THE EFFECTS OF HYDROSTATIC PRESSURE UPON THE NORMAL AND NARCOTIZED NERVE FIBER*

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INTRODUCTION

Recently, the author reported the results of an investigation on the effects of hydrostatic pressure upon the electrophysiological properties of the vertebrate myelinated nerve fiber (1). In the present paper are reported further studies along this line. Two types of experiments will be presented: (*a*) experiments on the effects of hydrostatic pressure upon the electrophysiological properties of the giant axon of the squid, and (*b*) experiments demonstrating a dependence of narcosis of the axon upon temperature and pressure.

The preliminary experiments on these problems were conducted in collaboration with other members of this laboratory and were presented at the Fourth Symposium of the Society of General Physiology (2).

Methods

The Measurement of the Resting and Action Potential.—The giant axon of the stellar nerve of the squid, *Loligo pealii*, was used. The major portion of the small fibers around the giant axon was removed with a small pair of scissors under darkfield illumination. The methods of recording the resting and action potentials were similar to those used by previous investigators (3, 4). Two types of intracellular electrodes were employed for studying the action potential of the giant axon. Both electrodes were inserted into the interior of the axon through the cut end. One electrode consisted of a 25 μ insulated nichrome steel wire wound on a 100 μ insulated silver wire. The insulated silver wire was scraped bare for a total length equal to 10 to 20 mm. In the middle of the scraped area of the silver wire, the steel wire was scraped bare for a length equal to 1 to 2 mm. The nichrome wire was used for recording the action potential and the silver wire was used for stimulation. A precaution was taken to avoid direct metallic contact between the two wires. The second type of intracellular electrode used was a glass tube (50 to 100 μ in diameter) filled with isotonic KCl solution. This electrode was used for recording both the resting and the action potential. The axon was stimulated near the cut

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end at a point several centimeters away from the tip of the pipette with two steel wire electrodes. The indifferent external electrodes with both methods of recording action and resting potentials were of the Ag–AgCl type. The method for recording the resting and action potentials with internal metal wire electrodes is illustrated in Fig. 1.

The Measurement of the Membrane Impedance during Activity.—The metal wire internal electrodes described above were used. The fiber was stimulated either through the long metal wire electrode in the axon or with a pair of external steel wire electrodes. The long internal electrode and the external Ag–AgCl electrode were connected to an arm of an A.C. impedance bridge. The ratio arms of the bridge were a pair of fixed resistors (one 10 ohms and the other either 100 or 300 ohms). The bridge was balanced

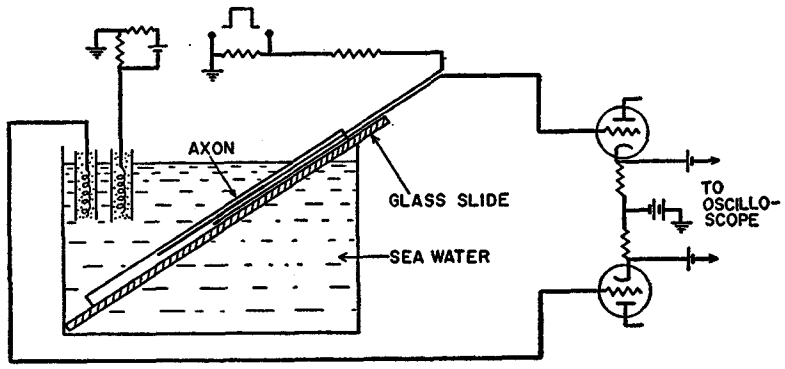

FIG. 1. Arrangement for recording action potentials using two internal metal wire electrodes. Axon was fixed to plate. Long metal wire (uninsulated for 10 to 20 mm.) was used for stimulation. Short metal wire (uninsulated for 1 to 2 mm.) was used for recording the action potential.

by varying the parallel capacity (0 to 200 $\mu\mu$f.) and the resistance (0 to 300 kilohms) in the third arm of the bridge. The frequency of the bridge A.C. was 10 to 20 kc. per sec. The output of the bridge, after filtering and amplification, was led to one beam of a dual channel oscilloscope. To the other beam was led the unfiltered response as recorded with the short internal electrode. In this manner simultaneous measurements were made of the membrane potential and impedance during activity.

