DYNAMICS OF POTASSIUM ION CURRENTS IN SQUID AXON MEMBRANE

A RE-EXAMINATION

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ABSTRACT The original experiments of Cole and Moore (1960. *Biophys. J.* 1:161–202.), using conditioning and test membrane potentials to examine the dynamics of the potassium channel conductance in the squid axon, have been extended to test voltage levels by the use of tetrodotoxin to block the sodium conductance. The potassium currents for test voltage levels from −20 to +85 mV were superposable by translation along the time axis for all conditions tested: (*a*) with depolarizing conditioning voltages; (*b*) with hyperpolarizing conditioning voltages; and (*c*) in normal and in high potassium external media. The only deviations from superposition seen were when the internal sodium concentration was abnormally high and the potassium currents showed saturation at high levels of depolarization. Some restoration toward normal kinetics could be obtained by rapidly repeated depolarizations.

INTRODUCTION

To test and discriminate among possible models for voltage-sensitive membrane ionic conductances, the most useful experiments often employ "conditioning" voltages before a standard test depolarization level.

Cole and Moore (1960 *a*) examined the dynamics of the potassium current in voltage-clamped squid axons pulsed to the sodium equilibrium potential, E_{Na}, starting from a wide range of initial conditions (hyperpolarizing as well as depolarizing). The observed currents could be superimposed with translation along the time axis, satisfying the definitive test[1] proposed for the currents by R. FitzHugh to evaluate the single-state variable description for the potassium conductance used by Hodgkin and Huxley (1952). However, they also found that the delay in the onset of the potassium current following strong conditioning hyperpolarization was much too large to be accounted for by the Hodgkin-Huxley (1952) equations. Nevertheless, the delayed onset still could be expressed as single-state variable raised to a much larger power (25th). This observation is frequently referred to as the "Cole-Moore effect."

Because these observations have been used to reject one model for the potassium

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[1]We are grateful to Dr. L. Goldman for pointing out to us that although a single voltage-sensitive step will always give superposition, under certain conditions multiple steps may do the same, e.g., R. Hahin and L. Goldman (1978).

conductance (Hill and Chen, 1972) and stand as tests that any other models must pass, it is important to know that they are general rather than restricted to the one test level originally used.

Another reason to repeat and extend the original observations on squid axon membranes was the report by Palti et al. (1976) that superposition of the Cole-Moore effect was not seen in frog node.

Therefore, we were moved to undertake experiments that would on the one hand extend the original observations and on the other hand try to uncover the basis of the reported differences seen after strong conditioning hyperpolarizations. We used the sucrose gap voltage clamp for squid axons and an axial wire voltage clamp (Moore, 1971) for crayfish axons. We blocked the sodium current with tetrodotoxin (TTX) (Moore and Narahashi, 1967) and examined the potassium currents over a wide range of conditioning and test potentials.

The squid axon experiments reported in this paper confirmed and extended the original observations. The crayfish axon, which gave similar results for depolarizating conditioning levels but nonsuperposition for hyperpolarizing, are described in the following paper (Young and Moore, 1981). In that paper we also show that such differing potassium channel dynamics can be subsumed under a single kinetic model with different constraints on rate constants.

METHODS

We used our standard sucrose gap method on squid giant axons (Moore and Narahashi, 1967) from *Loligo pealii* at the Marine Biological Laboratory, Woods Hole, Mass. The artificial node length used was $\leq \frac{1}{2}$ the axon diameter, short enough to assure a reasonable voltage uniformity (Moore et al., 1975). The experiments were carried out on an improved version of our previously described computer-controlled voltage clamp (Joyner and Moore, 1973) using a PDP8/E computer (Digital Equipment Corp., Marlboro, Mass.).

