MEMBRANE RESTING AND ACTION POTENTIALS FROM THE SQUID GIANT AXON

HOWARD J. CURTIS AND KENNETH S. COLE

Department of Physiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland; the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York; and the Marine Biological Laboratory, Woods Hole, Massachusetts

FOUR FIGURES

INTRODUCTION

The resting and action potentials of nerve and muscle have usually been measured between a "killed-end" and the region being investigated. These potentials would be the actual potential differences across the membrane if there were no external current flow and if the membrane potential at the "killed-end" had been reduced to zero, but such conditions are difficult to obtain experimentally. The potential drop developed by current flow in the transition region between the active and inactive portions reduces the measured resting potential below the membrane resting potential and the short-circuiting in the neighborhood of the active electrode reduces the measured action potential similarly, Cole and Curtis ('38). Also, the impulse may contribute to the measured action potential as it is extinguished in the transition region. The membrane potential of the injured region can certainly be eliminated but there is no direct measurement to indicate it has been reduced to zero.

The introduction of the squid giant axon preparation by Young ('36), made it possible to considerably reduce the errors in the "killed-end" technique of measurement. By working with well-cleaned fibers surrounded by as little sea-water as possible, Cole and Hodgkin ('39), and Webb and Young ('40), obtained action potentials of over 70 millivolts and Steinbach ('40), recorded resting or injury potentials as high as 65 millivolts. It became possible to avoid most of the difficulties of the "killed-end" technique by inserting a micro-electrode inside this axon and measuring these potentials directly across the active cell membrane. This was done at Plymouth by Hodgkin and Huxley ('39), with a very small silver-silver chloride electrode apparently in direct contact with the axoplasm; and at Woods Hole by Curtis and Cole ('40), with a micropipet filled with KCl. At both laboratories action poten-

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tials as large as 90 millivolts were found. Hodgkin and Huxley further reported measurements of the resting potential of about 50 millivolts. We could not obtain any reliable resting potential measurements because of electrode difficulties.

This finding of an action potential larger than the resting potential was quite unexpected because it showed that the potential difference across the membrane was actually reversed during the passage of the impulse. In these experiments the electrical characteristics of the micro-electrodes and the amplifiers were seen to be unusually important for an accurate record of the action potential. Also the contribution of the silver-silver chloride electrode to the resting potential might have been rather large because of the low chloride ion concentration of the axoplasm. Since the membrane potential reversal depended upon the difference between these two measurements, it seemed advisable to attempt to improve the technique for each.

In conjunction with these measurements, preliminary data were taken of the effects on the membrane action and resting potentials of changing the concentration of several of the ions in the external medium, particularly potassium.

METHOD

The material used was the giant axon from the hindmost stellar nerve of the North Atlantic squid, Loligo pealii. The method of dissection and teasing was essentially the same as previously described (Cole and Curtis, '39). The measuring cell was similar to the one used previously (Curtis and Cole, '40) and is shown in figure 1. The axon fitted snugly in a trough 540 μ square cut in the top of a sheet of polystyrene, with the tied ends of the fiber in the enlarged portions at A and B. The trough was filled with sea-water and covered with a cover slip. The micro-needles were about 15 mm. long and about 40 μ in diameter, and were pulled straight from the glass tubing which formed and shank of the needle. This shank had an outside diameter of nearly 540 μ and slid easily in the trough on both sides of C. After being pulled the needles were filled with KCl isosmotic with sea-water and stored in this solution.

With the axon in place, the needle was placed in the end of the trough at C and pushed along until its point encountered the axon where it entered the enlargement at B. A quick push usually sufficed to pierce the cell wall, and the needle was then pushed along until its tip was just opposite the capillary of the E electrode. As soon as the axon was placed in the cell fresh sea-water was siphoned in through one of the tubes at A and out the tube at B and a constant circulation maintained.

