SLOW CHANGES OF POTASSIUM PERMEABILITY IN THE SQUID GIANT AXON

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ABSTRACT A slow potassium inactivation i.e. decrease of conductance when the inside of the membrane is made more positive with respect to the outside, has been observed for the squid axon. The conductance-potential curve is sigmoid shaped, and the ratio between maximum and minimum potassium conductance is at least 3. The time constant for the change of potassium conductance with potential is independent of the concentration of potassium in the external solution, but dependent upon potential and temperature. At 9°C and at the normal sea water resting potential, the time constant is 11 sec. For lower temperature or more depolarizing potentials, the time constant is greater. The inactivation can be described by modifying the Hodgkin-Huxley equation for potassium current, using one additional parameter. The modified equation is similar in form to the Hodgkin-Huxley equation for sodium current, suggesting that the mechanism for the passive transport of potassium through the axon membrane is similar to that for sodium.

INTRODUCTION

A slow inactivation of potassium permeability in nerve has been reported for Ranvier nodes by Frankenhaeuser and Waltman (1). Lüttgau (2) found that the time constant for this inactivation is about 15 sec. Potassium inactivation has also been observed in other types of cells (3–7). The term "inactivation" is used here to characterize a process where the ionic conductance decreases as the inside of the membrane is made more positive with respect to the outside. Hodgkin and Huxley (8) have described a sodium inactivation process with a time constant of about 5 msec for the squid axon, but did not require a potassium inactivation process to fit their experimental data. If there is a potassium inactivation process in squid with a time constant comparable to that in the Ranvier node, then it would not influence experiments on relatively short duration phenomena, such as those of Hodgkin and Huxley. However, the presence of such a process would suggest a similarity in the sodium and potassium permeability mechanisms of the axon. We

found evidence for a slow potassium inactivation process in the steady-state current-voltage curves of squid axon in high external potassium solution. The high external potassium concentration causes a depolarization of the axon, and thus affords a convenient way of observing the inactivation. Further experiments were performed in high external potassium solutions to determine the time constants for this process. Other experiments were performed in artificial sea water to determine conductance changes and also to determine whether the inactivation is dependent on potassium concentration.

METHODS

Squid (*Loligo pealii*) axons of about 500 μ were carefully dissected out of the animals in flowing sea water. A length of about 30 mm was cleaned in cold artificial sea water [430 mM Na^+, 10 mM K^+, 10 mM Ca^{++}, 50 mM Mg^{++}, 560 mM Cl^-, and 0.5 mM tris(hydroxymethyl)aminomethane (Tris), which was used to buffer the solution at a pH of 7.4 $\pm$ 0.1]. The axon was then placed in an axon chamber, which contained cold flowing artificial sea water.

The point control voltage clamp system (9) was used. A microelectrode, having a resistance of about 1 meg and filled with 3 M KCl, was inserted through the membrane. The membrane potential, V_m, was recorded as the potential of this microelectrode minus the potential of a reference electrode in the external solution. V_m was corrected for a liquid junction potential by adding -4 mv (10). V_m was set to a predetermined command signal by the use of electronic feedback. A 5 mm central electrode, flanked on both sides by guard electrodes, was used to measure membrane currents.

All solutions unless otherwise stated contained 10 mM Ca^{++} and 50 mM Mg^{++}, and were buffered with 0.5 mM Tris to a pH of 7.4 $\pm$ 0.1. The other constituents of the 40 mM K solution were 40 mM K^+, 430 mM Na^+, and 590 mM Cl^-; of the 100 mM K solution were 100 mM K^+, 430 mM Na^+, and 650 mM Cl^-; and of the 440 mM K solution were 440 mM K^+ and 560 mM Cl^-. For the 100 mM K solution, the concentration of the Tris was 5 mM. When the concentration of Tris in the artificial sea water (ASW) was changed from 0.5 mM to 5 mM, there were no observed differences in any of the electrical measurements made. The relative osmolarities, as measured by the vapor pressure method at 37°C with reference to the ASW were 1.069 for the 40 mM K solution, 1.159 for the 100 mM K solution (this ASW contained 5 mM Tris), and 0.988 for the 440 mM K solution.

