Survival of K^+ Permeability and Gating Currents in Squid Axons Perfused with K^+-Free Media

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ABSTRACT K^+ currents were recorded in squid axons internally perfused with impermeant electrolyte. Total absence of permeant ions inside and out leads to an irreversible loss of potassium conductance with a time constant of ~11 min at 8°C. Potassium channels can be protected against this effect by external K^+, Cs^+, NH_4^+, and Rb^+ at concentrations of 100–440 mM. These experiments suggest that a K^+ channel is normally occupied by one or more small cations, and becomes nonfunctional when these cations are removed. A large charge movement said to be related to K^+ channel gating in frog skeletal muscle is absent in squid giant axons. However, deliberate destruction of K^+ conductance by removal of permeant cations is accompanied by measurable loss in asymmetric charge movement. This missing charge component is large enough to contain a contribution from K^+ gating charge movements of more than five elementary charges per channel.

INTRODUCTION

The study of gating currents (Armstrong and Bezanilla, 1974) has already given some insight into inactivation of sodium channels (Armstrong and Bezanilla, 1977) and has proved to be a useful new tool in studying pharmacological modification of sodium channels (Yeh and Armstrong, 1978; Cahalan and Almers, 1979 *a*, *b*). Similar advances may be expected in understanding K^+ channels once we know how to record K^+ channel gating currents. Such currents are a theoretical necessity and should, in squid axons, carry perhaps one-quarter to one-half as much charge as sodium channel gating currents. However, since K^+ channels respond more slowly to potential changes than Na^+ channels, their gating charge movements may take more time and hence produce currents of smaller amplitude. This fact, among others, may have prevented their discovery in nerve. On the other hand, asymmetric displacement current recorded from frog skeletal muscle (Chandler et al., 1976 *a*) has a relatively slower time-course, and the possibility that

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some (Adrian and Peres, 1977) or all of it (Almers, 1976, 1978; Chandler et al., 1976 *b*) is K^+ gating current has received much discussion.

One difficulty in studying K^+ channels is that they cease to function when internal K^+ is removed for prolonged periods (Chandler and Meves, 1970). It was therefore necessary to explore experimental conditions that maintain potassium channels in a functional state even though ion movement through them is largely prevented, i.e., under conditions suitable for measuring gating currents. The large and slow components of asymmetric displacement current seen in frog skeletal muscle are absent under these conditions and cannot, therefore, be necessary for K^+ channel gating in squid axons. K^+ gating currents remain undiscovered.

METHODS

Experiments were performed at the Marine Biological Laboratory in Woods Hole, Mass., on voltage-clamped, internally perfused giant axons of the squid *Loligo pealei*. The experimental procedures have been described in detail elsewhere (Bezanilla and Armstrong, 1977). Solutions employed in the experiments are given in Table I. The external solutions that contain Tris were made with Trizma 7.0 (Sigma Chemical Co., St. Louis, Mo.). All other solutions were buffered to pH 7.0–7.3 with 10 mM Tris. Many of them contained the pharmacologically inert but impermeant monovalent cation, tetramethylammonium (TMA). Unless otherwise indicated, all external solutions contained 0.5 μM tetrodotoxin (Sigma Chemical Co.) to block sodium channels. Temperature was 8° C. In identifying solutions, *x*//*y* means external solution *x* and internal solution *y*.

TABLE I

COMPOSITION OF SOLUTIONS

Solution External	Na^+	K^+	Ca^{++}	$Tris^+$	Cl^-	Other
				mM		
ASW	450	0	50	0	550	—
Tris-SW	0	0	50	480	580	—
*x*K-Tris-SW	0	x	50	(480−*x*)	580	—
Rb,Cs,Li, or NH_4-SW	10	0	50	0	550	440 Rb^+, Cs^+, Li^+,or NH_4
33% Na-SW	150	0	50	320	570	—

Internal	Na^+	K^+	Cs^+	TMA^+	F^-	Glutamate	PO_4	Sucrose
				mM				
SISA*	0	417	0	0	50	320	30	230
200 TMA	0	0	0	200	50	150	0	510
200 Na	200	0	0	0	50	150	0	510
200 Cs	0	0	200	0	50	150	0	0

* Standard internal solution A.

