brief communication

Volume expansion of nonmyelinated nerve fibers during impulse conduction

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ABSTRACT Nonmyelinated nerve fibers undergo rapid volume expansion while carrying an impulse. This volume expansion is incurred as a consequence of a lateral expansion of the excited portion of the fibers, where the superficial layer is transformed into a low-density structure.

INTRODUCTION

In a series of recent experiments, we have demonstrated that the production of an action potential in nonmyelinated nerve fibers is accompanied by rapid swelling (i.e., lateral expansion) of the fibers (1–3). In addition, we have shown that simultaneously with the swelling occurs longitudinal shortening of nerve fibers (3, 4). Although the shortening is far smaller than that of skeletal muscles, there is a certain similarity between the mechanical changes in the muscle and those in the nerve. In the muscle, the existence of another type of mechanical change, namely, expansion and dimunition of the volume, is known (5–9). In the present report, we show that there is also a rapid volume change during impulse conduction in nonmyelinated nerve fibers.

Previously, we favored the supposition that the volume of the nerve fiber could remain constant during impulse conduction because the swelling was shown to be accompanied by longitudinal shortening. In the present report, we demonstrate that the volume of the nerve does not remain constant but it increases in response to electric stimulation, and that this increase is brought about by lateral expansion of the constituent nerve fibers.

MATERIALS AND METHODS

Isolated and cleaned olfactory nerves of the garfish, *Lepisosteus osseus*, were employed because of their high membrane content (3, 10). A bundle of three nerves of 13–16 mm in length, tied together with fine thread at the ends, was introduced into a water-tight nerve chamber and rapid changes in the hydrostatic pressure in the chamber were measured by using a mechanoelectric transducer. The schematic diagram in Fig. 1 illustrates the experimental setup employed.

The nerve chamber was made from a 4.5-cm cube block of plastic. The chamber had three cavities, each having a size of ~0.3 cm^3. These cavities were interconnected with a hole of 0.9 mm in diameter drilled through the 2–3-mm-thick partitions separating the cavities. Four large silver electrodes were installed on the walls of the cavities, two electrodes in the middle cavity. The upper cavity was directly connected to an opening of 5 mm in diameter at the top of the chamber. The cavities and the interconnecting holes were filled with oxygenated saline solution for garfish nerve (3). A nerve bundle was passed through the holes, and its ends were fixed to small silver hooks in the top and bottom cavities. In the early stage of the present study, the holes were aligned in such a way that the nerve bundle was held vertically in the chamber, as shown in the figure. In later stages, however, the positions of the holes were staggered and the nerve was bent at every partition. This was done for the purpose of preventing lengthwise displacement of the nerve bundle associated with action potential production (see Fig. 3 in reference 3). As a rule, the holes in the partitions were sealed with vaseline. Then, the front surface of the chamber, covered with a 100-μm-thick silicon-rubber gasket, was completely sealed with a thick plastic plate (not shown). Precautions were taken to remove all visible bubbles in the upper cavity. Finally, the hole on the top of the chamber was covered with a 6-μm-thick stainless-steel diaphragm and was rendered leak-proof by pressing the diaphragm against the top surface of the chamber with a rubber O-ring (not shown). Small pressure (0.5–1 atm) was applied to the diaphragm by means of a screw penetrating the wall of the chamber.

The mechanoelectric transducer employed was a G-1195 piezo-ceramic bender purchased from Gulton Industries Inc. (Metuchen, NJ). One end of the bender was immobilized and connected to the input of an operational amplifier (OPA 128) which had a feedback, capacitor (usually 0.5 nF) and a parallel resistance (0.5 or 10 GΩ). To the other end of the bender, a stylus (14 mm in length and ~15 mg in weight) was attached. The box housing the bender was attached to a rack-and-pinion device and was lowered from above toward the steel diaphragm on top of the nerve chamber. After bringing the stylus in light contact with the surface of the diaphragm, the bender was lowered further by 100 μm.

The transducer was calibrated by measuring changes in the output voltage produced by movements of a screw penetrating the chamber wall. In a wide range of volume increase and decrease in the chamber, the voltage change varied linearly with the volume. In the absence of air bubbles in the cavities, a volume change of 0.14 mm^3 in the chamber generated a change of 1 V in the output of the operational amplifier. The resonant frequency of the bender was 380 Hz when the stylus was free in air; it was markedly enhanced when the stylus was brought in contact with the steel diaphragm. The output was amplified further with a capacity-coupled amplifier with a gain of 1,000 and then led to a signal-averager (model 1070, Nicolet Instrument Co., Madison, WI). Pressure changes in the chamber were evoked by delivering brief (0.5 ms) voltage pulses (15–20 V) to the nerve across the lower partition using electrodes E_3 and E_4.

