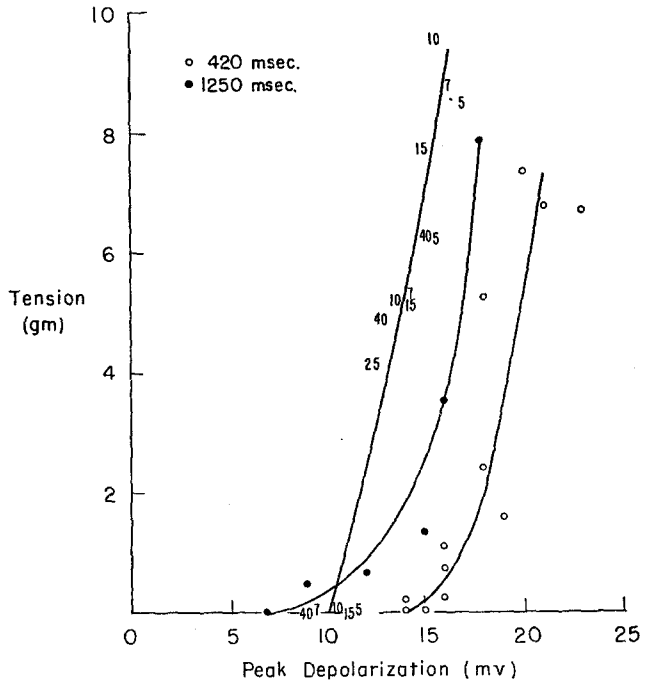

FIGURE 7. Maximum tension as a function of a peak membrane depolarization during (*a*) AC stimulation at the frequencies whose values are used to mark the points, and (*b*) square current pulses of the indicated durations (open circles and filled circles).

Fibers treated as above with solutions containing 66 mM K produced tension in response to depolarizing currents. The tension-potential curve was quite steep, and there apparently was a threshold depolarization for tension production (Figs. 8 and 9).

The tension of muscle fibers treated in this way was also sensitive to hyperpolarizing currents. Termination of a hyperpolarizing current pulse initiated tension. A similar finding in frog muscle has been reported by Curtis (1963). The magnitude of the tension was a function of the strength of the hyperpolarizing potential change (Fig. 8). For a given duration and magnitude of potential change, the magnitude of the tension resulting from the depolarizing current was greater than from the hyperpolarizing current. Furthermore, the time course of the tension change following a hyperpolarizing pulse was slower, both for the onset of tension and especially for the decay of tension (Fig. 9). After depolarizing currents the tension returned to about the initial level in

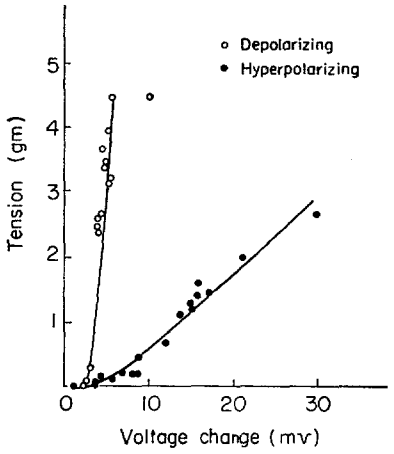

FIGURE 8. Tension produced in muscle in elevated potassium (66 mM). Maximum tension produced by depolarizing current pulses and after termination of hyperpolarizing current pulses plotted as a function of the membrane potential change, for pulses of constant width (630 msec.).

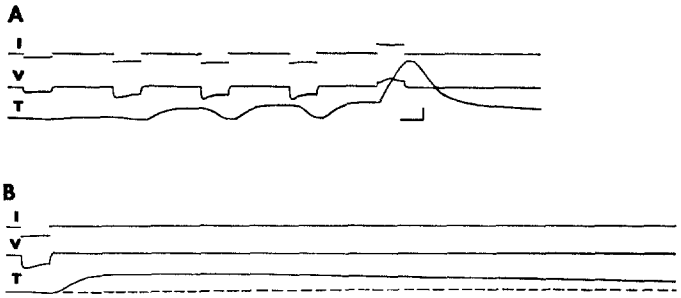

FIGURE 9. Time course of tension and potential change produced by square current pulses in elevated potassium (66 mM). A, four hyperpolarizing pulses followed by one depolarizing pulse. B, one hyperpolarizing pulse. Calibration, horizontal, 0.5 sec.; vertical, current (I) 2×10^{-4} amps, voltage (V) 50 mv, tension (T), 1.3 gm.

several seconds, while the tension after the hyperpolarizing pulse showed no sign of decay from the maximum level reached in the 1.3 seconds between the hyperpolarizing pulses (Fig. 9 A) and decayed to half its maximum value in about 25 seconds (Fig. 9 B). Decrease of tension was produced, however, by a second anodal current pulse.