RESULTS

Irreversible Loss of K^+ Channel Conductance by Exposure to K^+-Free Solutions

In physiological saline, a squid axon depolarized under voltage clamp produces membrane currents similar to those in Fig. 1 (upper record). As all other records in this paper, Fig. 1 has been corrected for linear capacitive and leakage admittances at −140 mV and shows only excess (or asymmetry) currents produced by the depolarization. There is first a transient outward current lasting ~200 μs; it is capacitive in nature and mostly gating current associated with the sodium channel. Outward gating current is followed by inward sodium current and, as sodium channels inactivate, by a large outward current through the K^+ channel. Current records of this kind can be obtained for many hours after perfusion is initiated, indicating excellent survival of the two ionic channels in physiological or near-physiological solutions.

When internal K^+ is replaced with an impermeant cation such as TMA^+,

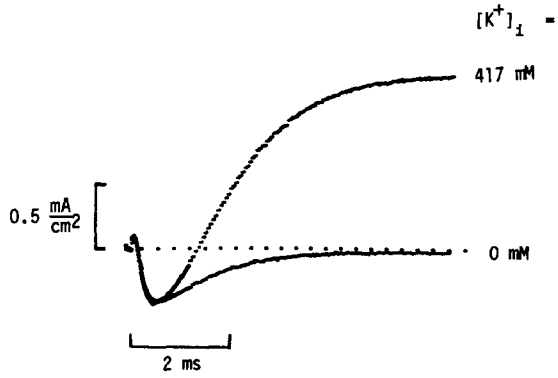

FIGURE 1. Membrane currents with and without internal K^+. The traces are for a depolarization from −70 to 30 mV in 33% Na-SW//SISA or 33% Na-SW//200 TMA. Axon AU297A. No tetrodotoxin.

outward current is abolished and only gating current and the inactivating inward sodium current remain (Fig. 1, lower trace). Outward current can be abolished also by adding internal tetraethylammonium (TEA) or other substances (not shown). But, whereas block by TEA is readily reversible, complete withdrawal of permeant ions inside and out leaves lasting damage (cf. Chandler and Meves, 1970). This is shown in Fig. 2 (top) where final K^+ outward currents during repeated depolarizations are plotted against time. The depolarization was of fixed amplitude and large enough to open nearly all K^+ channels. A K^+-free artificial seawater (ASW) was present externally. Twice during the experiment, there was a period of ~10 min where internal K^+ was exchanged for a mixture of sucrose and TMA^+ (200 TMA, see Table I); both times, recovery upon readmitting K^+ was incomplete.

Some of this effect is due to a spontaneous decline of K^+ currents ("rundown"), which is often unavoidable during such a long experiment; in Fig. 2, for example, some rundown was visible even at the beginning where the

internal solution, standard internal solution A (SISA), was of nearly physiological composition. To correct for rundown, currents were plotted on a semilogarithmic ordinate so that a straight line could be fitted to the initial points. If rundown is a first-order process, the line defines its rate and can be extrapolated to provide a reference for estimating completeness of recovery. The dashed lines in Fig. 2 have the same slope which corresponds to a rundown time-constant of 168 min derived from the first 13 min of the experiment.

