LETTERS TO THE EDITOR

What Is the Source of the Calcium That Activates Contraction of Barnacle Muscles under Physiological Conditions?

Dear Sir:

The source of the calcium necessary to activate the contractile response of muscle fibers may be either internal stores or a Ca\(^{++}\) ions that enter during the change in membrane potential initiating the contraction. In a recently published study of contractile activation in barnacle by voltage clamp, the magnitude of the tension produced by depolarizing pulses was found to reach a saturating level at a membrane potential of about +10 mV and to be unchanged at potentials up to 230 mV (Caputo and DiPolo, 1978). Since the equilibrium potential for Ca is between 157 and 186 mV, the constancy of the tension response for membrane potentials between 10 and 230 mV argues strongly against a significant contribution of entering calcium in activating tension under the conditions of the experiment. The authors conclude: "These results suggest that under physiological conditions the contractile activator is probably released from an internal store. . . ."

The extrapolation from the conditions used in the experiment to physiological conditions may not be completely justified. The physiological activation of the barnacle muscle is initiated by endplate potentials of up to 40 mV in size, which may set up graded local responses. The magnitude of these is reported never to exceed that of the resting potential (Hoyle and Smyth, 1963), so that under physiological conditions, contraction is activated at negative membrane potentials. Therefore, the range of potentials used by Caputo and DiPolo (1978) to demonstrate the absence of a relationship between tension and membrane potential, i.e., 10–230 mV, may not be physiological.

Caputo and DiPolo (1978) found with voltage clamp that depolarizations of less than the magnitude of the resting potential produced tension with only an outward current. However, the amount of inward current necessary for sufficient Ca\(^{++}\) to enter to initiate contraction would likely not be detectable in their experiments. The Ca\(^{++}\) threshold for contraction is about 5–9 \(\times 10^{-7}\) M (Ashley, 1967). For a fiber of 2-mm diameter, the current density to move sufficient Ca\(^{++}\) across the membrane to reach threshold in 50 ms is 1–2 \(\times 10^{-4}\) A/cm\(^2\). Tetraethylammonium, which inhibits the outward K\(^+\) current, was found by Caputo and DiPolo (1978) to reduce the outward current during voltage clamp by something of the order of this amount. Therefore, the inward Ca\(^{++}\) current necessary to initiate contraction could have been masked by a larger outward K\(^+\) current.
There is much indirect evidence to suggest the Ca\textsuperscript{2+} entry may be important physiologically in barnacle muscle. If the ionic mechanism underlying the endplate potential in barnacle muscle is similar to that in the frog (Takeuchi, 1963), there will be some Ca\textsuperscript{2+} entry during the endplate potential, and this could be significant since the innervation is multiterminal (Hoyle and Smyth, 1963). Addition of glutamate, the putative excitatory transmitter, produces tension; the response is lost within 30 s after removal of Ca\textsuperscript{2+} from the bath solution (Ashley and Campbell, 1978). The rising phase of any abortive action potential is likely due to Ca\textsuperscript{2+} entry, since channels showing a voltage-dependent increase in Na\textsuperscript{+} permeability seem to be absent (Hagiwara and Naka, 1964). Furthermore, Ashley (1967) has shown that the amount of EGTA that has to be injected to suppress the contractile response in the presence of caffeine, which presumably acts to release Ca\textsuperscript{2+} from the sarcoplasmic reticulum, is similar to the estimated muscle fiber calcium content. The block of contractures initiated by high K\textsuperscript{+} requires considerably more EGTA; the additional Ca present under these conditions likely enters across the cell membrane. K\textsuperscript{+} contractures are probably a good model for physiological activation, since the membrane potential is not reversed in high K\textsuperscript{+}. Additional evidence for the entry of Ca\textsuperscript{2+} during K\textsuperscript{+} contractures is the fact that the development of tension in high K\textsuperscript{+} requires the presence of Ca\textsuperscript{2+} and Sr\textsuperscript{2+} in the bathing fluid; Mg\textsuperscript{2+} is without effect (Edwards and Lorkovic, 1967). In crab muscle, injections of Ca\textsuperscript{2+} and Sr\textsuperscript{2+} produce tension and Mg\textsuperscript{2+} is without effect (Caldwell and Walster, 1963). It might be noted that Ca\textsuperscript{2+} and Sr\textsuperscript{2+} are able to activate the actin-myosin-tropomyosin-troponin, ATPase of muscle, and that Mg\textsuperscript{2+} is without effect (Seidel and Gergely, 1963).