The Measurement of the Time Constant of the Membrane.—The intracellular metal wire electrodes were used. The long internal electrode was used for passing a current pulse through the axonal membrane and the short electrode for recording the change in membrane potential brought about by the current flow. Two methods were employed for measuring the time constant of the membrane. In one method a long weak rectangular current pulse was passed through the membrane; the initial rate of change in membrane potential was a function of the membrane capacity and the plateau level of the potential was a function of the membrane resistance. In the other method a brief strong rectangular pulse of inward current was applied to the membrane charging its capacity. The extent of the initial rise in potential was an index of the mem-

brane capacity and the rate of exponential decay of the potential, an index of the time constant of the membrane.

The Measurement of the Threshold Membrane Current.—The threshold membrane current was determined using the arrangement of Fig. 1. The long metal wire was used for passing the test current pulse through the membrane. The short metal wire was used for recording the response.

The Measurement of the Conduction Velocity.—The axon was placed inside a plexiglass tube 30 to 40 mm. long and about 1 to 1.5 mm. inside diameter. At one end of the tube the axon was in contact with two platinum wires that were used for stimulation. The axon mounted in the tube was immersed in sea water. In this manner direct contact with the mineral oil was avoided (*cf.* reference 1). An estimate of the conduction velocity was obtained from the shock response interval.

Variation of Pressure.—Hydrostatic pressure was varied from 14.7 to 16,000 psi. The reaction vessel employed was of the "outside cap compression closure type." (For more details of the assembly for increasing the pressure and of the need for surrounding the fiber with large volumes of aqueous solutions to prevent heat change: *cf.* reference 1.)

The Temperature.—The results to be described were obtained at 10°–15°C. unless special mention is made.

Other Instruments.—Other instruments employed included a General Radio Company beat-frequency oscillator, a Spencer-Kennedy variable electronic filter, a DuMont dual beam cathode ray oscillograph, a Grass kymograph camera, a Grass stimulator, a Tektronix stimulator, and a Tektronix preamplifier. The cathode follower utilized a Z729 Emitron tube.

RESULTS

The study of the effects of pressure upon the electrophysiological properties of the giant fiber was complicated by the finding that at pressures of 3000 to 7000 psi the fiber fired spontaneously. Reliable measurements of most properties could be made only at pressures below the threshold for spontaneous discharge.

Resting Potential.—In the range of 14.7 to 5000 psi the resting potential as determined with the internal glass pipette was found to be independent (within 5 per cent of pressure.

Membrane Resistance and Capacity.—At pressures above atmospheric but below 4000 to 6000 psi the resistance of the membrane was slightly increased (Fig. 2). At 5000 psi the resistance was 10 to 20 per cent higher than at 14.7 psi. At pressures above 4000 to 6000 and below 12,000 psi the membrane resistance often decreased to as much as one-half to one-third the resistance at 14.7 psi. Under these conditions the recovery of the original resistance upon decompression was poor. These changes in the membrane resistance are shown by the variations in the plateau level of the membrane potential in Fig. 2. Since the intensity of the rectangular current pulses applied through the membrane was held constant, the change in the (D.C.) potential level was a direct measure

of the membrane resistance. In the record of Fig. 2, the change in the membrane potential caused by the current pulse was (before any compression) about 45 mv. Essentially the same results were obtained in the experiments in which the current intensity was reduced so that the potential variation was within a few millivolts below or above the resting potential (recorded after higher amplification).