In order to correct for the independence principle (11), at each value of membrane potential, the current density was multiplied by the following factor:

$$\frac{\exp\left(\frac{ZFV}{RT}\right) - \frac{[K]_o'}{[K]_i}}{\exp\left(\frac{ZFV}{RT}\right) - \frac{[K]_o}{[K]_i}}$$

where Z is the valence of the potassium ion; F is the Faraday; V is the membrane potential; R is the gas constant; T is the absolute temperature; $[K]_o'$ is the external potassium concentration in ASW; $[K]_o$ is the external potassium concentration in the actual solution; and $[K]_i$ is the internal potassium concentration, which was assumed to be 400 mM (12).

In those cases where the external potassium concentration is 440 mM, corrections due to local variations in the potassium concentration in the region near the membrane–the Frankenhaeuser-Hodgkin space (13)–are negligible, since internal and external concentrations of potassium are almost the same and very high.

In order to determine the time constants in Figs. 3 and 4, we used a computer program (14) to fit the experimental points to a single exponential term using a least-squares criterion.

The currents obtained after step voltages of about 40 msec duration were designated as the steady-state currents. Previous authors have defined steady-state current as that after step voltages of 5 to 10 msec. In almost all cases, the current is essentially constant between 10 msec and 40 msec, and so the choice is quite arbitrary. But for hyperpolarizing pulses of about 70 mv, the time constant for current to reach a constant value is about 6 msec, as determined by experiments where an axon was clamped back to its resting voltage in artificial sea water (15). Since hyperpolarizing pulses of this magnitude were used in connection with Fig. 1, the time for steady state was taken as 40 msec. The steady-state current is the sum of the potassium and leakage currents (16). In this paper, the leakage currents have been neglected.

It will be demonstrated that the steady state referred to above, whether at 5 to 10 msec or at 40 msec, is really a quasi steady-state, since there are processes with time constants of tens of seconds. In order to conform to present usage, however, we have continued to use the term "steady-state."

In several experiments relating to Fig. 8, the axon was held at a potential somewhat different from the resting potential. The resulting small constant current was neglected in these cases. The corresponding conductances were determined from measurements of slope, and hence are unaffected by a constant translation along the current axis.

RESULTS

When the external solution bathing a squid axon was changed from ASW to one containing 440 mM K, normal Ca and Mg, and no Na, the resting potential changed to +6 mv. The axon was clamped at this voltage and a series of 40 msec pulses of various voltages was applied. The steady-state currents obtained are plotted as a function of membrane voltage in Fig. 1. Steady-state current as a function of membrane voltage for two other runs in this solution and for control runs in ASW are also plotted in Fig. 1. The leakage current for ASW is given, but could not be accurately determined for the experiments with the solution containing 440 mM K because of the depolarized resting potential.

The 440 mM K results differ from the ASW results in Fig. 1 because of differences in both holding potential and concentration. In order to correct for the latter, an independence principle correction described in the Methods section was applied to the 440 mM K curves. Fig. 1 shows that the first and third 440 mM K curves, even when corrected for the independence principle, are markedly smaller in amplitude than the curves for the normal resting potential. It also shows that the second 440 mM K curve is greater in amplitude than the first. Furthermore, at least for positive potentials, the ratio of the amplitude of the second curve to that of the