The sodium current was blocked by the addition of tetrodotoxin (TTX) (300–500 nM) to artificial sea water (450 mM NaCl, 10 mM KCl, 50 mM $CaCl_2$ buffered with Tris to a pH of 7.0–7.5). The linear capacitive and leakage currents were balanced by an active electronic bridge. The remaining pure potassium current was digitized by an Explorer II oscilloscope (Nicolet Instrument Corp., Madison, Wis.) and stored on magnetic tape for later analysis. To see whether accumulation of potassium in the extracellular space with outflow of potassium during depolarizing steps (Frankenhaeuser and Hodgkin, 1956; Adelman et al., 1973) affected the onset of the potassium conductance, we carried out some experiments with K^+ replacing the 450 mM Na^+ in the external medium.

We examined the potassium currents under a wide range of initial and test conditions. The holding potential was usually −70 mV. The initial state was varied by changing the amplitude of a constant-duration conditioning pulse or by changing the duration of a constant-amplitude conditioning pulse. The test level was varied over the full range of voltages where significant potassium current could be seen. Except for the experiment shown in Fig. 4 at 11°C, the temperature was held at 7 ± 0.7°C.

Tests for superposition were carried out by having the computer shift the curves for coincidence at the chosen level shown by the plus sign on the ordinate.

RESULTS

Variable Amplitude, Constant-Duration Conditioning

Test pulses to several voltage levels were preceded by conditioning pulses 1 ms long and varying from −150 to +85 mV. Fig. 1, giving representative examples of currents at test levels at +5 and +30 mV, shows that temporal translation of the potassium currents results in

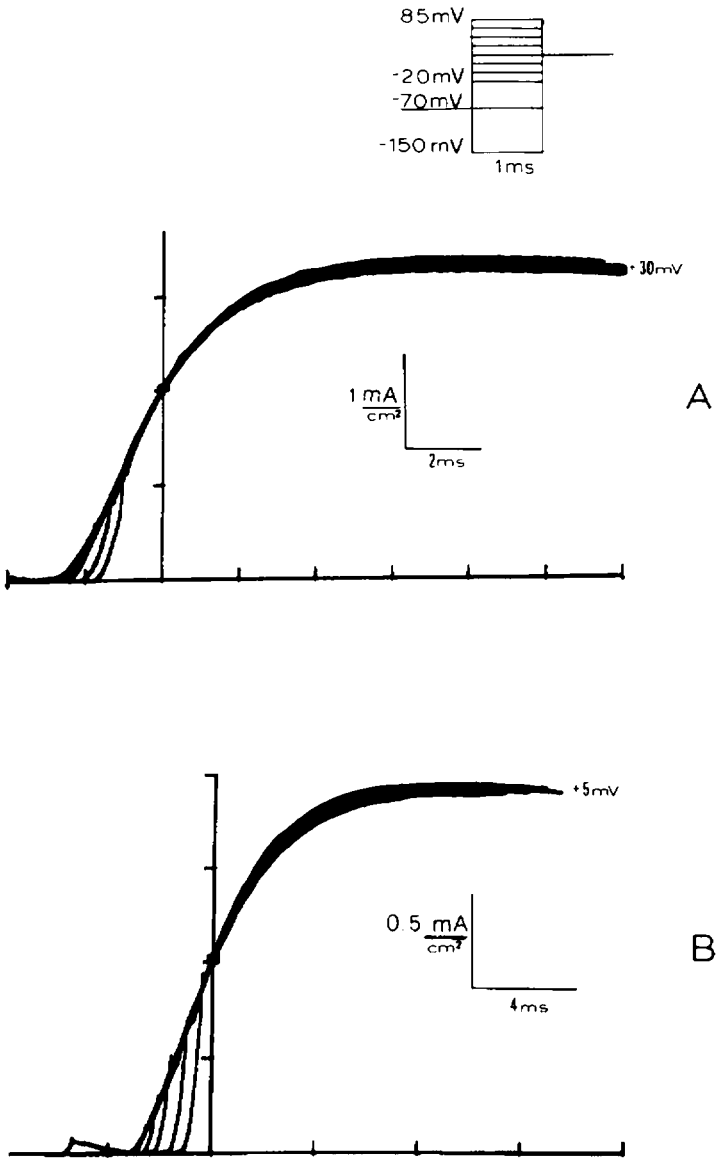

FIGURE 1 Superposition of currents from squid axons at +30 mV (*A*) and +5 mV (*B*) after 1-ms conditioning pulses ranging from −150 to +85 mV in 15-mV increments. (The thin vertical traces are from fast current increases at conditioning potentials above the test level.)

