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

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 aferpotential 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 glycerol might directly inhibit contraction, we elicited action potentials in muscle fibers during their exposure to the glycerol 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 glycerol 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.

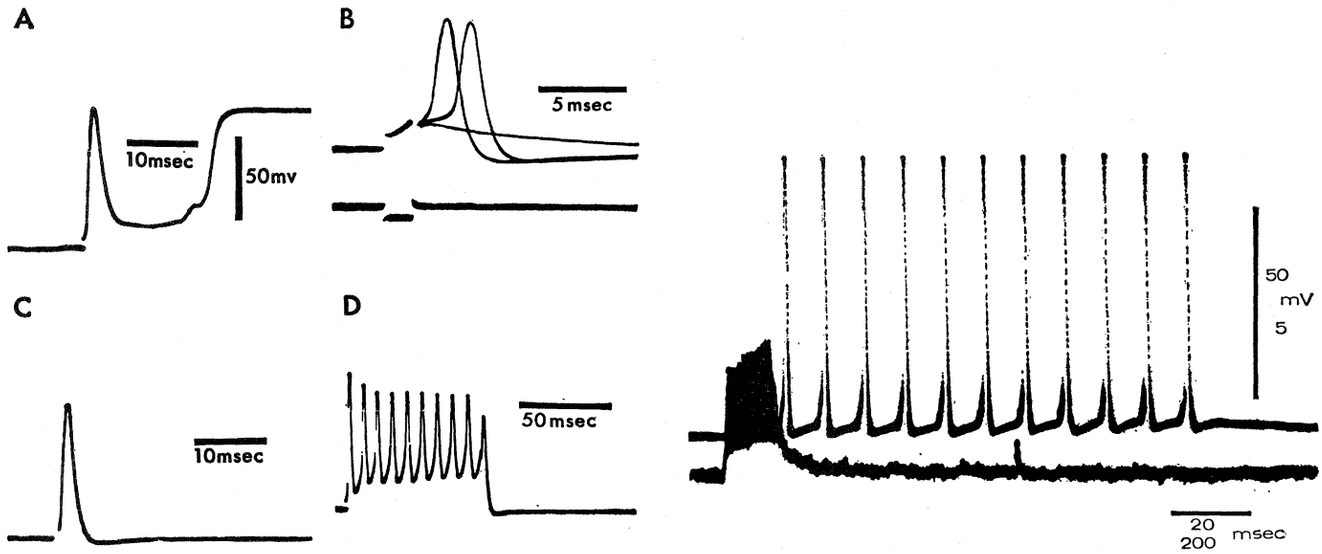

Fig. 1 (left). Action potentials in surface muscle fibers. (A) An action potential followed by a loss of membrane potential caused by movement (40 minutes in glycerol-Ringer solution). (B) Action potentials in a treated fiber (after 1 hour in Ringer solution) in response to three depolarizing current pulses (6×10^{-7} ampere) 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, 20 msec). Lower trace is at higher gain and slower sweep speed and shows that there is no late afterpotential. Note miniature endplate 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 afterpotential) 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 afterpotential. The disappearance of the late afterpotential 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.

PETER W. GAGE
ROBERT S. EISENBERG
Department of Physiology, Duke University Medical Center, Durham, North Carolina

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Haptoglobin Levels in Serum of Various Strains of Mice

Abstract. *Only a single type of haptoglobin was found in the serum of 11 strains of mice, but there were wide variations between strains with respect to the amount of haptoglobin found. In the AKR and C3H strains, in which haptoglobin was low or absent, various agents stimulated production of high levels of haptoglobin. Serum haptoglobin rose in association with the development of leukemia in AKR mice, but remained low when C3H mice developed mammary tumors.*

In man, the relative risk of developing leukemia may be associated with haptoglobin type (*1*), and accordingly it was of interest to determine whether an analogous situation might exist with respect to certain strains of inbred mice for which the relative risk of acquiring leukemia or another malignancy is under known genetic control. Genetically controlled variations in the molecular structure of serum haptoglobins, well documented in man, are not known to exist in other species where only a single protein has been described (*2*).

We have found that the quantitative level of serum haptoglobin differs markedly in several strains of mice. A preliminary examination of the serum of eight mice from each of 11 strains of inbred mice showed considerable variation with respect to the numbers in each strain which had haptoglobin levels exceeding the sensitivity of the analytical method (*3*) as follows: AKR, 0; C3H, 2; BALB/c, 3; A, 3; SWR, 3; NBL, 3; C57BL/6, 4; C57BL/An, 6; C58, 7; DD, 7; DBA/2, 8. More extensive studies were carried out on female AKR, C3H, and DBA/2 mice with the following result: AKR, 0/100; C3H, 31/183; DBA/2, 23/24 mice possessed clearly demonstrable haptoglobins.

The AKR and C3H strains are similar in having very low values for serum haptoglobin; indeed, most mice in these two strains have haptoglobin levels beneath the detectable limit. They differ clearly from the DBA/2 strain, where high values (50 to 150 mg of hemoglobin bound per 100 ml) are most common. The AKR and DBA/2 strains represent extremes with respect to percentage of mice having haptoglobins; other strains studied appear to occupy intermediate positions.

When found at all, the presence of haptoglobin in the serum of C3H mice is transient. Fifteen C3H mice, which on first examination had some haptoglobin, were reexamined 3 to 4 weeks later. Eight had small (approximately 30 mg of hemoglobin bound per 100 ml) amounts of haptoglobin in their serum; but the other seven had lost their haptoglobin and were negative.

Subcutaneous injections of turpentine regularly cause rises in serum haptoglobin (*2*). All of the animals that were tested by subcutaneous injections of 0.1 ml of turpentine responded by a marked increase in the amount of serum haptoglobin, whether or not the particular mouse's haptoglobin had been detectable prior to treatment. The stimulating effect of turpentine was studied in both AKR and C3H mice, where the levels rose from 5 to 10 mg of hemoglobin bound per 100 ml to a maximum of 150 to 250 mg of hemoglobin bound per 100 ml and persisted for 5 days. Other agents that provoked a rise of serum haptoglobin level were talcum powder, India ink, corn oil, and paraffin oil. These latter agents were not effective on all mice tested, nor was the response as long lasting as with turpentine. Injections of 0.85 percent NaCl did not result in changes in the serum levels. In all cases where haptoglobin could be observed, there was a single major protein, analogous to the human 1-1 protein, migrating with the same mobility in all strains. In most cases where mice had elevated haptoglobin levels, there were several additional slower moving bands (Fig. 1). The electrophoretic patterns of the mice which normally have higher levels

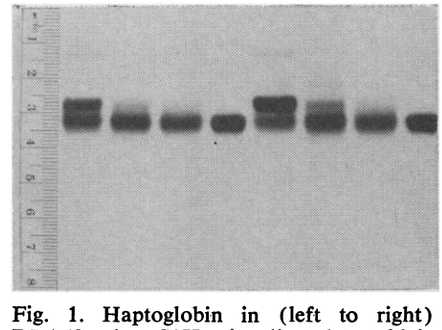

Fig. 1. Haptoglobin in (left to right) DBA/2 mice, C3H mice (just detectable), AKR mice (negative), human hemoglobin, AKR (turpentine stimulated), DBA/2, C3H (negative), human hemoglobin. The origin is at the zero index, and the migration is towards the anode at the bottom of the figure. Human hemoglobin was added to each serum to form the haptoglobin-hemoglobin complex; guaiacol was used to stain the complex (major band at 2.8 cm). Unbound hemoglobin migrated at 3.3 cm.