The findings just summarized suggest that Ca\textsuperscript{2+} entry may play a role physiologically. They, and the above comments on the experiments of Caputo and DiPolo (1978), warrant the conclusion that the source of the activator Ca\textsuperscript{2+} remains to be firmly established.

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Reply to Edwards's Letter:
Does External Calcium Play Any Role in Contractile Activation?

Dear Sir:

Edwards's letter commenting upon our article on contractile activation in voltage-clamped barnacle muscle fibers (Caputo and DiPolo, 1978) calls for some general and specific responses. The demonstration of calcium currents during depolarizing voltage-clamp pulses (Hagiwara et al., 1968; Keynes et al., 1973; Hidalgo et al., 1979), plus other lines of indirect evidence quoted in detail in the letter, have led to the widely accepted view that external calcium entering the fiber during depolarization acts directly to activate contraction. However, the results of our experiments argue against such a direct role; in fact, we have shown that, in untreated (nonperfused) fibers, the contractile activator is probably released from an internal store, and that the calcium entering the fiber as inward current does not play a major role in contractile activation.

Edwards argues that an extrapolation from the conditions used in our experiments to physiological conditions may not be justified; in particular he notes that the range of potentials used by us might not be physiological (depolarizing pulses from resting potential up to +230 mV). It is appreciated that most experiments, including those using voltage clamps, are carried out under circumstances quite different from physiological conditions. On the other hand, we wanted to test the hypothesis held by several authors that the external calcium that enters the fiber as calcium current during membrane depolarization activates contraction. This hypothesis was clearly disproved by our results (Caputo and DiPolo, 1978). It is worthwhile to recall that our experiments were performed on fibers that were under voltage-clamp conditions but were otherwise untreated. Thus, except for the insertion of compound electrode, the composition of the internal medium was not altered. This
contrasts with the conditions necessary to observe calcium action potentials or calcium currents in these fibers. In these cases, it is necessary to inject or perfuse the fibers with EGTA, tetraethylammonium etc. (Hagiwara et al., 1969; Keynes et al., 1973; Atwater et al., 1974), treatments which markedly reduce the tension output of these fibers. The treated fibers give tensions of 0.2 kg/cm², whereas the untreated fibers give tensions of more than 2 kg/cm². Furthermore, treatment of these fibers with tetraethylammonium or other agents (Hidalgo et al., 1979) alters the tension voltage relationship in a way similar to the effect produced by procaine (see Fig. 14, Caputo and DiPolo, 1978). Under these circumstances tension, besides being diminished, does not remain unchanged up to potentials of +230 mV but declines, contrary to what happens in untreated fibers.

It is not clear why Edwards thinks that depolarization associated with an endplate potential or with a potassium contracture is to be considered physiologically, i.e., mechanistically different from a similar depolarization during a voltage-clamp pulse. If membrane depolarization causes a change in the membrane conductance to calcium ions, it should not matter in which way such depolarization is achieved. It must be stressed that, if the sarcoplasmic reticulum of these fibers is capable of releasing calcium during caffeine contractures, as shown in experiments carried out with no external calcium (Caputo and DiPolo, 1978), it could also release calcium during depolarization. Evidence for this view has been recently obtained by Desmedt and Hainaut (1977).

Finally, the tension reduction that points to the necessity of extracellular calcium, as observed in low-calcium media, could be interpreted in terms of a stabilizing role of this cation in one of the steps of the excitation-contraction coupling process. We have no arguments against the view that calcium entry in barnacle fibers may play a physiological role, in fact, an important role, for calcium entry could serve for replenishment of the intracellular calcium stores.

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