In the range of 14.7 to 6000 psi, the membrane capacity was found to be independent of pressure. This is shown by the coincidence of the initial rate of potential fall in the two lower traces in Fig. 2. A change in capacity over

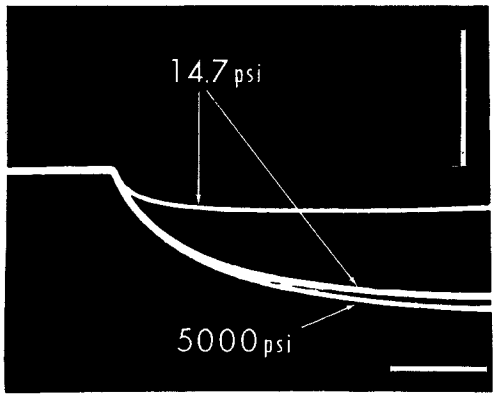

FIG. 2. Records showing the effect of hydrostatic pressure upon the membrane resistance and capacity. The membrane potential was modified by passing a constant current through the membrane using the arrangement of Fig. 1. Middle tracing at 14.7, lower tracing at 5000 and upper tracing at 14.7 immediately after subjecting the axon to 12,000 psi. Vertical bar at right subtends 50 mv. The duration of the current pulse was 20 msec. Pulse amplitude 3×10^{-6} amperes. Time marking 5 msec.

20 per cent would have been detected. Even after the membrane resistance suffered an irreversible reduction under high hydrostatic pressure, it was not possible to detect a measurable change in the membrane capacity.

Threshold Membrane Current.—The threshold membrane current and the threshold membrane potential were found to be significantly dependent upon pressure. The threshold membrane current as well as the threshold membrane potential decreased with an increase in pressure. A decrease in threshold was apparent at pressures below 100 psi. At pressures from 3000 to 7000 psi the threshold approached zero, and the fiber started to discharge nerve impulses spontaneously.

Conduction Velocity.—The conduction velocity was found to be only slightly affected by pressure. At 5000 psi the conduction velocity as evidenced by the shock response interval was decreased by about 5 to 15 per cent.

Action Potential.—At 5000 psi the amplitude of the action potential was found to be relatively unaffected. The duration of the falling phase of the response was markedly increased by an increase in pressure. At 4000 psi the duration was increased by about 40 to 60 per cent. The duration of the rising phase was also increased though always with a considerably lower coefficient than that of the falling phase. At 4000 psi it was increased by about 20 to 35 per cent. The effects of pressure upon the action potential are illustrated in Fig. 3. The record at the left was obtained at 14.7 psi and that at the right at 5000 psi. The duration of the rising phase was increased by about 35 per cent and that of the falling phase by about 65 per cent; there was no appreciable change in the amplitude of the response.

Membrane Impedance during Activity.—With the present system of recording

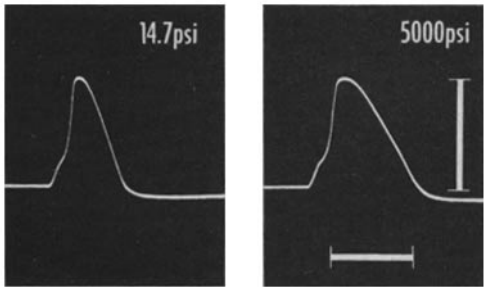

FIG. 3. Membrane action potentials of a squid giant axon recorded at 14.7 psi and 5000 psi. The arrangement of Fig. 1 was used. Vertical bar at right subtends 100 mv. Time marking 5 msec.

simultaneously the action potential and the membrane impedance loss, it was found that the peak of the impedance changes followed the peak of the potential changes by 0.5 to 3 msec. depending upon temperature and pressure. At 5000 psi the amplitude of the maximal bridge unbalance was in most experiments decreased by about 15 per cent or less. At the same pressure the duration of the bridge unbalance was increased by a factor of about 1.7 to 2.0. The transition from the unbalanced state to the balanced was very gradual so that an accurate determination of the duration of the changes in impedance was not possible. For this reason the pressure coefficient of the duration given above should be considered as being somewhat arbitrary. The impedance measurements were carried out at 5°–10°C.