Similar experiments were done in 124 mM K. These fibers responded to hyperpolarizing currents in the two ways mentioned above. However, only 1 fiber of 4 was responsive to depolarizing currents. In 472 mM K, no mechanical response was found to either hyperpolarizing or depolarizing currents. Membrane potentials were not measured in these experiments, but from data of Hagiwara, Chichibu, and Naka (1964), the potentials should have been approximately: 66 mM K, −26 mv; 126 mM K, −14 mv; 462 mM K, +25 mv.

DISCUSSION

The general relation between tension and voltage (both magnitude and duration) is as would be expected. The magnitude and the duration of the pulses

used were too small to reach the maximum tension possible. This was demonstrated in the case of AC stimulation, where a steady tension was reached only after a period of 5 seconds or so. Further, a given tension level can be initiated by a large number of combinations of voltage and duration.

The penetration by the electrode obviously destroys much of the internal organization of the muscle fiber, and yet contraction is still possible. This is probably due to the regions still intact. Appreciable tension values were found, however, the maximum being 28 gm.

An important problem in muscle physiology is the relation between the time course of the exciting process and the tension changes. Difficulties in determining this relation are illustrated by the hysteresis phenomena found with AC stimulation. Once a certain steady level of tension was reached, it apparently could be maintained by a lower level of displacement of membrane voltage. A similar hysteresis during AC stimulation of frog muscle has been found by Sten-Knudsen (personal communication).

The findings are interpretable in terms of a system in which depolarization releases a substance which diffuses a short distance, initiates tension, and is then removed. The rate of release is presumably a function of the magnitude of the depolarization. The latency between depolarization and onset of tension is, under some circumstances at least, greater than can be explained by mechanical factors alone. There is much evidence to suggest that the substance released may be calcium, *e.g.*, injection of Ca^{++} in small amounts produces tension in muscle fibers of crab (Caldwell and Walster, 1963) and injection of calcium-binding agents, such as EGTA, into barnacle muscle fibers abolishes the tension change following depolarization (Hagiwara and Naka, 1964). Once the substance was released by depolarization, hyperpolarization was without effect.

The calcium threshold to initiate contraction in crab muscle fibers following injection is probably about 10^{-5} mole/liter (Caldwell and Walster, 1963). Thus to activate a region 1.5 cm long of a fiber of diameter 1.5 mm, 2.6×10^{-10} mole of calcium must be released, if these two muscles are similar. If the current itself moved, or released, calcium, 5.2×10^{-5} coulomb would be required. For threshold in the normal barnacle muscle, the total charge passing through the muscle membrane was of the order of 10^{-5} coulomb.

The state of calcium in the muscle fiber remains to be determined. The calcium content of the cell is most likely higher than the threshold level for contraction, but much of the calcium is likely bound by some cellular constituents. However, there presumably is some free calcium within the cell, for injection of calcium-binding agents alters the properties of the cell membrane and also eliminates the mechanical response following depolarization (Hagiwara and Naka, 1964).

In fibers which had undergone contraction and relaxation following partial depolarization by increased potassium, tension was produced by depolarizing

voltage pulses as well as following termination of hyperpolarizing pulses. This suggests that at certain potentials tension is set up which is a function of the potential, and that there is a slow process of accommodation present. The accommodation, which leads to relaxation after exposure to elevated potassium, is reversed by hyperpolarization; the degree of reversal is dependent upon the magnitude and duration of the hyperpolarizing pulse. The relaxation of tension in these depolarized fibers during hyperpolarizing pulses would be expected from this scheme. The behavior is consistent with the model proposed by Hodgkin and Horowicz (1960), where depolarization releases an activator which initiates contraction; this activator is destroyed, and can be regenerated by hyperpolarization. The slow decay of the tension set up by hyperpolarizing pulses could reflect the time course of the inactivation of the activator. In crab muscle, injection of Ca^{++} into fibers depolarized by high external potassium produces contractures, just as in the absence of depolarization (Caldwell and Walster, 1963). Thus, the difficulty in producing tension in depolarized muscle fibers lies presumably in the system activating contraction, rather than in the contractile system itself.

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