After correction for rundown in this manner, recovery still appears incom-

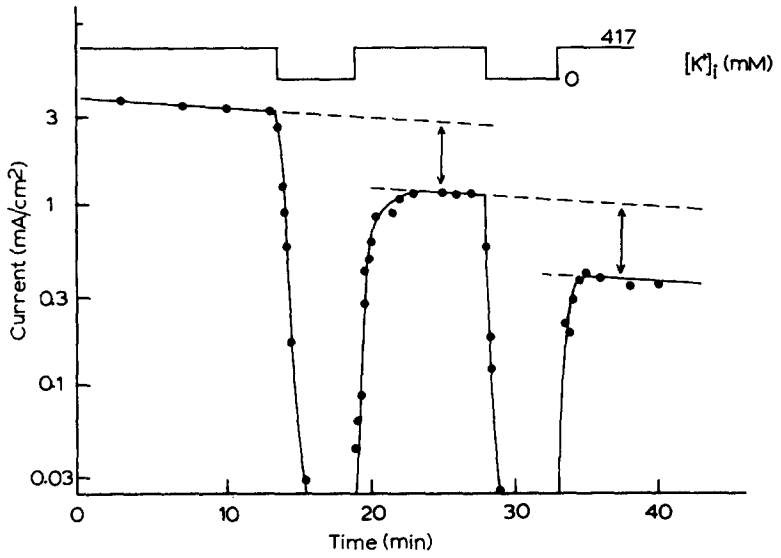

FIGURE 2. Loss of K current in K^+-free medium. Ordinate: maximum I_K at 90 mV on a logarithmic scale. External solution was ASW + 0.2 μM tetrodotoxin throughout; internal solution was SISA (420 mM K^+) or 200 TMA (no K^+) as indicated. A straight line was fitted to the first 13 min of the experiment, minimizing the largest deviation from the data. Its slope defines the time constant of rundown, 168 min in this experiment. Parallel lines (---) were drawn through later data points by eye; their vertical distances show that 40% of all K^+ channels survived the first, and 38% the second period of K^+ deprivation. Axon MA 186C.

plete. After each 10-min period without K^+, about 40% of the K^+ current was irreversibly lost. Fig. 3 summarizes other experiments similar to that of Fig. 2, plotting the percentage of K^+ current that recovered (ordinate) against the duration of K^+ deprivation. In the absence of permeant cations, loss of K^+-current proceeds with a time constant of about 11 min, more than ten times faster than the rate of spontaneous rundown with K^+ inside.

The following experiments show that the effect is due to the absence of K^+ rather than the presence of TMA^+. (*a*) When the internal fluid contained 200 mM TMA^+, as in Figs. 2 and 3, but in addition 100 mM K^+-glutamate

instead of sucrose, reintroduction of SISA produced full recovery (one experiment). (*b*) Loss of K^+ current occurs also if Na^+ instead of TMA^+ replaces internal potassium; in one experiment, a 30-min internal perfusion with a solution containing 200 mM Na^+ instead of K^+ resulted in loss of all but 10% of the K^+ current. This experiment confirms previous observations of Chandler and Meves (1970). From their data on NaF-perfused axons one can calculate a time constant of 8–9 min (1–4°C) for the loss of K^+ current. Chandler and Meves (1970) have also shown that the remaining K^+ currents have normal kinetics.

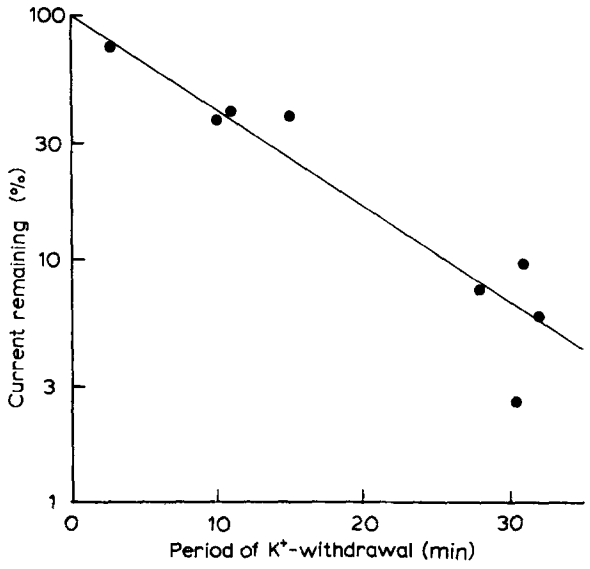

FIGURE 3. Loss of K current during K^+ deprivation. Ordinate: the percentage of the original I_K that remains after K^+ deprivation for the period given on the abscissa. When rundown was appreciable, ratios were obtained on semilogarithmic plots as in Fig. 2. Measurements from six axons. Details as in Fig. 2. 8°C.