The Temperature and Pressure Dependence of Drug Action.—Application of 3 to 7 per cent ethanol–sea water at room temperature (20°–23°C.) reduced the amplitude of the spike by about 20 to 50 per cent. Occasionally, under alcohol, the response obtained (when stimulated with an internal electrode)

varied with the intensity of the stimulus. When the temperature of the narcotized axon was lowered to 6°–10°C. the amplitude of the action potential was almost completely or partially restored. A restoration of the heightened threshold was also observed at low temperatures. In those instances in which the response of the alcohol-treated axon was not all-or-none at room temperature, lowering of the temperature restored the all-or-none property.

The temperature dependence of narcosis in the giant axon is illustrated in Fig. 4. In this experiment two metal wires were inserted into the interior of the axon, as shown in Fig. 1. One wire was used for stimulation and the other

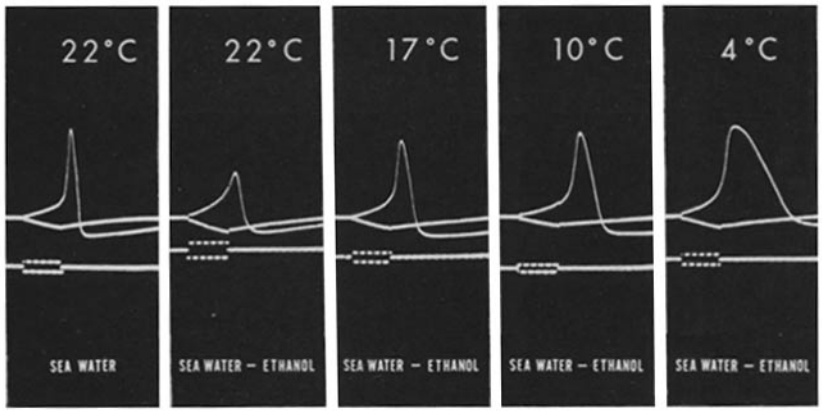

FIG. 4. Records showing the counteraction of ethanol narcosis with low temperatures. The amplitude of the response of the giant axon treated with ethanol at 22°C. varied with the intensity of the stimulus. At 4°C. the fiber fired spontaneously. The record in this figure (at 4°C.) was obtained by electrical stimulation with the internal wire electrode. The arrangement of Fig. 1 was used. Concentration of ethanol 3 per cent. Time marking 5 kc.

for recording the action potential. The record at far left (obtained at 22°C. in normal sea water) is the control. The second record from the left was obtained at 22°C. in sea water containing 3 per cent ethanol. The response obtained was not all-or-none. In the record presented in the figure the peak of the change in potential was reached following the end of the stimulating pulse. Under the same conditions action potentials of a much higher amplitude were obtained with higher stimulus intensities. The three records at the right were obtained at low temperatures (17°, 10°, and 4°C.) in the presence of ethanol in the sea water. At these low temperatures the response was larger and all-or-none.

The increase in the amplitude caused by lowering the temperature was far greater in the narcotized than in the unnarcotized axon. For a drop in tempera-

ture from 22° to 4°C. the amplitude of the response of a normal fiber increases only by about 5 to 10 per cent. For the same decrease in temperature, the amplitude of the response of the narcotized fiber indicated in Fig. 4 was almost doubled. The temperature coefficient of the duration of the response of the ethanol-treated axon is approximately the same as that of the unnarcotized axon.

