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FLUORESCENCE CHANGES IN DYE-TREATED NERVE FOLLOWING ELECTRIC STIMULATION

Ichiji Tasaki

Laboratory of Neurobiology
National Institute of Mental Health, Bethesda, Maryland

Last year, following up a suggestion made some time ago by Dr. Morales, my collaborators and I found that the fluorescence of an ANS-treated nerve does actually change when the nerve is stimulated (1). This finding is consistent with the view that the process of nerve excitation and conduction involves reversible and cooperative changes in the conformation of the macromolecules in the nerve membrane. Later, we found that several fluorochromes in addition to ANS can be used to demonstrate fluorescence changes during nerve excitation (2,3).

The experimental arrangement for this purpose is shown diagrammatically in Figure 1 (top). Symbol S represents a light source, L_1 and L_2 are quartz lenses; F_1 is an interference filter for the wave length at which the particular fluorochrome used has its absorption maximum; F_2 is a high cut-off filter which passes only the portion of light much longer in wave length (at least 40 nm) than the incident light (through F_1). The nerve chamber was made of black lucite and was provided with two pairs of platinum electrodes, one pair (E) for stimulation and the other (V) for externally recording action potentials. A 3-4 mm long portion of a nerve (N) was vitally stained with a fluorochrome. Changes in the intensity of the flourescent light were recorded with a photomultiplier (P) used in conjunction with a CAT computer. In some instances fluorescence changes were recorded directly with an oscilloscope (without a computer).

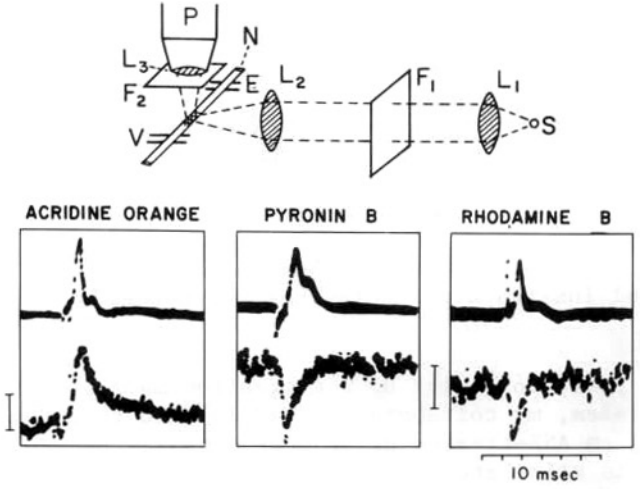

Figure 1. Fluorescence changes associated with nerve excitation. (Adapted from ref. 3)

The upper traces in the figure represent the action potential recorded from the nerve near the site of optical recording. The lower traces show changes in fluorescence associated with the action potentials. The intensity of fluorescence was found to increase in crab nerves stained by immersion in sea water containing ANS. Similarly, an increase in fluorescence was observed in nerves stained with acridine orange. In the case of nerves stained with either pyronin B or rhodamine B, there was a decrease in fluorescence at the moment when the nerve impulse reached the site of optical recording. The duration of the optical signal was comparable to that of the action potential.

In a series of experiments, fluorochromes were introduced into the protoplasm of squid giant axons, keeping the connective tissue and Schwann's cells free of fluorescent dyes. Intracellularly applied acridine orange gave rise to an increase in fluorescence during nerve excitation (2).

The effect of electric currents through the nerve membrane upon the flourescence was examined in nerves stained with various fluorochromes (3). An outward-directed current through the membrane (depolarization) produced an increase in

fluorescence in nerves stained with acridine orange and a decrease in nerves stained with pyronin B. The effect of a hyperpolarizing current was opposite to that of a depolarizing current. In nerves stained with rhodamine B, a diphasic optical signal (with an initial decrease followed by an increase in fluorescence) was observed during passage of a depolarizing current. This finding obtained with rhodamine B indicates that there is no simple relationship between the membrane potential and the intensity of the flourescent light.

References

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