Protection of K^+ Channels by External K^+ and Other Cations

Loss of K^+ current can be greatly slowed or even prevented by external K^+. The experiment of Fig. 4 tests the survival of K^+ current after two 30-min periods of perfusion with 200 TMA^+. During the entire first period, $[K]_o$ was raised to 100 mM; recovery of K^+ outward current upon reintroduction of internal SISA was nearly complete. During the second period, $[K] \approx 0$ inside and out, and only 2.6% of the K^+ current survived. This and similar experiments are summarized in Table II. Shown are the fractional currents surviving a 30-min internal perfusion with K^+-free TMA^+ solution, first in the presence of an external test cation such as K^+, and then in K^+-free ASW. Besides K^+,

the cations Rb^+, Cs^+, and NH_4^+ seem effective in slowing or preventing loss of K^+ conductance; Li^+ and, of course, Na^+ are relatively ineffective.

The results in Table II might suggest that the ability of small cations to protect K^+ is correlated with their permeability. Compared with Na^+ and Li^+, K^+, Rb^+, and NH_4^+ are all highly permeant (Hille, 1973) as well as being effective protecting agents. A possible exception is Cs^+, which is regarded as impermeant (Hille, 1973) yet seems much more effective than Na^+ in protecting K^+ current both inside (Chandler and Meves, 1970) or out (Table II). Factors other than permeability may contribute to the superiority of Cs^+ over

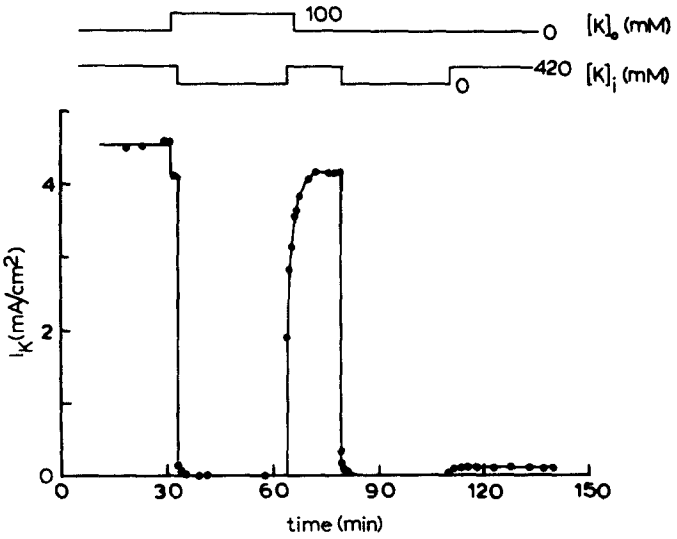

FIGURE 4. Protection of K^+ current by external K^+. I_K is the maximum current during a 5-ms pulse from −70 to 90 mV. The holding potential of −70 mV was maintained throughout the experiment. External solutions were Na-ASW ($[K]_o$ = 0) and 100 K-ASW ($[K]_o$ = 100 mM), as indicated; tetrodotoxin (0.2 μM) was present throughout. Internal solutions were SISA (417 mM $[K]_i$) or 200 TMA (0$[K]_i$) as indicated. In this experiment, no rundown was detectable during the first 12 min with internal SISA; therefore, an "infinite" rundown time constant was assumed in Table II. If the slight loss of conductance after the first challenge with internal TMA^+ is entirely due to rundown, one obtains a lower limit of 769 min for the rundown time-constant. Axon MA 136A.

Na^+ in protecting K^+ current. For instance, external Cs^+ blocks (and therefore binds to) K^+ channels much more strongly than Na^+ (Adelman and French, 1978).

Dependence of Inward K^+ Current on $[K]_o$

It appears that K^+ channels do not readily survive in the absence of K^+ unless some other permeant ion is present. A related question is whether K channels continue to function in the absence of K, even when the period of deprivation is too brief to damage the channels permanently. Na channels do gate in the absence of Na, as evidenced by the presence of gating current (I_g). Is this also

true for K channels, for which no I_g has been recorded? We approached this problem by steadily lowering the external K^+ concentration in the absence of internal K, and measuring the amplitude of inward tail currents which accompany repolarization after all K^+ channels have been opened by a large depolarization. Trace A in Fig. 5 was recorded with $[K]_i = [K]_o = 0$; the inward transient is presumably capacitive and mostly Na^+ gating current. Transients at elevated $[K]_o$ were larger; correcting for capacitive or gating currents by subtracting trace A from such transients resulted in traces B–D which should be pure K^+ current. Initial amplitudes were measured (see legend of Fig. 5 for details), normalized with respect to similar measurements at $[K]_o = 44$ mM, and plotted against $[K]_o$. Fig. 6 summarizes experiments on three axons. The currents are proportional to $[K]_o$ as would be predicted by the "independence principle" (Hodgkin and Huxley, 1952 *b*).