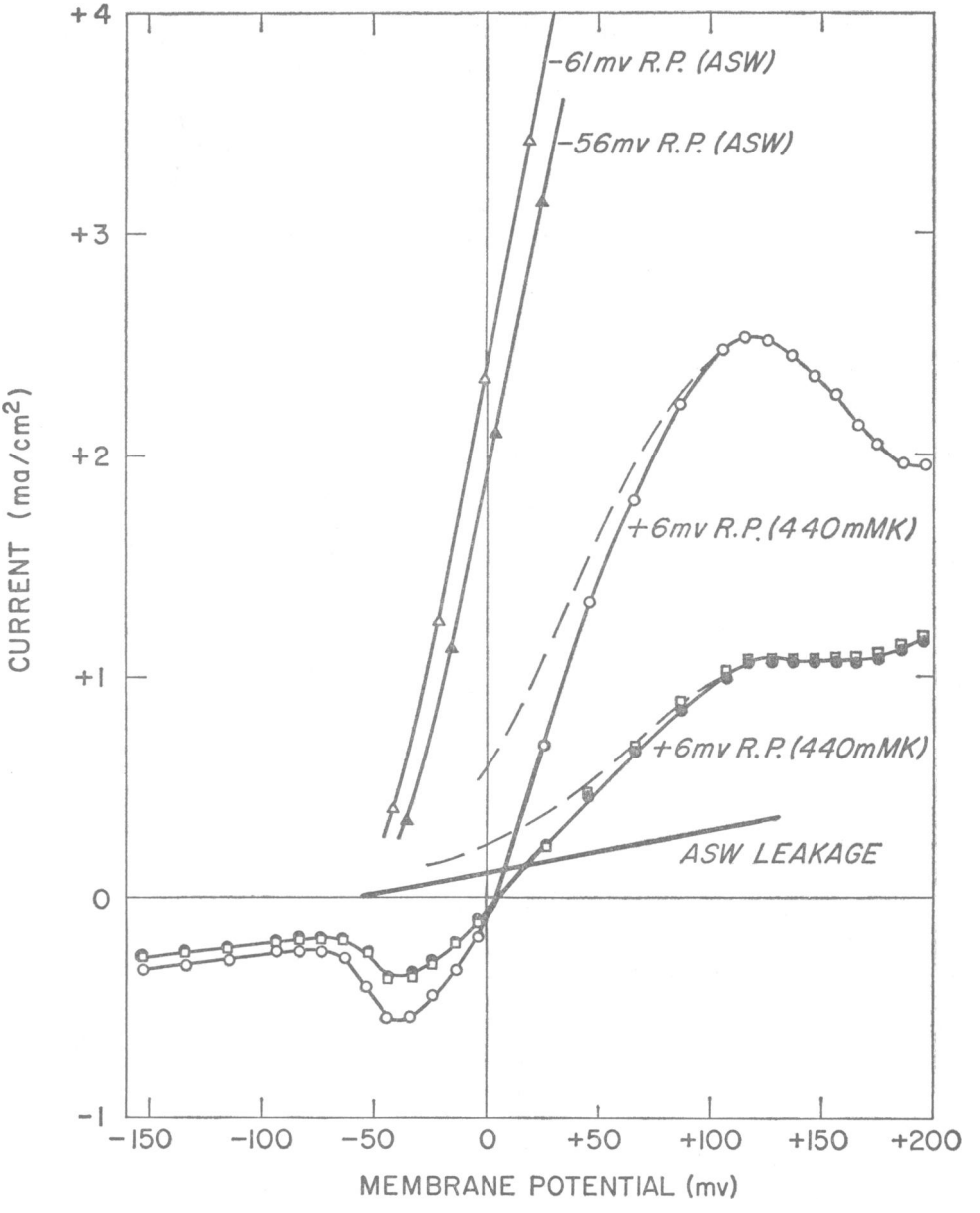

FIGURE 1 Steady-state current-voltage curves in artificial sea water (ASW) and in a solution (440 mM K) where all the sodium is replaced by potassium, but the other components of ASW are not changed. For each run, axon was clamped at its resting potential (R.P.). △, ASW run before 440 mM K runs; ▲, ASW run after 440 mM K runs; ●, first 440 mM K run; ○, second 440 mM K run, immediately after first; □, third 440 mM K run, after 10 min wait; - - - - corrected for independence principle.

first decreased with increasing potential. For the experimental conditions used, this corresponds to a ratio decrease with increasing time.

These results suggest a potassium inactivation process where the conductance decreases with increased depolarization and the time constant for the effect is relatively long. Currents for the first run are quite small because the axon had been held at a depolarizing potential for a long time. Currents for the second run are somewhat greater, because the hyperpolarizing pulses applied in the first run increased the conductance, and this partially compensated for the decrease in conductance caused by the depolarizing holding potential. This compensation should become less important as the time interval following the last hyperpolarizing pulses increases. Thus, currents would approach those of the first run for the final points in the second run and for all the points in the third run, in agreement with experiment.

In one check of this hypothesis, steady-state current-voltage curves were obtained for depolarizing pulses for two consecutive runs. No hyperpolarizing pulses were applied. According to the above hypothesis, currents for the second run should be smaller than or equal to those of the first. Fig. 2 shows that the currents were actually equal.

In another check, an axon was placed in a solution containing 440 mM K and clamped at its resting potential. Then a hyperpolarizing conditioning pulse of 100 mM amplitude and 300 msec duration was applied in order to increase the conductance. After that, depolarizing monitor pulses of +50 mv amplitude and 300 msec duration were applied every few seconds and the steady-state currents determined. This procedure was continued for several minutes until the steady-state current for several successive monitor pulses did not change. The sequence of voltages for the first minute is shown on the bottom of Fig. 3. The monitor steady-state current decreased for successive pulses, in agreement with the inactivation hypothesis.

