MECHANICAL CHANGES IN SQUID GIANT AXONS ASSOCIATED WITH PRODUCTION OF ACTION POTENTIALS

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SUMMARY: The action potential of squid giant axons is accompanied by a quick and small swelling, about 0.5 nm in displacement of the surface, and about 1 dyne/cm^2 in pressure increase.

INTRODUCTION

Hill et al. (1) reported that a small rapid contraction in diameter of a crayfish axon was observed when an action potential traveled along the axon. By measuring two mechanical quantities, displacement and pressure changes, however, we have demonstrated that swelling is the primary event which takes place in giant axons of the squid, Loligo pealei, concomitantly with the action potential. The phase of swelling was found to be followed by a slower contraction phase.

METHODS

(A) For detecting small changes in the pressure at the axon surface, as in our recent study (2), we used a piezo-ceramic bender (type R050S, Gulton Industries, Inc.) in conjunction with a voltage follower (AD515, Analog Devices). The output of the voltage follower was led to a signal averager (model 1072, Nicolet Instrument Corp.) after amplification by a factor of 1,000. The sensitivity of the bender as a mechano-electric transducer was calibrated by hanging weights at the tip; its frequency response was examined by connecting a calibrated condenser microphone with a thin thread. The frequency response was found to extend up to about 10,000 Hz under our experimental conditions.

The experimental setup employed is illustrated schematically in Fig. 1, top. The plastic nerve chamber consisted of three seawater-filled compartments connected with two 6-mm long, narrow grooves. The chamber had a curved surface so that the bottom surface of the axon was making firm contact with the solid surface. A portion of an axon devoid of small fibers was introduced into the 6-mm wide middle compartment. The upper surface

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of the axon was pressed with the flattened end of a stylus (made of bristles) attached to the bender. Electric shocks were applied to the axon through a pair of platinum electrodes on one side of the middle compartment and the action potentials evoked were recorded on the other side. Mechanical responses were recorded after averaging over 1024 or 4096 trials.

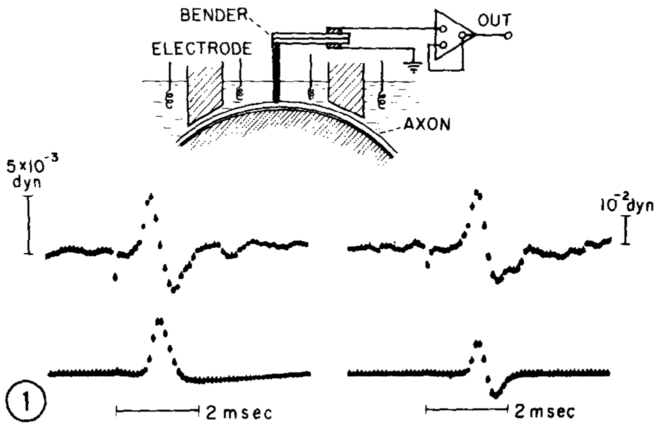
(B) To measure small displacements of the axon surface (see Fig. 2), we employed a system of fiber optics including a Fotonic sensor (Mechanical Technology, Inc.). The sensor consists of two bundles of optic fibers, one for transmitting light from a source (100 W quartz-iodine lamp) to the light-reflecting target (gold particles) on the surface of the axon, and the other for carrying the reflected light to a photo-diode (Pin-10, United Detector Corp.). The fibers in the two bundles are mixed at the sensing end. The photo-current was measured by the use of FET operational amplifier (Model 1011, Teledyn Philbrick). With this device, the intensity of the detected light increases sharply with the distance between the target and the tip of the sensor and reaches a maximum at about 150μm from the target.

RESULTS AND DISCUSSION

In the records of the mechanical responses presented in Fig. 1, an upward deflection represents a rise in the pressure exerted by the axon to the bender. The peak value of the force observed ranged between 7 and 13 μg. The flattened surface of the stylus had an area of about 0.01 cm^2; therefore, the rise in pressure was roughly 1 mg/cm^2 or 1 $dyne/cm^2$.

The period of an elevated pressure (about 0.6 msec) was close to the depolarizing phase of the action potential. The peak of the mechanical response was found to precede the peak of the action potential by 0.1-0.2 msec; this period corresponds roughly to the time required for the impulses to travel from the site of mechanical recording to the wall of the middle compartment. We thus find that the peak of the mechanical response was reached roughly at the moment when the peak of the action potential reached the site of mechanical recording. The period of an elevated pressure was followed by a slightly longer period of a depressed pressure.

The mechanical record in Fig. 2 was obtained by keeping the sensor at a position where the light intensity varied very



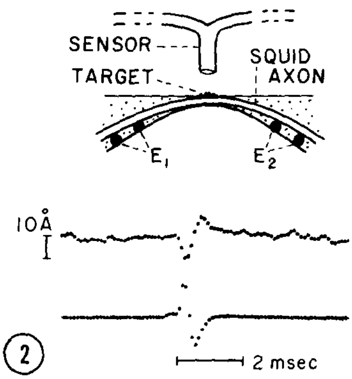


Fig. 1. Top: Schematic diagram of the setup used for measuring pressure changes in squid axon during action potentials. A bender provided with a bristle is held by a micromanipulator. Bottom: Records of pressure changes (upper trace) and extracellularly recorded action potentials (lower trace). An upward deflection of the upper traces represents a pressure increase. Stimulating shocks are delivered at the beginning of the time marker. Temperature 21.5°C.

Fig. 2. Top: Setup used for measuring surface displacements. E's represent electrodes. Bottom: An example of mechanical records. A downward deflection in the upper trace represents an upward displacement of the target, gold particles, placed on the axon. The lower trace is extracellularly recorded action potential. Temperature 23°C.

sharply with the distance. The downward deflection of the upper trace represents a reduction in the light intensity. The observed deflection indicates that there was a transient upward movement of the axon surface at the time when action potential was generated. The time-course of the surface movement was very similar to that of the tension developed. The observations described above strongly suggest that squid giant axons swell concomitantly with the production of the action potential.

The magnitude of the displacement of the surface of the squid axon during the action potential was close to the value reported by Hill et al.(1). However, the mechanical responses we observed had a rising phase far shorter than those described previously. Furthermore, the mechanical responses in crayfish axons were said to represent a "contraction" of the diameter followed by a slow expansion. It was difficult for us to understand why crayfish axons contract whereas squid axons expand. Therefore, we examined mechanical responses of crab and crayfish axons using our recording device. The results of those experiments, which will be described elsewhere, indicated that crustacean axons also expand during the depolarizing phase of the action potential.

REFERENCES

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2. Tasaki, I., Iwasa, K. (1980) Biochem. Biophys. Res. Commun. in press.