superposition to within about the line width—the repeatability of any one curve. Other data show this to be true also for other test levels up to +80 mV.

Variable Duration, Constant-Amplitude Conditioning

The duration of conditioning pulses ranging from −155 to +85 mV were varied from 0 to 6 ms preceding the test level, which ranged from +5 to +80 mV. Fig. 2, showing representative

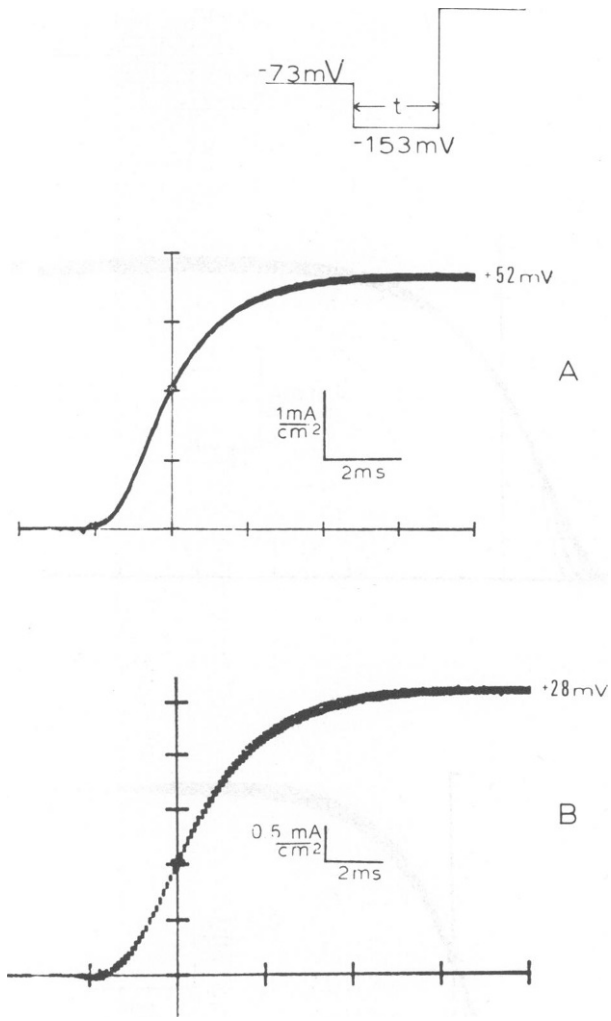

FIGURE 2 Superposition of currents from squid axons at +52 mV (*A*) and +28 mV (*B*) after a −153-mV conditioning pulse ranging in time from 0 to 6 ms in 1-ms increments.

examples for a conditioning pulse of −153 mV and test levels of +52 (*A*) and +28 mV (*B*), also demonstrates that temporal translation of the potassium currents provides excellent superposition.

High Potassium

To extend the range of test levels to lower depolarizations, it was necessary to have a larger ionic driving voltage (E–E_K) to observe significant currents. For this we used the high (450 mM) K^+ medium. Fig. 3 shows superposition of inward currents at a test level of −10 mV after conditioning at −180 mV for 0–5 ms. Superposition of other potassium currents at test potentials of +80, −10, and −20 mV was also seen in the high potassium medium.