The electrode wells at E, F, G, and H contained silver-silver chloride electrodes and were kept filled with sea-water. Small glass capillaries, filled with sea-water, joined these electrode wells with the trough. The D electrodes were used for stimulation, and the action potential could be recorded between any pair of the other electrodes. A vaseline seal was placed around the needle between B and C, so that the H electrode could only respond to potential changes near the tip of the needle. The other electrodes were also well insulated.

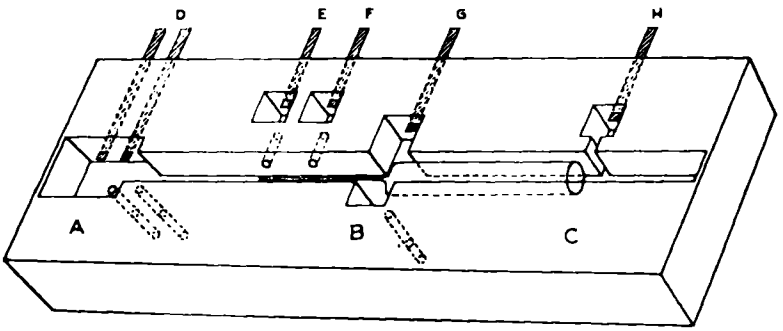

Fig. 1 Schematic drawing of the cell used for measuring membrane action and resting potentials. A clean axon was placed in the trough with its ends in the enlargement at A and B. The micropipet, filled with KCl, was placed in the trough with its tip in the B enlargement, and, after piercing the cell wall, was pushed along to the position shown. All chambers were then filled with sea-water, and covered with a glass plate. Fresh sea-water was siphoned in the tubes at A and out the tube at B. The axon was stimulated at the D electrodes, and membrane potentials measured between the E and H electrodes.

Since the resistance of the needles was 5 to 20 megohms, small external capacities introduced relatively large errors in the size and shape of the action potential. These were minimized by an "impedance changer" placed close to the measuring cell. There remained, however, the capacity of the needle itself, and a variable inductance in the amplifier was used to compensate for its effect. Before an experiment was started, the needle to be used was placed in the measuring cell and the inductance set for the optimum response to a potential suddenly applied to the needle tip. In this way, needle action potentials were recorded with a response time of 10 microseconds or less.

The amplifier was direct coupled and drew an input current of less than 10^{-11} amperes and therefore resting potentials could be measured directly on the face of the cathode ray oscillograph tube. However, it was found that the small silver-silver chloride electrodes which were necessary for action potential measurements did not maintain as con-

stant a potential as was desired, so a pair of large silver-silver chloride electrodes was used. These electrodes were sealed in glass tubes filled with sea-water and made contact with the electrode wells by means of wicks. They maintained a constant potential to within 0.5 millivolt for many hours. A string soaked in sea-water was placed between the E and H electrode wells to short-circuit the resting potential and establish the zero for the resting potential measurements. This was done before and after each resting potential measurement.

When the medium surrounding the fiber was changed the fluid was siphoned in through one of the tubes at A and out the other, thus quickly flushing out this chamber, while the flow out the tube B continued. In this way it was usually possible to change the fluid surrounding the entire fiber in less than 15 seconds.

In order to vary the potassium ion concentration, solutions isosmotic with sea-water were made in which the calcium ion concentration was the same as that of sea-water, and potassium was substituted for sodium, or vice versa. All solutions were buffered with phosphate buffers at pH 8.03, the pH of Woods Hole sea-water. In making a potassium concentration curve, a sea-water value was taken, then the solution was changed and kept circulating until the potential became constant (usually 2 to 3 minutes), and the solution immediately changed back to sea-water. All effects reported here were entirely reversible. When the resting or action potentials did not return to their original values after the fiber was returned to sea-water, the results were discarded.

Results obtained from axons in which the potentials did not remain constant for at least an hour after impalement were discarded. It was further required that the action potential recorded between the F and G electrodes be substantially the same as that measured between the E and G electrodes at the time the measurements were made. In all, fifteen axons fulfilled these requirements. Seventeen others showed substantially the same characteristics but were not sufficiently constant and reproducible.