The time course of this current decrease is the same as the time course of the inactivation process, since monitor voltage was constant. Fig. 3 shows that on a semilog scale, the inactivation is linear with time, and hence the inactivation is a single exponential.

For experimental convenience, the durations of conditioning and monitor pulses were always the same–in this case 300 msec. The entire procedure was repeated for conditioning and monitor pulses of the same amplitude as above but with the duration changed first to 75 msec and then to 1.5 sec. The results for these cases are also plotted in Fig. 3.

The monitor pulses did not influence the measurements as indicated by the fact that the time course of current decay was about the same for all three curves in Fig. 3. The decay of monitor current has an average time constant of about 33 sec for a potential of −3 mv at 9°C.

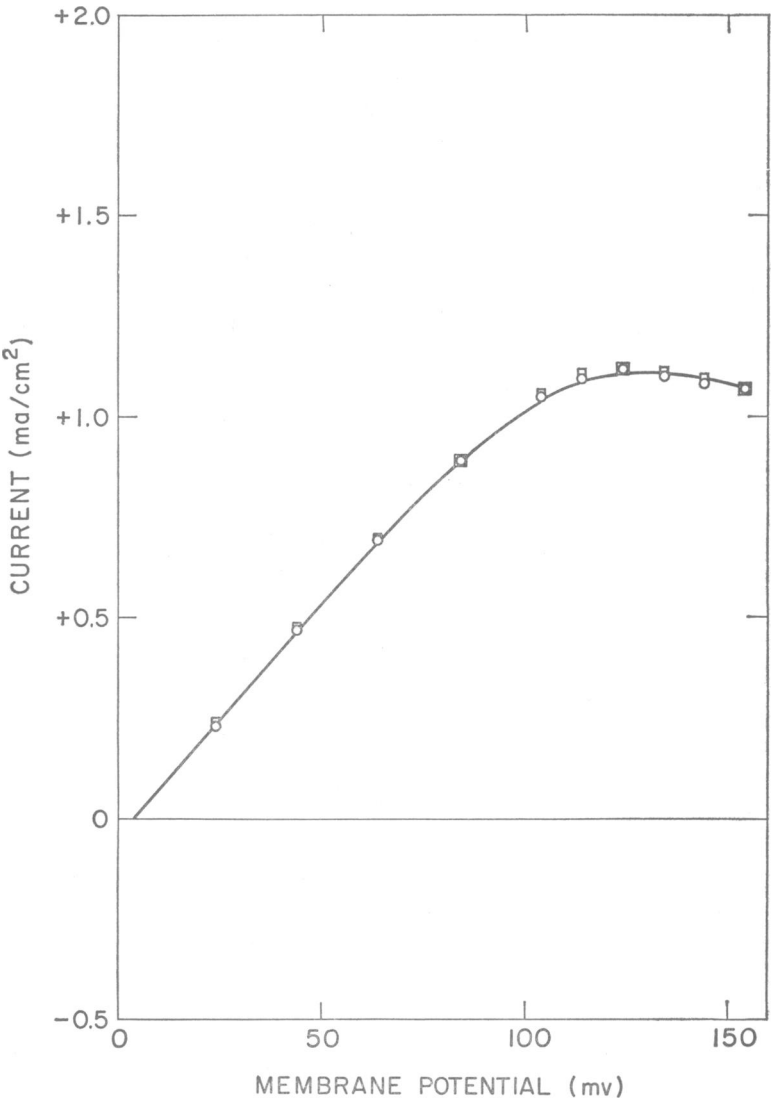

FIGURE 2 Steady-state current-voltage curves for depolarizing pulses only in a solution where all the sodium is replaced by potassium, but the other components of artificial sea water are not changed. For each run, axon was clamped at its resting potential, +4 mv. ○, first run; □, second run, immediately after first.

Experiments were performed with a different axon to determine the variation of this time constant with temperature. It was found that as temperature was lowered from 19°-8°C, the time constant increased from 18 to 52 sec. This temperature dependence corresponds to a Q_{10} of 2.6 at 15°C.