It was found necessary to suppress the sensitivity of the recording device to extraneous mechanical disturbances which could produce irregular distortions of the base line of the records obtained. This was accomplished by placing both the nerve chamber and the operational amplifier on a heavy metal plate and suspending them from the top of a metal rack by means of rubber bands. In addition, the metal plate and rack assembly were placed on a pneumatic isolation bench (Barry Controls, Watertown, MA). All the experiments were carried out at room temperature (20–22°C).

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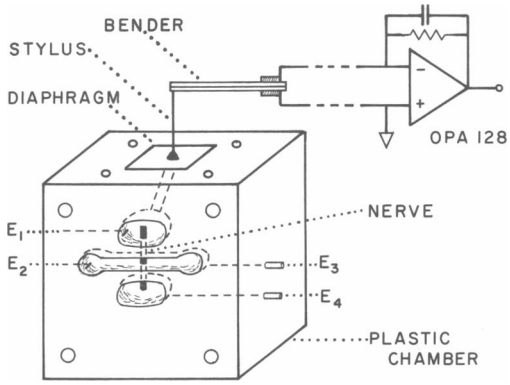

FIGURE 1 Schematic diagram illustrating the plastic nerve chamber employed for measuring volume expansion of the garfish olfactory nerve during impulse conduction. The chamber has three cavities separated by two partitions. The upper cavity is connected to an opening (covered with a metal diaphragm) at the top of the chamber. E_1 and E_2 represent silver electrodes on the wall of the upper and middle cavities (used for recording action potentials of the nerve bundle): electrodes E_3 and E_4 were employed to deliver stimulating voltage pulses across the partition between the middle and lower cavities, E_3 being used as the cathode. All the cavities were with filled preoxygenated Ringer and were closed in a water-tight manner with a thick plastic plate (not shown). See the text for details.

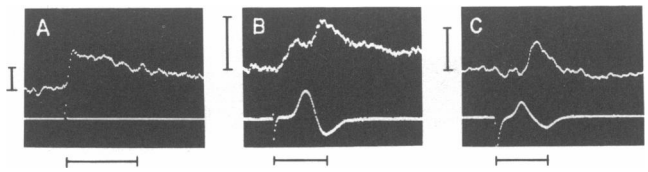

FIGURE 2 (*A*) Record showing a rise in the hydrostatic pressure in the cavities of the nerve chamber (*upper trace*) after the delivery of a maximal stimulating pulse to a bundle of nerve fibers (*lower trace*). The nerve was held vertically in the nerve chamber. (*B*) Record taken from a nerve bundle bent at the upper and lower partitions in a nerve chamber. The holes were carefully sealed with vaseline. Note that the pressure rise in the upper cavity occurred in two steps. The lower trace represents the action potential recorded with the electrodes placed across the upper partitions (~6 mV in amplitude). (*C*) Record obtained from a nerve bundle bent at the partitions of the chamber which had holes in staggered positions. Time markers, 30 ms.

RESULTS

Fig. 2 shows examples of the records obtained. Record A was taken using the nerve chamber in which the nerve was held vertically. An upward deflection of the upper trace in the record represents the development of a force tending to displace the piezoceramic bender upwards, indicating that there was an expansion of the volume in the chamber. It is seen that the volume expansion started almost immediately after the delivery of a stimulating pulse. The volume expansion observed under these conditions lasted for a period of 50 ms or longer. It is known that the conduction velocity in this nerve is ~0.2 m/s at room temperature (3); hence, the long duration of volume expansion is considered to reflect the time required for the nerve impulse to reach the ends of the 15-mm-long nerve bundle (3). The magnitude of expansion observed was usually between 1.0 and 2.6 times 10^{-6} mm^3.

Record B was taken using the nerve chamber with staggered holes (see Methods). Only a short (3–4 mm) portion of a nerve bundle was introduced into the upper cavity. After introduction of the nerve bundle into the chamber, the holes were carefully sealed with vaseline. It is seen in the record that the upper trace shows two humps separated by ~20 ms. Obviously, the first hump is associated with the impulse initiated in the middle cavity. The lower trace in the record shows the action potential recorded across the upper partition (with electrodes E_1 and E_2 in Fig. 1). The second rise of the upper trace is seen to start at the time of arrival of the nerve impulse at the upper cavity, as indicated by the onset of the falling phase of the diphasic action potential. Thus, we find that the deflection of the upper trace after the "notch" represents the volume expansion of the portion of the nerve in the upper cavity.