Application of high pressures (3000 to 7000 psi) upon a narcotized axon had an effect similar to that of low temperatures, though not as dramatic. The effects of hydrostatic pressure upon ethanol narcosis are illustrated in Fig. 5. In this experiment a glass pipette was introduced into the interior of the giant axon through the cut end. Near the cut end the axon was stimulated with two steel wires. The response recorded through the tip of the glass pipette was

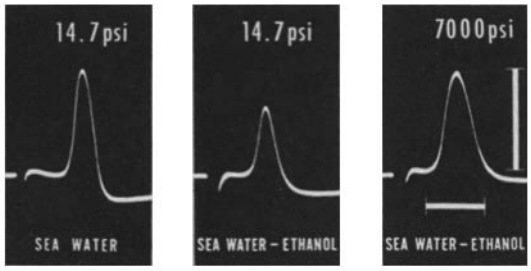

FIG. 5. Records showing counteraction of ethanol narcosis with high pressure. A glass pipette internal electrode was used. The axon was stimulated near its cut end with two steel wires. The glass pipette recorded the conducted response. Ethanol concentration 3 per cent. Temperature 22°C. Vertical bar at right subtends 100 mv. Time marking 1 msec.

a conducted response. The record at left represents the response that was obtained at 14.7 psi in normal sea water. The response shown in the middle record was obtained at 14.7 psi in sea water containing 3 per cent ethanol. The response shown in the record at the right was obtained at 7000 psi in ethanol–sea water. Although the amplitude was increased appreciably, it was still slightly subnormal.

DISCUSSION

In general the effects of pressure upon the giant axon are similar to those upon the myelinated nerve fiber. In both types of fibers one of the most marked effects of pressure involved the duration of the response. In both types of fibers the conduction velocity and the amplitude of the response were relatively unaffected by pressure. A marked reduction in threshold and a spontaneous discharge of nerve impulses were two effects of pressure observed only in

the giant axon. In the myelinated nerve fiber at high pressures the rheobase was only slightly affected and no spontaneous firing was encountered.

Most of the effects of high pressures upon narcosis and upon the electrophysiological properties of the fiber are similar to the effects of low temperatures (for review *cf.* reference 2). The temperature dependence of the pressure effects upon the nerve fiber is under investigation.

A pronounced temperature and pressure dependence of drug action in a variety of chemical and biological systems is well known (for review *cf.* reference 5). An example of this phenomenon is the counteraction by low temperatures or high pressures of narcosis in whole animals. It was very surprising to find that this problem had not been investigated either on the nerve fiber or on the nerve trunk. The experiments presented in this paper on the temperature and pressure dependence of drug action may provide the basis for an explanation of low temperature or high pressure counteraction of some types of narcosis in animals.

The approach of measuring a process at different temperatures, pressures, pH, and concentrations of inhibitors has been used extensively in the study of a number of chemical and biological systems (5). Perhaps further studies on the nerve fiber may prove of some value in the elucidation of the mechanism of excitation.

The sensitivity of the axon to pressure makes it feasible that similar or different properties of other elements in the nervous system may also be sensitive. This in turn would indicate that the *direct* effects of pressure on processes in the nervous system should not be overlooked in the consideration of problems of underwater high pressure physiology.

SUMMARY

The properties of the giant axon of the squid *Loligo pealii* were studied at different hydrostatic pressures from 14.7 to 16,000 psi. At 4000 psi the resting potential, the membrane resistance, membrane capacity, the conduction velocity, the amplitude of the action potential, and the maximal change in the membrane impedance during activity were only slightly affected. At the same pressure the duration of the falling phase of the action potential was increased by about 40 to 60 per cent and the duration of the rising phase by about 20 to 35 per cent. The duration of the membrane impedance change during activity was increased by 50 to 100 per cent at 4000 psi. At pressures even slightly above atmospheric the threshold membrane current was appreciably reduced. At about 3000 to 7000 psi the fiber fired spontaneously. At pressures considerably above 5000 psi the membrane resistance decreased to about one-half to one-third the original value. The narcotizing effect upon the nerve fiber of 3 to 7 per cent ethanol was partly or almost completely opposed by low temperatures or high pressures.

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