For each pulse duration shown in Fig. 3, the currents were extrapolated to zero

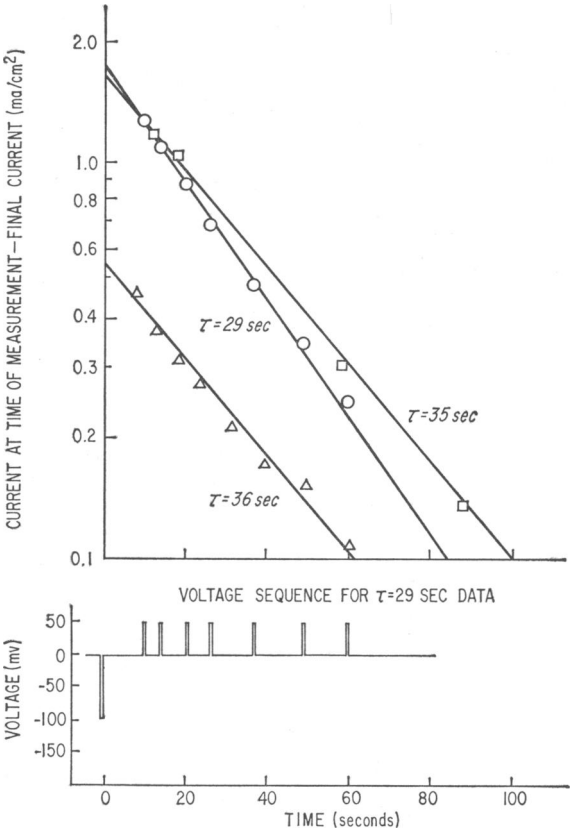

FIGURE 3 Change of monitor current following −100 mv pulses of various durations. Axon in solution where all the sodium is replaced by potassium, but the other components of ASW are unchanged. Resting potential = −3 mv; Final current = 0.46 ma/cm²; △, pulse duration = 75 msec; ○, pulse duration = 300 msec; □, pulse duration = 1.5 sec.

time. It can be seen that an increase of conditioning pulse duration from 75 msec to 300 msec resulted in a considerable increase in monitor current at zero time, but that a further increase in conditioning pulse duration from 300 msec to 1500 msec did not significantly change the monitor current at zero time. Hence, the increase in conductance caused by a pulse of −100 mv has a time constant somewhat less than 300 msec.

A method of obtaining the time constant with ASW external solution was also tried. An axon with a resting potential of about −65 mv was clamped at a depolarized potential, and the decay of conductance was monitored. Clamp potentials ranging from −45 mv to 0 mv were tried, but in every case the axon did not recover.

In order to obtain information about the dependence of the time constant on

concentration, axons were placed in solutions with external potassium concentrations of 40 and 100 mm. The resting potentials were about −45 mv and −25 mv respectively. In each case the axon was clamped at about −65 mv, and the steady-state currents of a series of monitor pulses were measured.

Fig. 4 is a semilog plot of these currents versus time from the beginning of the clamp, and the slopes of these curves were used to determine the time constants. The procedure was similar to that employed for the experiments described in Fig. 3, except that in one case the time constant for conductance increase during hyperpolarization was measured and in the other the time constant for conductance decrease during depolarization was measured. All the time constant results are summarized in Table I.

The potassium inactivations described so far were for axons in external solutions where the potassium concentration was higher than normal. In order to find out whether the inactivation process occurs in ASW and also to determine the variation