We also carried out a more direct test of whether or not accumulation of potassium in the

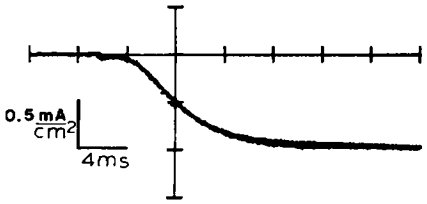

FIGURE 3

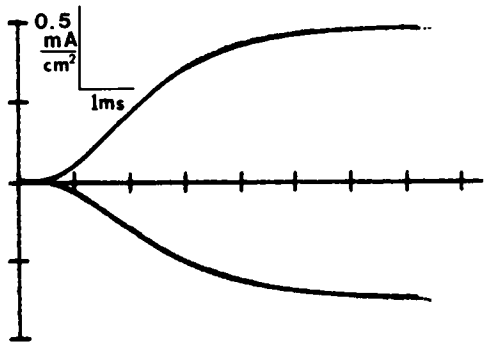

FIGURE 4

FIGURE 3 Superposition of currents in squid axons at a test level of −10 mV in high potassium seawater. Conditioning pulses to −180 mV were applied for 0–5 ms in 1-ms intervals.

FIGURE 4 Comparison of time-courses of potassium currents in squid axon in ASW and in high potassium seawater for the same membrane test depolarization to +52 mV. τ_{ASW} = 1.18 ms. $\tau_{high\ K}$ = 1.24 ms.

extracellular space affected the kinetics of the potassium conductance. The time-course of the turn on of the potassium current, I_K, was compared on the same area of membrane in normal artificial seawater (ASW) and in high potassium. Fig. 4 shows one example of such an experiment. The currents were fitted by the Hodgkin and Huxley (1952) expression $I_K = I_\infty (1\text{-}\exp(-t/\tau)^4$ and the time constants in the two solutions were found to be equal within the reproducibility of the fitting method (τ_{ASW} = 1.18 ms; $\tau_{high\ K}$ = 1.24 ms).

In the high potassium experiment, accumulation of potassium in the Frankenhaeuser-Hodgkin space (Frankenhaeuser and Hodgkin, 1956; Adelman et al., 1973) should be minimal. Because the time constant of the rising phase of g_K was the same as in ASW, it is evident that K^+ accumulation does not play a role in our present studies.

Nonsuperposition under a Pathological Condition

On occasion, we tested axons that had deteriorated somewhat and were loaded with sodium; that is, having low sodium equilibrium potentials (+15 to +30 mV instead of the normal +50

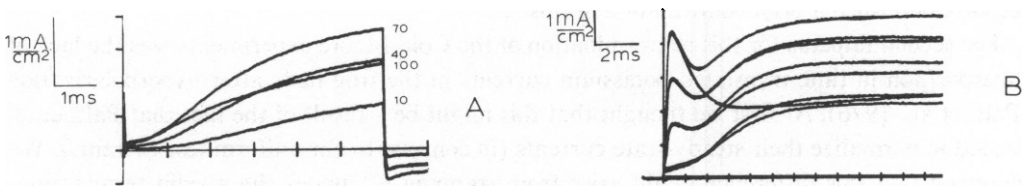

FIGURE 5 (*A*) Slowing of potassium kinetics in squid axons with high internal sodium at strong depolarizations. I_K resulting from four depolarizing steps from a holding potential of −70 mV to levels of +10, +40, +70, and +100 mV. (*B*) Relief from I_K saturation by conditioning depolarization. Four pairs of current traces are shown for test voltages of +40, +55, +70, and +85 mV. The lower trace in each pair is the first and the higher trace in the last of 25 depolarizing steps delivered at the rate of 6/s. (TTX-free seawater).

mV). We found that strong test pulses, carrying the inside of these axons beyond the sodium equilibrium potential, caused a saturation of I_K. At still stronger depolarizations, I_K decreased, revealing a negative slope conductance region. In many respects, these observations are similar to those of both Bezanilla and Armstrong (1972) and French and Wells (1977), whose axons were internally perfused with high Na^+ or Cs^+ concentrations.