TABLE II

PROTECTION OF K^+ CHANNELS BY SEVERAL EXTERNAL TEST CATIONS IN THE ABSENCE OF INTERNAL K^+

Axon	Rundown time-constant	Test cation X, *mM*	g_K remaining with test cation	g_K remaining with Na^+-ASW
	min		%	%
MA 186B	(∞)	Li^+, 440	2	—
MA 136A	(∞)	K^+, 100	>92	3
MA 156A*	98	K^+, 100	99	10
MA 216B†	612	K^+, 10	24	3
MA 146A	(∞)	Rb^+, 440	68	6
MA 186A	96	NH_4^+, 440	75	7
		Cs^+, 440	102	

The Table compares survival of K^+ conductance after a 28–32-min internal perfusion with K^+-free solution (200 TMA) first in the presence of an external protecting ion (test cation) of indicated concentration, then in the K^+-free ASW. Substitution of test cation for Na^+ occurred on an equimolar basis. Only few channels survive internal K^+ deprivation when the only univalent external cation is Na^+ (last column) or Li^+ (first row, fourth column). All results from experiments as in Fig. 4. Where necessary, correction for rundown was applied (see Fig. 2 for details) and rundown time-constants are given. Otherwise, rundown was too slow to measure.

* Internal perfusion with 200 Na instead of 200 TMA.

† Exposure to 200 TMA only lasted 20 min; the value given allowed for this by assuming exponential loss of K^+ conductance.

In other experiments,[1] instantaneous current-voltage curves were measured at physiological $[K]_i$ after opening all K^+ channels with a large depolarizing prepulse. When $[K]_o$ was changed over the range from 0 to 100 mM, the new current-voltage curve agreed well with the prediction from the independence principle. Since (*a*) K^+ channels remain fully functional at $[K]_o = 0$ when $[K]_i$ is in the physiological range (see, e.g., Fig. 1), and (*b*) the membrane obeys the independence principle for changes in external $[K]_o$ no matter whether $[K]_i = 0$ (Fig. 6) or in the physiological range,[2] we suggest that all channels remained functional in Fig. 5.

[1] Armstrong, C. M. Manuscript in preparation.

[2] Almers, W., and C. M. Armstrong. Unpublished observations.

A linear relation between $[K]_o$ and K^+ inward current under conditions of large negative displacements from the K^+ equilibrium potential was previously observed on the inwardly rectifying K^+ channel in frog muscle (Almers, 1971). Thus net charge flux (or current) may agree with the independence principle even though tracer fluxes (delayed rectifier: Hodgkin and Keynes, 1955 *b*; inward rectifier: Horowicz et al., 1968) do not.

Absence of Large K^+ Channel Gating Currents

In the search for K^+ gating currents, it is important to abolish ionic currents through K^+ channels, as can be achieved, for instance, by removing all permeant ions for brief periods. Fig. 7 illustrates such an experiment and

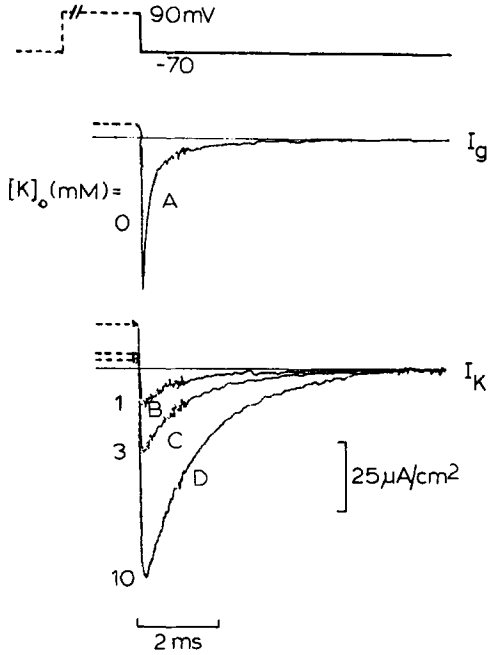

FIGURE 5. I_K tails during repolarization from +90 to −70 mV. *x*K-Tris-SW//200 TMA with the value of *x* (K_o in millimolar) indicated next to each trace. The top trace is assumed to be purely I_g and to contain no ionic current through K^+ channels; it was subtracted from all other traces to obtain I_K. Amplitudes of tails were obtained by fitting declining exponentials to the lowermost three traces and determining their values at the moment of repolarization. Axon JN156A.