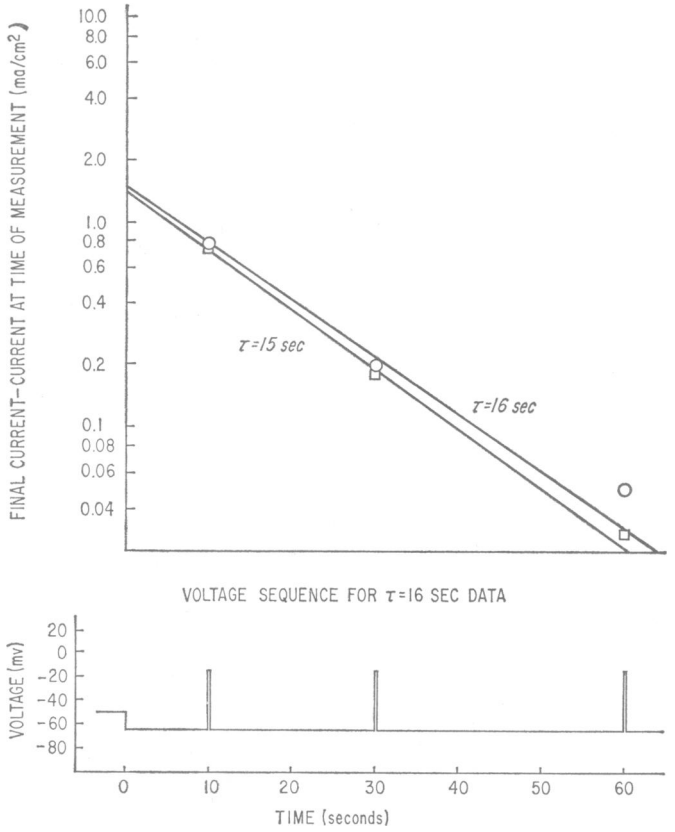

FIGURE 4 Change of monitor current following voltage clamp at −65 mv. ○, external [*K*] = 40 mM. Resting potential = −45 mv. □, external [*K*] = 100 mM. Resting potential = −25 mv.

TABLE I

TIME CONSTANT OF POTASSIUM SLOW INACTIVATION

Axon	Potential	Ext [K]	Temperature	Time constant Observed	Time constant Corrected to 9°C
	mv	*mM*	*°C*	*sec*	*sec*
63A34	−100	440	9	<0.3	<0.3
64A22	−65	40	5	16	11
64A22	−65	100	5	15	10
63A34	−3	440	9	33	33

of conductance with potential for the slow inactivation process, the following experiment was performed: an axon was clamped at its resting potential in ASW, and the steady-state current-voltage curve was obtained. Then various experiments in high external potassium solutions were performed. Between runs in high potassium solutions, the axon was placed in ASW and clamped at its new resting potential. The steady-state current-voltage curve was again obtained. In the course of these experiments, the resting potential in ASW decreased owing to natural deterioration of the axon, thus affording an opportunity to compare the axon conductance for different resting potentials with the same external solution. The conductance was found to decrease as the resting potential decreased. To test the possibility that this

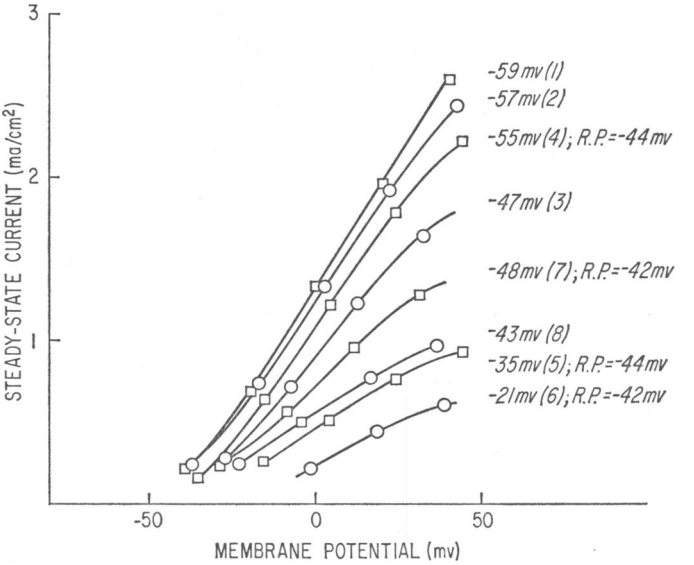

FIGURE 5 Potassium current-voltage curves in ASW for various holding potentials. Each curve is identified by its holding potential and by a number in parentheses showing the order of the curves. For cases where the resting potential and holding potential differ, the resting potential (R.P.) is also shown.

change in conductance was primarily an aging effect not causally related to the resting potential, the axon was clamped in several cases at potentials different from the resting potential at the time of the clamp. Steady-state current-voltage curves were again obtained. Fig. 5 shows the curves for ASW at the various holding potentials. The slopes of these curves are a measure of potassium conductance, and are plotted as a function of holding potential in Fig. 6. The order in which the runs were made is indicated by the number next to each point. If a simple smooth curve is drawn through the experimental points, then point 3 is clearly above the curve and points 7 and 8 are below it. This is evidence of an aging effect. In many other experiments, it was also observed that for a given holding potential, conductance decreased in later runs. Hence, a smooth curve was drawn through the experimental points with lower numbered points slightly above and higher numbered points slightly below the curve. If the curve were drawn for a best fit of all the points without the aging bias, its shape would differ from Fig. 6 only slightly.