Below saturation test voltage levels, such axons displayed normal I_K characteristics, increasing in speed and amplitude with depolarization. However, as the saturation voltage level was approached, there was a dramatic slowing of the current onset to rates below that for lower depolarizations, as can be seen in Fig. 5 *A*. This slowing of I_K onset also appears in Fig. 3 of Bezanilla and Armstrong (1972).

Our normal rates of application of voltage-clamp pulses are low enough (0.5–1/s) to give identical currents from pulse to pulse. At these normal rates, voltage pulses beyond the level of saturation show so much slowing that temporal translation cannot provide superposition. Furthermore, even when the amplitude was normalized according to Palti et al. (1976), superposition could not be achieved.

Such pathological currents could be restored to nearly the normal higher rate and amplitude by conditioning with depolarization. A single conditioning pulse provided partial restoration but repeating this pattern at 6 pulses/s was much more effective, as can be seen in Fig. 5 *B*. Bezanilla and Armstrong (1972) have shown that high external potassium levels relieve the blockage by high internal sodium. This relief of the partial blockage of the potassium current may be the result of accumulation of potassium in the Frankenhaeuser-Hodgkin (1956) space with the multiple bursts of outward I_K with rapidly repeated depolarizations. Although it appeared that such relief of the blockage of the potassium channel would also restore superposability to the currents, we did not think it worthwhile to pursue this point further.

DISCUSSION

The confirmation that potassium currents resulting from a wide range of test levels after a wide range of conditioning levels and times can be superimposed by translation along the time axis is important in the building and testing of g_k models. The fact that these observations, made with the sucrose gap technique, are similar to those using an axial wire voltage clamp gives assurance of the validity of both sets of observations. Keynes and Kimura (1980) recently published a brief confirmation of this.

The second impetus for this re-examination of the Cole-Moore experiments was the lack of superposition in time-translated potassium currents in the frog node after hyperpolarization (Palti et al., 1976). At first we thought that this might be a result of the fact that Palti et al. needed to normalize their steady-state currents (in contrast to our uniform test currents). We imagined that this difference might arise from trapping K^+ under the myelin terminations near the node, leading to a non-uniform membrane current. However, Dr. T. Begenisich told us of (and later published, 1979) his studies on the node in which the steady-state currents did not need to be normalized. Otherwise, his results were in close agreement with Palti et al. At that point, we began to think that the resolution of the differences was in the species difference.

We do not think that the possible accumulation of K^+ in the Frankenhaeuser and Hodgkin (1956) space around the squid axon can account for the difference in results. Although it is known that E_K and g_K can change significantly with a long and strong depolarization (Adelman et al., 1973), these changes are not significant until after the steady-state level of I_K is reached. We found the same time-course of the rising phase in our normal ASW as in a high potassium solution, where the current was flowing in the opposite direction and K could not accumulate in the Frankenhaeuser-Hodgkin space. Furthermore, we observed superposition with temporal translations in the high potassium solution as well. With a hyperpolarizing conditioning level, the potassium current flow is very small and cannot effect a change in the Frankenhaeuser-Hodgkin space or the E_K seen by the membrane. Therefore, we do not see how the Frankenhaeuser-Hodgkin space surrounding the squid axon contributes in any way to our observations.

Our results on the crayfish reported in the next paper (Young and Moore, 1981) differ from those on the squid and are similar to those from the node as far as we can tell. Begenisich (1979) gives a single result for depolarizing conditioning pulses and finds superposition with temporal translations. Schauf et al., (1976) reported that in *Myxicola* axons superposition holds under all initial conditions.