RESULTS

The resting potentials of these fifteen axons varied from 46 to 59 millivolts, and averaged 51 millivolts. The potential of any given axon would remain remarkably constant and one axon maintained a constant potential for more than 8 hours. Furthermore the potentials would not fall appreciably for at least half an hour after the axon became inexcitable. No correlation was observed between the magnitude of the resting potential and the length of survival.

The effect on the resting potential of changing the potassium concentration while the calcium concentration was maintained constant is shown in figure 2 where the resting potential is plotted against the potassium ion concentration for a typical experiment. All curves taken showed the same form; they tended to become parallel to the concentration axis at low values of concentration, and to approach a straight line at high concentrations. The slope of the line at high concentrations is about 50 millivolts for a tenfold change in concentration. It will be noted that the potential is zero at a concentration of about 18 times the normal, and is reversed in sign by about 15 millivolts at 40 times (isosmotic KCl). The same effect of potassium was observed when both calcium and magnesium concentrations were held constant.

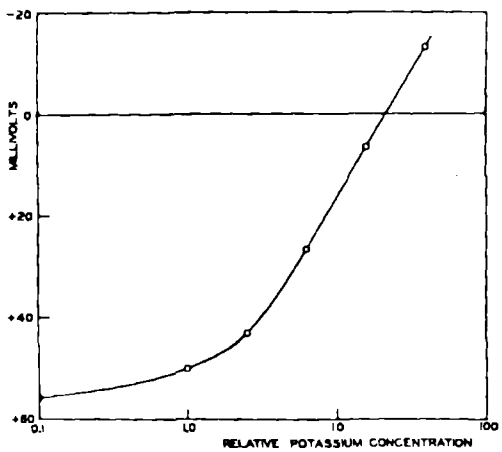

Fig. 2 Resting potential in millivolts *vs.* potassium concentration of the surrounding fluid. The concentration scale is in multiples of the potassium concentration of sea-water, 13 millimolar, and is logarithmic. At high potassium concentrations the curve is a straight line, the slope of which is nearly that of the potassium electrode. In the physiological range of concentrations the potential is nearly independent of the concentration.

Action potentials recorded with the needle electrode are somewhat diphasic and tend to be oscillatory (Curtis and Cole, '40; Hodgkin and Huxley, '39) with the first positive phase about 15% of the spike height. The maximum negative variation from the resting potential (spike height) varied from 77 to 168 millivolts, and averaged 108 millivolts. A typical action potential record is shown in figure 3.

Action potentials were also recorded as a function of potassium concentration, and the spike height is plotted in figure 4 as a function of the potassium concentration. The fibers ceased to conduct at a potassium concentration between 2.5 and 6 times normal. No sudden cessa-

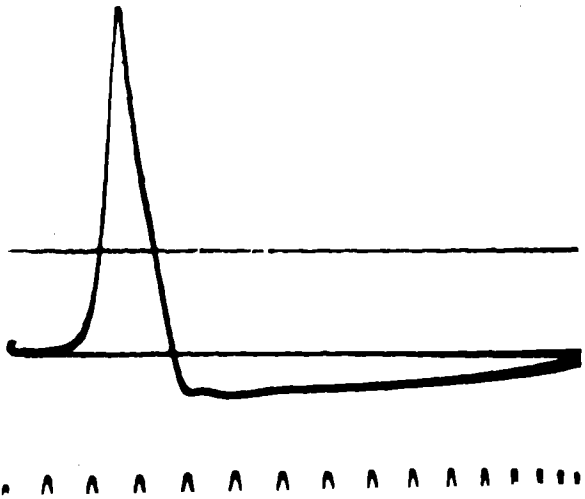

Fig. 3 Membrane action potential of the squid axon. The two horizontal traces are 50 millivolts apart. The resting potential in this axon was 58 millivolts. Thus the upper horizontal line approximately represents zero potential difference across the membrane, the lower line the resting potential (outside positive) and the action potential, starting from the resting potential, swings to 110 millivolts (outside negative). Time intervals at the bottom are 0.2 msec.

