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Turbidity, Birefringence, and Fluorescence Changes in Skeletal Muscle Coincident with the Action Potential

Abstract. *Electrical stimulation of frog striated muscle was found to produce transient changes in turbidity, birefringence, and fluorescence (of fibers stained with pyronine B). The initial phase of these optical changes was coincident with the action potential. These findings suggest that there is a macromolecular conformational change in muscle membrane during excitation.*

Contraction in skeletal muscle is known to be preceded by propagation of membrane depolarization (excitation) along the sarcolemma and the transverse tubular system (*1*). The mechanism of excitation in the muscle fiber membrane is considered to be similar to that in the nerve fiber membrane. It was recently found that the excitation process in nerve is associated with changes in fluorescence, turbidity, and birefringence (*2*, *3*). It appeared reasonable, therefore, to investigate whether there are similar optical changes in muscle fibers during excitation.

In the present studies of the optical properties of muscle fibers, semitendinosus muscles of frogs (*Rana pipiens*) were used. Under dark-field illumination, microdissection was carried out to reduce the number of muscle fibers to approximately 50. The preparation was mounted in a black acrylic chamber which allowed simultaneous electrical and optical recordings (see upper part of Fig. 1). The 2-mm long segment of muscle in the middle pool (C_1) was stimulated by a pulse of electric current (0.01 msec in duration) with the stimulus cathode in the middle pool and the anode in the lateral pool (C_2). The action potential was recorded externally with separate electrodes in the middle and lateral pools (C_1 and C_3). All these electrodes were of the silver–silver chloride type. At room temperature, 22°C, the conduction velocity of the fibers is roughly 2 m/msec; therefore, the time required to excite the entire portion of the muscle in the middle pool by a suprathreshold stimulating pulse was less than 1 msec. The pools were filled with amphibian Ringer solution.

The muscle fibers were illuminated with quasi-monochromatic light obtained with a d-c operated quartz-iodine lamp (L) and an interference filter (F_1). The incident light was focused on the muscle segment in the middle chamber from below by a × 20 objective. Changes in the intensity of light scattered by the muscle fibers were detected with a photomultiplier placed at 0° or 90° to the incident light (see D or D' in Fig. 1). In birefringence experiments, a polarizer (P) was inserted between the light source and the muscle fibers; an analyzer (A) and the photomultiplier were aligned at 0° relative to the incident light.

In fluorescence studies, a secondary filter (F_2) was placed between the muscle and the photomultiplier; light was detected at 90°. The output of the photomultiplier was connected to a Tektronix oscilloscope via a Bak electrometer stage. To increase the signal-to-noise ratio, a computer of average transients (Mnematron) was used in conjunction with a Tektronix 122 preamplifier.

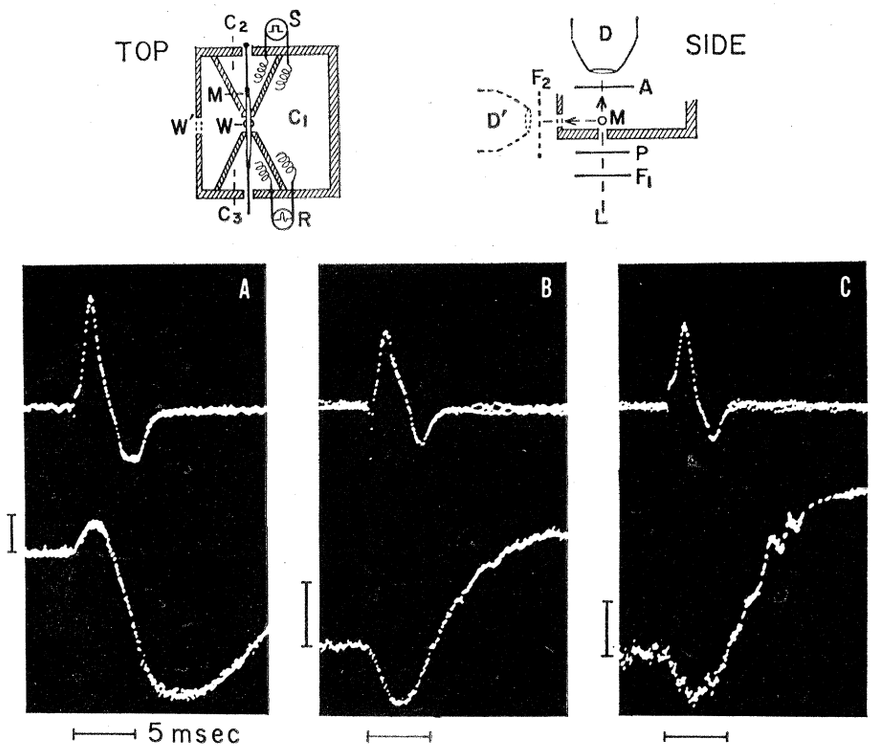

Fig. 1. (Upper) Schematic diagram (top and side views) of recording chamber and optical arrangement used for detection of changes in turbidity, birefringence, and fluorescence of skeletal muscles following electric stimulation. Abbreviations: M, muscle fibers; S, stimulating electrodes; R, recording electrodes; W and W', windows for illumination and detection; L, light source; F_1, interference filter; F_2, sharp cut filter; P and A, polarizer and analyzer, respectively; and D (or D'), photomultiplier at 0° (or 90°). (Lower) Records of the changes in light intensity (lower traces) coincident with the externally recorded action potentials (upper traces) in frog striated muscles. The records show the optical signals representing changes in light scattering (A); changes in birefringence (B); and changes in fluorescence of muscle fibers stained with pyronine B (C). Different muscle preparations were used in each record. An upward deflection of the lower trace represents an increase in light intensity. A CAT computer was used to record both the action potentials and the optical signals. The vertical bars represent an increase of 2×10^{-4} (A and B) or 10^{-4} (C) times the resting level of illumination.