This kinetic difference does not seem to arise from problems with methods or techniques. For example, similar observations were obtained on the squid axon using axial wire and sucrose gap voltage-clamp techniques. On the other hand, a single technique (the axial wire voltage clamp) gave similar results for *Loligo* and *Myxicola* but different results for crayfish. Internal perfusion of squid giant axons does not affect these kinetic phenomena; Keynes and Kimura (1980) confirm the original Cole and Moore (1960*a*) observations with this technique. We are left only with the species difference as the underlying source of this one kinetic difference.

It appears that all membranes tested give superposition for depolarizing conditioning. However, the frog node and crayfish axon membranes differ from the *Loligo* and *Myxicola* axon in their response after hyperpolarizing conditioning levels. Although this difference seems subtle, it appears to be reproducible. Therefore, it is to be considered carefully in building any model for the potassium conductance in axon membranes.

In this respect, we are gratified to find that both kinds of dynamics can be encompassed in a linear kinetic reaction scheme by different constraints on rate constants. This is described in the following paper (Young and Moore, 1981).

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REFERENCES

Adelman, W. J. Jr., Y. Palti, and J. P. Senft. 1973. Potassium ion accumulation in a periaxonal space and its effect on the measurement of membrane potassium ion conductance. *J. Membr. Biol.* 13:387–410.

Begenisich, T. 1979. Conditioning hyperpolarization delays in the potassium channels of myelinated nerve. *Biophys. J.* 27:257–266.

Bezanilla, F., and C. M. Armstrong. 1972. Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axons. *J. Gen. Physiol.* 60:588–608.

Cole, K. S., and J. W. Moore. 1960 *a*. Potassium ion current in the squid giant axon: dynamic characteristic. *Biophys. J.* 1:161–202.

Cole, K. S., and J. W. Moore. 1960 *b*. Ionic current measurements in the squid giant axon membrane. *J. Gen. Physiol.* 44:123–167.

Frankenhaeuser, B., and A. L. Hodgkin. 1956. The aftereffects of impulses in the giant never fibres of *Loligo*. *J. Physiol. (Lond.)* 131:341–376.

French, R. J., and J. B. Wells. 1977. Sodium ions as blocking agents and charge carriers in the potassium channel of the squid giant axon. *J. Gen. Physiol.* 70:707–724.

Halin, R., and L. Goldman. 1978. Initial conditions and the kinetics of the sodium conductance in *Myxicola* giant axons. I. Effects on the time-course of the sodium conductance. *J. Gen. Physiol.* 72:863–898.

Hill, T., and Y.-D. Chen. 1972. On the theory of ion transport across the nerve membrane. V. Two models for the Cole-Moore K^+ hyperpolarization delay. *Biophys. J.* 12:960–976.

Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.)*. 117:500–544.

Joyner, R. W., and J. W. Moore. 1973. Computer controlled voltage clamp experiments. *Ann. Biomed. Eng.* 1:368–380.

Keynes, R. D., and J. E. Kimura. 1980. The effect of starting potential on activation on the ionic conductances in the squid giant axon. *J. Physiol. (Lond.)*. 308:17P.

Moore, J. W. 1971. Voltage clamp methods. *In* Biophysics and Physiology of Excitable Membranes. W. J. Adelman, editor. Van Nostrand Reinhold Co.

Moore, J. W., and T. Narahashi. 1967. Tetrodotoxin's highly selective blockage of an ionic channel. *Fed. Proc.* 26:1655–1663.

Moore, J. W., F. Ramon, and R. W. Joyner. 1975. Axon voltage clamp simulations. Ii. Double sucrose gap. *Biophys. J.* 15:25–36.

Palti, Y., G. Ganot, and R. Stämpfli. 1976. Effect of conditioning potential on potassium current kinetics in the frog node. *Biophys. J.* 16:261–273.

Schauf, C. L., T. L. Pencek, and F. A. Davis. 1976. Potassium current kinetics in *Myxicola* axons. *J. Gen. Physiol.* 68:397–403.

Young, S. H., and J. W. Moore. 1981. Potassium ion currents in the crayfish giant axon; dynamic characteristics. *Biophys. J.* 36:723–733.