Action Potentials without Contraction in Frog Skeletal Muscle Fibers with Disrupted Transverse Tubules

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Abstract. Action potentials, with no accompanying contraction, were recorded from muscle fibers in which the transverse tubular system had been disrupted. The results show that action potentials require an intact transverse tubular system to cause contraction. Furthermore, both the after-depolarization following a single action potential and the slower, late afterpotential following a train of action potentials were absent in this preparation. Therefore, both phenomena must normally involve the transverse tubular system.

It is generally believed (1) that the transverse tubular system is an essential link between the action potential and activation of the contractile apparatus. A method which removes transverse tubules would permit this hypothesis, which seems very likely from the results of Huxley and Taylor (2), to be tested directly.

We have used the method of Howell and Jenden (3) to disrupt the transverse tubular system (4). The solutions used in these experiments were the same as those previously described (4), but they did not contain curare or tetrodotoxin. Two microelectrodes were inserted into surface muscle fibers; one passed current, the other recorded potential.

After the muscles had been treated (3, 4) and the transverse tubules were disrupted, depolarizing currents passed through the fibers caused action potentials. These had a distinct threshold (Fig. 1B) and were propagated. There was never movement of any kind (Fig. 1, B and C), even with action potentials at a high frequency. For example, a long (75 msec) depolarizing current produced repetitive action potentials (Fig. 1D), but there was no evidence of movement as judged by microscopic examination or from oscilloscope traces (compare Fig. 1A). The shape of the action potential was quite different from that of normal muscle in that no after-depolarization could be seen. In fact, an after-hyperpolarization was evident in most fibers (Fig. 1, B and C).

To exclude the possibility that glycine might directly inhibit contraction, we elicited action potentials in muscle fibers during their exposure to the glycine solution. It has been shown that in this solution the transverse tubular system is intact (3, 4). Action potentials were accompanied by twitches like those described by Fujino et al. (5). Figure 1A shows an action potential of a fiber in the glycerc solution followed by an upward deflection caused by dislodgement of the microelectrodes during the twitch. These action potentials had normal after-depolarizations (Fig. 1A) in contrast to those of treated fibers, which have after-hyperpolarizations.

The absence of an after-depolarization in treated fibers strongly suggests that the normal after-depolarization is produced by the circuit elements which characterize the transverse tubular system. Furthermore, if the after-hyperpolarization in the treated fibers is caused by a persistent increase in potassium conductance, as it is in the squid axon, the potassium channel responsible for it must be in the surface membrane.

Contraction following an action potential was abolished only in muscle fibers in which transverse tubules were destroyed (3, 4). The unexplained observation of Fujino and his co-workers (5) that excitation and contraction were uncoupled after a similar treatment can probably be attributed to disruption of the transverse tubular system in their preparations. It has been shown that these treated muscle fibers retain the ability to contract since they will do so when exposed to caffeine (4). Therefore the uncoupling of action potentials and contraction can be attributed to the absence of transverse tubules.

Fig. 1 (left). Action potentials in surface muscle fibers. (A) An action potential followed by a loss of membrane potential caused by movement (40 minutes in glycerc-Ringer solution). (B) Action potentials in a treated fiber (after 1 hour in Ringer solution) in response to three depolarizing current pulses (6 x 10^{-7} amperes) at threshold strength. (C) An action potential with an after-hyperpolarization in a treated muscle fiber (after 1 hour in Ringer). (D) A train of action potentials elicited by a long (75 msec) depolarizing current pulse which terminated during the rising phase of the last action potential (treated fiber). Fig. 2 (right). Upper trace shows repetitive action potentials in a muscle fiber with disrupted tubules (voltage calibration, 50 mv; time calibration, 200 msec). Lower trace is at higher gain and slower sweep speed and shows that there is no late afterpotential. Note miniature end-plate potential. Voltage calibration, 5 mv; time calibration, 200 msec.
Another phenomenon which has been thought to depend on the transverse tubular system was also noted to be absent in these treated fibers. The large, prolonged after-depolarization following a train of action potentials (the late after-potential) has been attributed to an accumulation of potassium in an extracellular compartment which was thought to be the lumen of the transverse tubules (6). In muscle fibers with disrupted transverse tubules, no late afterpotential was seen.

In the experiment illustrated in Fig. 2 a series of action potentials was elicited by a train of short depolarizing pulses at 100 pulses per second. The upper trace of Fig. 2 shows a train of eight action potentials displayed at low voltage gain and high sweep speed. The same record is shown below at ten times the voltage gain and one-tenth the sweep speed. Even at this high voltage gain there is no sign of the normal late after-potential. The disappearance of the late after-potential in muscle fibers with disrupted transverse tubules indicates that the extracellular compartment thought to be responsible for the potential is indeed the lumen of the transverse tubules.

The lower trace of Fig. 2 shows a miniature endplate potential which suggests that release of the transmitter is unimpaired. In fact, in muscle fibers with disrupted transverse tubules, endplate potentials with a time course that is shorter than normal can still be elicited by nerve stimulation (see 7). The treatment with glycerol apparently does not damage the nerve trunk or the nerve terminals, nor does it disrupt the mechanism for transmitter secretion.

References and Notes
7. S. Machnik and P. W. Gage, unpublished observations.
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