The experiment described above was carried out for a range of voltages between about −60 and −20 mv. Experiments on other axons were performed in order to determine the over-all slope of the conductance-voltage curve. The conductance of an axon with a resting potential of −65 mv was found to remain practically constant

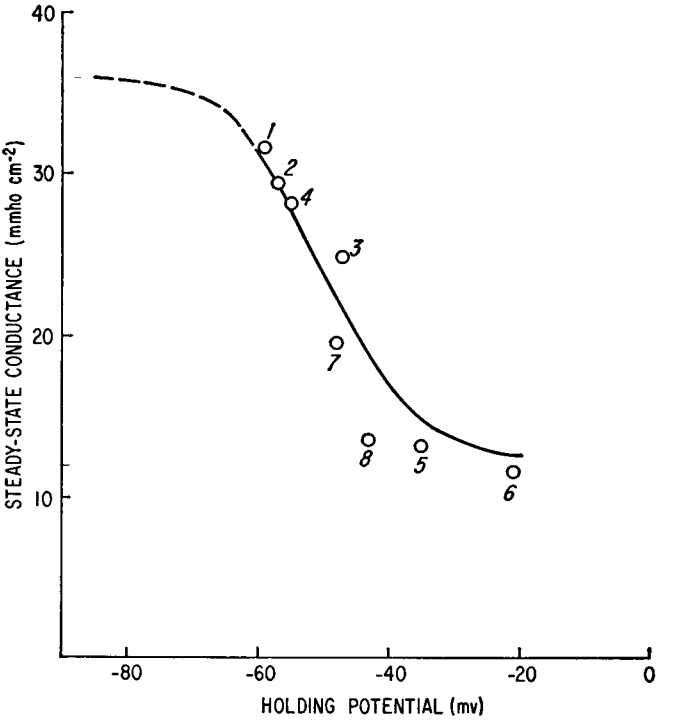

FIGURE 6 Dependence of potassium conductance on holding potential. The numbers next to the points indicate the order in which they were obtained.

when the holding potential was changed to −75, −85, and −95 mv. In particular, the conductance increased about 5% when the holding potential was changed from −65 to −85 mv. The effect was reversible. This demonstrates a leveling off of the conductance vs. holding potential curve on the hyperpolarizing side. The leveling off is shown in Fig. 6 by a dashed line.

Because of the slow potassium inactivation, current-voltage curves based on pulses taken immediately after each other may be in error. In order to check this, a steady-state curve with intervals of 5 min between hyperpolarizing pulses was obtained in high external potassium solution. This curve is shown in Fig. 7, and is

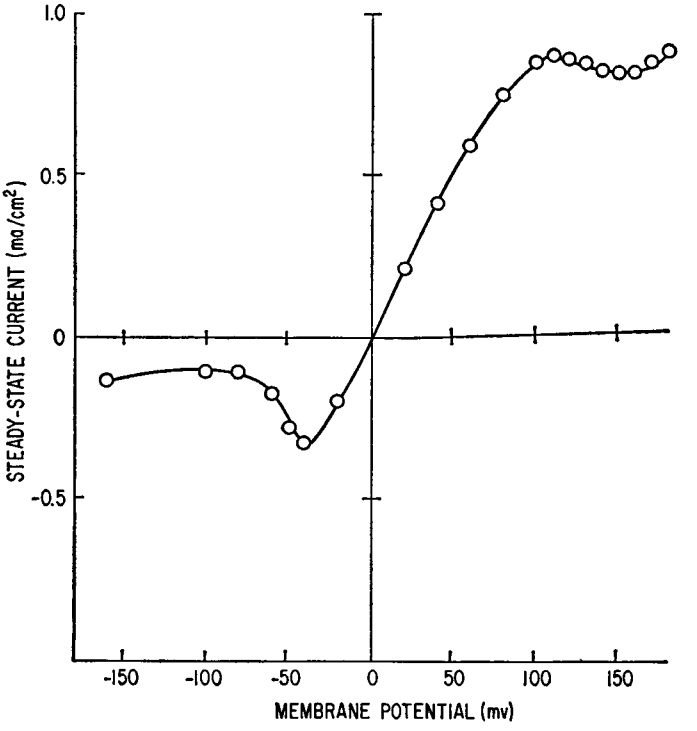

FIGURE 7 Steady-state current-voltage curves with 5 min intervals between hyperpolarizing pulses in a solution where all the sodium is replaced by potassium, but the other components of ASW are not changed.

only slightly different from the curve with closed circles in Fig. 1, which was obtained from pulses with very short intervals between them.