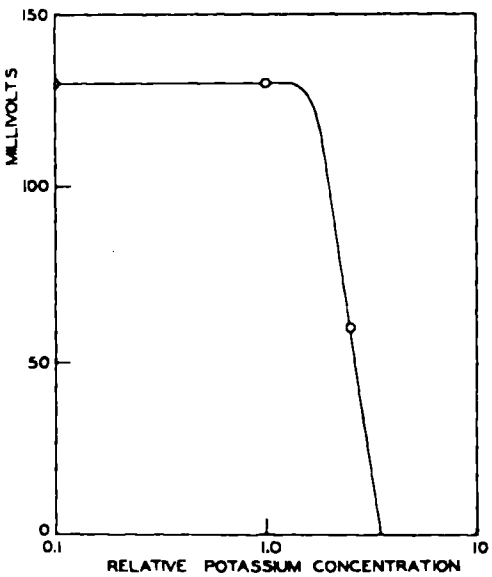

Fig. 4 Spike height in millivolts *vs.* relative potassium concentration as in figure 2. The action and resting potentials both start to decrease rapidly at about twice the normal potassium concentration.

tion of the action potential was observed, although when the potential became less than a millivolt it was difficult to distinguish the all or none response from the electrotonic potential. On being returned to sea-water, the action potential returned to its normal height even after 3 minutes immersion in isosmotic KCl.

Some preliminary observations were made on the effects of changing the other ions in the circulating fluid. Changing the calcium ion concentration from zero to 4 times normal produced practically no effect on the resting potential. Removing all ions by circulating isosmotic dextrose increased the potential only slightly (3 to 5 millivolts) higher than it was raised by removal of potassium alone. Likewise, the height of the action potential was not appreciably affected by these procedures.

Thus in a conducting fiber it was found that the action potential might vary from over 150 millivolts to nearly zero under various conditions of ionic environment, deteriorations, etc., but that the resting potential would remain relatively constant in the neighborhood of 50 millivolts.

DISCUSSION

The entire process of excision, teasing, and impaling these fibers would not be expected to be conducive to long survival. The various techniques were all improved over the previous summer but probably the most important factor was the circulation of fresh sea-water past the axon in the cell. Some of these fibers were probably no more abnormal than other excised fibers. The process of impalement did not measurably change the height of the action potential as recorded by means of an inactive end before and after impalement. An excised fiber must be in very good condition before it is possible to elicit spontaneous activity by lowering the calcium ion concentration but this phenomenon was demonstrated with a few of these impaled fibers. We therefore feel that the process of impalement produced no drastic change in the axon as a whole. The improved condition of the fibers and the increased fidelity of the electrical equipment are primarily responsible for the larger action potentials observed.

It is rather surprising that the range of the observed resting potentials is not greater than 13 millivolts, but on the other hand the constancy of the resting potential in each axon during the periods of observation suggests that the resting potentials of the axons in situ may have had a similar variation.

The reasons for believing that the potentials recorded here are true membrane potentials have been discussed (Curtis and Cole, '40). However, there is undoubtedly some error in the absolute values of the

resting potentials caused by liquid junction potentials. These potentials are between (1) the axoplasm and the KCl of the micropipet, and (2) the KCl of the micropipet and the sea-water communicating with its silver-silver chloride electrode, H of figure 1. The second of these can be quite accurately calculated (MacInnes, '38), and amounts to 4.5 millivolts. The first cannot be accurately calculated since the composition of axoplasm is not known but an estimate can be made. Using the chemical analysis of axoplasm by Bear and Schmitt ('39), the mobilities of the cations and 25% of the anions may be assumed to be known. The remaining 75% of the anions are unknown, but let us assume they are monovalent, and have average mobilities such as to make the known conductivity of axoplasm (Cole and Hodgkin, '39) approximately correct. These values may then be substituted in the equation of Henderson ('07), which gives 6 millivolts. This value is probably of the correct order of magnitude. The total correction is then the sum of these two, or about 10 millivolts. Since these potentials are of opposite sign to the resting potential, the true resting potentials are larger than the measured values by this amount, and we conclude that the average value of the resting potential is about 61 millivolts.