It was found possible to reduce the pressure change in the middle cavity by introducing a small amount of air into the cavity (see Record C). The nerve chamber with staggered holes in the partitions was employed. By rotating a screw in the hole on the chamber wall, a ~5 mm^3 air bubble was introduced into the fluid medium in the middle cavity. The partitions were sealed with vaseline after introducing the nerve bundle into the chamber. Then, the pressure in the chamber was raised slightly and a record was taken of a response to a stimulating voltage pulse. It is seen that the deflection of the upper trace in Record C consists predominantly of a pressure rise (slightly reduced in size) associated with the volume expansion taking place in the upper cavity. The small downward deflection of the trace seen immediately after stimulation is interpreted as being brought about by a lengthwise shortening of the nerve bundle associated with the onset of a nerve impulse in the middle cavity.

It may be noted in this connection that the compressibility of water at 20°C is ~4.6×10^{-5} per atm and also that the pressure rise in the nerve chamber associated with the production of a propagated action potential is ~10^{-6} atm. Hence, the water in the chamber may be regarded as being incompressible under the present experimental conditions. The volume change, ΔV, of an air bubble with volume V is related to the change in

pressure, Δp, by the relation $\Delta V/V = -\Delta p/p$, where p denotes the pressure maintained inside the chamber (1.5–2 atm). Hence, the presence of a small bubble ($V \approx 10^{-3}$ mm^3) does not interfere with the measurements.

DISCUSSION

We have seen that, associated with impulse conduction in the garfish olfactory nerve, there is a volume expansion of the order of 2×10^{-6} mm^3. The wet weight of the 15-mm-long nerve bundle employed in these experiments was usually between 5 and 8 mg. There is little doubt that the observed volume expansion takes place only in the excited region of the nerve which is ~2 mm (3). This excited region weighs only ~1 mg. The volume expansion occurring in this region must then be of the order of 10^{-6} cm^3 per gram of the nerve. This value is only slightly smaller than that observed in the frog muscle (8, 9).

The present measurements give information only about changes in the sum of the volume of the nerve and the volume of the external fluid medium. Therefore, the observed volume expansion cannot be ascribed to simple translocation of water (or other) molecules from outside to the interior of the nerve fibers (In the case of simple translocation, an increase in the volume of the nerve proper should be accompanied by a decrease of the same magnitude in the outside medium.) For the same reason, the observed volume change cannot be explained in terms of the well-known Na^+-K^+ exchange process associated with action potential production. Our finding indicates that there is a decrease in the density of the excited portion of the nerve fibers while carrying an impulse.

It is to be noted that this decrease in the density is not a phenomenon associated with a rise of the temperature. The temperature rise associated with action potential production is about 2.3×10^{-5} degrees (3). Thermal expansion of water is known to be 2.1×10^{-4} deg^{-1} (see Dictionary of Chemistry and Physics, CRC Press). Hence, the volume expansion associated with the temperature rise is probably smaller than 10^{-8} times the volume of the excited portion of the nerve. This is too small to account for the phenomenon described in this paper.

Previously, we have shown that the swelling of the nerve fiber rises and falls simultaneously with the action potential recorded from the site of mechanical recording (1, 3). For this and other reasons, we have proposed that the mechanical (and electrical) changes in the nerve fiber during excitation arise from replacement of divalent cations, Ca^{2+}, bound to multianionic sites of the membrane macromolecules with univalent cations (Na^+ and K^+). It appears possible that such a cation-exchange process converts compact layers in and near the membrane into swollen, low-density structures. Such a cation-exchange process may give rise to an enhancement of repulsive electrostatic force between fibrous macromolecular elements near the membrane, thus contributing to a rise in lateral expansion of the nerve fiber. It has been suggested that longitudinal contraction in the muscle may arise from an enhancement of electrostatic repulsion between actin filaments (11). In the nerve fiber, there is little doubt that lateral expansion of the excited region of the nerve fiber is at the base of the longitudinal shortening, as well as the phenomenon of volume expansion described in this report. This finding is significant because it throws new light on the mechanism whereby the mechanical (and electrical) changes in the nerve fiber are generated.

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