DISCUSSION

The permeability of the squid axon to sodium and potassium has been described by Hodgkin and Huxley (16) by means of the following equations:

$$I_{Na} = g_{Na} m^3 h (V - V_{Na})$$

$$I_K = g_K n^4 (V - V_K)$$

where I_{Na} and I_K are sodium and potassium currents, g_{Na} and g_K are constants, V is the membrane potential, V_{Na} and V_K are the sodium and potassium equilibrium potentials, and m, n, and h are voltage-dependent parameters. The *"n"* parameter for potassium is qualitatively similar to the *"m"* parameter for sodium. Both parameters increase with increasing depolarization. The sodium *"h"* parameter decreases with increasing depolarization and has a time constant about 20 times longer than that for m. A potassium analogue for h has not previously been described for the squid axon. Fig. 6 shows a potassium slow inactivation process somewhat similar to that associated with the sodium h parameter. Frankenhaeuser (17) has observed a similar effect in myelinated nerve fibers of *Xenopus laevis,* and has referred to the corresponding potassium parameter as *"k."* We shall follow his nomenclature, and propose that the Hodgkin-Huxley equation for potassium be modified to:

$$I_K = g_K n^4 k (V - V_K)$$

where k is a parameter with the voltage dependence shown in Fig. 6. This equation reduces to that of Hodgkin and Huxley under the conditions they considered; i.e., with the holding potential fixed. The similarity of the proposed potassium equation to the Hodgkin-Huxley sodium equation suggests that there is a similar type of mechanism for the passive transport of both potassium and sodium. Despite the over-all similarity, there are some differences. One difference is that the potassium inactivation appears to be only partial, as shown in Fig. 6, whereas the sodium inactivation is complete. Another difference is the much greater ratio of inactivation-to-activation time constant for potassium than for sodium. This ratio is about 20 for sodium and is at least 300 for potassium. However, it is possible that there is another potassium process with a time constant intermediate between about 10 msec and about 30 sec. Our experiments did not determine whether or not there is such an intermediate process.

Table I shows the time constant results we have obtained. There are no results for ASW, but the results for other potassium concentrations at −65 mv indicate that the time constant is independent of concentration. If so, then the three time constants in Table I can be considered valid for ASW. With only three time constants it is not possible to draw an accurate curve of time constant as a function of potential. However, the trend of these points is consistent with the view that the relation between the τ_k curve and the k curve is similar to the relations between the curves of τ_h and h, τ_m and m, and τ_n and n.

As indicated in the Methods section, leakage currents have been neglected. The leakage conductance for the axon in Fig. 6 could not be accurately measured, but the leakage conductance for the axon in Fig. 1 is less than 0.8 mmho cm^{-2}. If this correction were applied to Fig. 6, the resultant change would be negligible.

In most of the experiments described, the axon was held at its resting potential.

Changes in resting potential were due either to solution change or to the slow deterioration of the axon. This eliminated the errors that might be caused by passing currents for an extended period of time, such as errors due to electrode polarization or electrode chemical reactions. Four of the curves in Fig. 5, however, were obtained with the axon held at potentials somewhat different from the resting potential. These curves are subject to this type of error, but the manner in which they fit into the set of curves with resting and holding potentials equal suggests this is not a serious difficulty.

Moore (18) has reported that a squid axon in 0.5 M potassium chloride will give an "action potential" response when a depolarizing current pulse is superimposed on a constant hyperpolarizing current. In order to explain this, he postulates a shift of the current-voltage curve in the direction of decreasing current amplitude. The potassium inactivation process described above would give such a shift following a depolarizing pulse. Moore also reported minor variations in his current-voltage curves, depending on past history. This is similar to the difference between runs 1 and 2 in Fig. 1, which can also be explained by the potassium inactivation process.

The main purpose of this paper is to demonstrate the slow potassium inactivation for squid axon. In the course of this work, however, information has also been obtained on the properties of squid axons in high external potassium solution. Moore (18) has previously reported a negative resistance region on the hyperpolarizing side of the steady-state curve for squid axon in a solution containing isosmotic potassium. In Fig. 7, the steady-state curve is extended to the depolarizing side, where there is another negative resistance region. A negative resistance on the depolarizing side has also been reported for reconstituted membrane with 0.1 M NaCl solution on both sides (19), for eel electroplaques (7), for myelinated nerve (20), and for frog skin epithelium (21).

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