These liquid junction potentials will change as the potassium concentration of the surrounding fluid changes, but assuming that the composition of the axoplasm is unchanged, the maximum change in the junction potentials would be 4.5 millivolts. All these estimates of the junction potentials are rather uncertain. For this reason it seems advisable to report only the measured values of potential. The above calculations were included only to indicate that the corrections which must ultimately be made to these measurements will probably be relatively small.

Thus during the passage of an impulse the membrane potential is momentarily reversed in sign, so that the outside may be as much as 110 millivolts negative with respect to the inside. This fact throws doubt on the simple explanation of the action potential as a passive depolarization of the membrane or abolition of the resting potential. With the further observations of wide variability in the size of the action potential with little if any change of the resting potential, it is reasonable to suppose that a separate mechanism is responsible for the production of each. Thus the resting potential may be an electrical measure of the energy made available by metabolism and the action potential an index of the ability of the membrane to utilize this energy for propagation.

However, there may be an explanation of this phenomenon on the basis of a passive depolarization. A membrane inductance has been observed, (Cole and Baker, '41) in this fiber of 0.2 henries per cm.^{2} and this, in conjunction with the membrane capacity of 1 microfarad per cm.^{2} (Curtis and Cole, '38) forms a resonant circuit. It has been possible to explain several phenomena of peripheral nerve on the basis of an equivalent membrane circuit involving capacity, resistance, and inductance (Cole, '41). The explanation of the present phenomenon in terms of this equivalent circuit is not available, but it seems possible that a complete solution of the problem on the basis of the cable equations may yield an adequate explanation.

The curve of figure 2 indicates that the potassium concentration difference between axoplasm and sea-water is not a complete explanation of the resting potential, but undoubtedly is an important factor. At high potassium concentrations the curve is approximately a straight line. The limiting slope of 50 millivolts for a ten-fold concentration change is near the value which it would theoretically have if the membrane were permeable to potassium alone, i.e., 58 millivolts. The measured potential is zero for about a twenty-fold increase in potassium concentration above normal, or at about the point where the potassium concentration is the same inside as outside (Bear and Schmitt, '39). However, the curves flattened out in the neighborhood of the normal concentration and below, so it appears that some other and perhaps more important factor is operative. It seems improbable that this other factor could be some of the other ions normally present, since removal of all ions from the surrounding fluid by the addition of isosmotic dextrose caused no more change in potential than was caused by the removal of potassium alone. The fact that the size of the action potential falls sharply in the concentration range where the slope of the resting potential curve is changing most rapidly (figs. 2 and 4) is undoubtedly very important in this connection.

SUMMARY

The action and resting potentials from the membrane of the squid giant axon have been measured between one electrode inside and another electrode outside the axon. The resting potential of these axons varied from 46 to 59 millivolts and averaged 51 millivolts, but these potentials are probably about 10 millivolts too low because of liquid junction potentials. Action potentials varied from 77 to 168 millivolts and averaged 108 millivolts. Thus during the passage of an impulse the membrane potential is momentarily reversed in sign so that the out-

side may be as much as 110 millivolts negative with respect to the inside. The observed membrane inductance may explain this reversal.

The resting potential was measured as a function of the potassium ion concentration of the fluid surrounding the axon. It was found that in the region of the normal potassium concentration there was a relatively small change of potential with concentration, but at higher concentrations the potential fell sharply, reached zero at about 20 times normal concentration, and was about 15 millivolts negative at 40 times (isosmotic KCl). The spike height was very sensitive to increases in potassium concentration, and was reduced to only a few millivolts by a concentration which left the resting potential practically unchanged.

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