In all the experiments, the tendinous ends of the semitendinosus muscles were tied with silk thread and the resting length between the ends was measured *in situ*. After mounting the muscle fibers at resting length in the chamber, the thread from one of the tendons was fixed to a micromanipulator and the fibers were stretched until the length of a segment in the zone of optical recording was increased by 80 to 100 percent. At this degree of stretch, the light scattering associated with contraction was abolished (*4*). The excitability of the muscle fibers was not significantly affected by this stretch. In preliminary experiments in which hypertonic sucrose-Ringer solution was used as another means of dissociating excitation from contraction results similar to those described below were obtained. Optical signals during excitation could be observed when contraction was present, but elimination of contraction permitted more rapid signal averaging and higher amplification.

By the method described above, reproducible and consistent changes in turbidity, birefringence, and fluorescence (of fibers stained with pyronine B) were found in muscle fibers coincident with the action potential. Record A in Fig. 1 shows that the earliest change in the intensity of light scattered by muscle fibers at 90° (lower trace) is coincident with the externally recorded action potential (upper trace). In order to compare the time course of the electrical and optical signals, intracellular recordings from single muscle fibers in the middle pool (C_1) were made by use of micropipettes filled with KCl. The peaks of the intracellularly recorded action potentials occurred 0.2 to 0.8 msec after the peaks of the extracellularly recorded action potentials, which indicates that the peak of the initial upward deflection of the optical trace roughly coincides with the peak of the internally recorded action potential. (That the optical signal coincident with the action potential is due to an increase in turbidity and not to a change in absorption could be shown by reversal of the optical signal at 0°.) Neither the time course nor the magnitude of the optical signal was found to vary significantly with the wavelengths of the incident light between 450 and 650 nm.

To detect birefringence, a polarizer was positioned below the chamber with the plane of polarization at 45° to the longitudinal axis of the fibers and an analyzer was placed above the muscle in the crossed polar position. Under these conditions, there was a decrease in light intensity concurrent with the action potential (see record B). This decrease in the birefringence of the fibers could be observed with all the wavelengths examined between 450 and 650 nm. No optical signal was obtained with the polarizer and analyzer in the crossed polar position and the plane of polarization either parallel or perpendicular to the long axis of the muscle.

In fluorescence studies, the middle chamber was initially filled with Ringer solution containing 0.05 mg of pyronine B per milliliter. The portion of muscle in the middle pool was stained with dye solution for 10 minutes and then washed with dye-free Ringer. Neither the amplitude nor the duration of the action potential was affected by staining with this fluorochrome. The interference filter (F_1), inserted between the muscle and the light source, had a peak of transmission at a wavelength of 550 nm, which corresponded to the absorption maximum of pyronine B (*5*). A secondary filter (F_2) placed between the muscle and the photomultiplier cut off all light shorter than 610 nm in wavelength. Record C shows a transient decrease in fluorescence beginning at the onset of the action potential.

The following observations show that the fluorescence signal was not due to artifacts such as imperfect transmission characteristics of the filters or the effects of electric current on the dye molecules. (i) If the muscles were not stained, but the rest of the experimental procedure remained unchanged, no optical signal was obtained. (ii) When the cut-off filter (F_2) was positioned between the interference filter and the muscle, no early optical signal was observed. (iii) When the stimulating electrodes were reversed, there was no change in the fluorescence signal after stimulation until the action potential was conducted from the lateral to the middle chamber.

The earliest changes in the optical properties of the muscle fibers described in this report are coincident with the action potential. The variations in light intensity subsequent to the early optical signals in records A, B, and C are most likely due to changes in the optical properties of the fibers during the period of latency relaxation (*4, 6*). The early increase in turbidity, decrease in birefringence, and decrease in fluorescence (of fibers stained with pyronine B) following electrical stimulation of muscle are similar to the changes in the optical properties observed in nerve during excitation (*2, 3*). In other experiments to be detailed elsewhere, similar changes in fluorescence during the action potential of giant barnacle muscle fibers stained with pyronine B, in which excitation was dissociated from contraction by internal injection of ethylenediaminetetraacetate, have been detected. This similarity may be an indication that the excitation process is due to comparable conformational changes in the membrane macromolecules of nerve and muscle.

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Unexpected Adrenergic Effect of Chlorpromazine: Eating Elicited by Injection into Rat Hypothalamus

Abstract. *Although chlorpromazine is believed to block adrenergic transmission, injection of this drug into the hypothalamus of satiated rats does not block norepinephrine-elicited eating, but instead mimics norepinephrine by eliciting eating. The amount of eating elicited by norepinephrine and by chlorpromazine is reliably correlated. These results suggest that endogenous norepinephrine mediates eating elicited by centrally injected chlorpromazine.*

Chlorpromazine (CPZ), a tranquilizing agent, is probably the most widely used drug in the treatment of mental illness. Its mechanism of action on the central nervous system, however, still