shows membrane currents during depolarizing pulses large enough (from −70 to +90 mV) to drive all K^+ channels from closed to open states. Initially, internal K^+ was present, and the large K^+ current in trace B shows the kinetics of channel opening as well as giving an estimate of $\bar{g}_K$, the K^+ conductance with all channels open. For the remainder of the experiment, we set $[K]_i = 0$ and $[K]_o = 44$ mM. Outward K^+ currents were now absent, but inward "tail" currents as in Fig. 5 were present and could be used to estimate the fraction

of functional K^+ channels remaining after, e.g., brief periods in complete absence of internal and external K^+.

Trace A in Fig. 7 was recorded during such a period of total K^+ withdrawal. It shows a large outward current transient during, and an inward transient after the depolarization to +90 mV. Presumably, these transients are mostly Na^+ gating current and do not readily reveal the relatively smaller and unknown amount of K^+ gating current that should also be present. They can be used, however, to place constraints on the size of K^+ gating currents predicted from mathematical models of K^+ gating. Suppose that a portion of K^+ gating current, I_n, is given by dn/dt where n is the well-known gating parameter of Hodgkin and Huxley (1952 *a*):

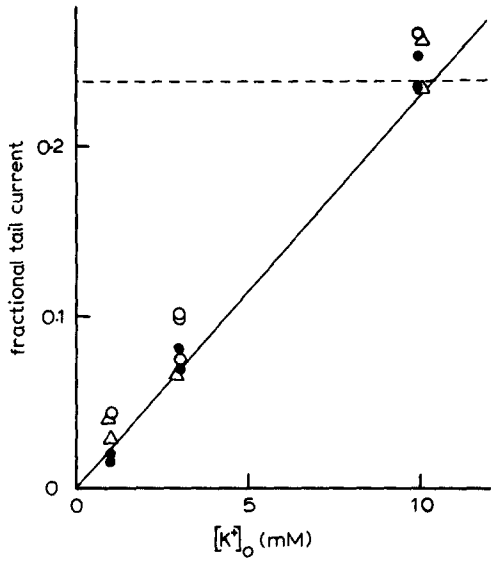

FIGURE 6. Tail amplitudes as a function of $[K]_o$. Values are normalized with respect to measurements as $[K^+]_o$ = 44 mM. Solid line is predicted by the independence principle. Data from experiments as in Fig. 5 on three axons; the axon of Fig. 5 yielded data shown by open circles.

$$I_n = Q_{n,\max} \frac{dn}{dt}. \tag{1}$$

From analysis of trace B, the time-course of I_n should be that of a single exponential with time constant τ_n = 0.7 ms. The largest such exponential which could be contained in trace A is given by trace C; it carries a charge of $Q_{n,\max}$ = 9.9 nCi/cm^2. Alternatively, suppose that another component of K^+ gating current, I_γ, is given by the time derivative of K^+ conductance

$$I_\gamma = Q_{\gamma,\max} \frac{d(g_K/\bar{g}_K)}{dt}, \tag{2}$$

as discussed by Adrian and Peres (1977). This slow component of gating

current would contain only charge movement accompanying the opening of K^+ channels during the final, or one of the final, steps in the sequence of K^+ channel gating reactions. The time-course of this component is given by trace C′; any such component contained in trace A must be at least five times smaller ($Q_{\gamma,max}$ <8 nC/cm^2). Evaluation of tail amplitudes after restoring $[K]_o$ = 44 mM (now shown) suggests that $\bar{g}_K$ with normal $[K]_i$ would have been 16 mS/cm^2 after trace A was recorded. Thus, gating current components with time-course dn/dt must carry <0.6 nC/mS or four elementary charges

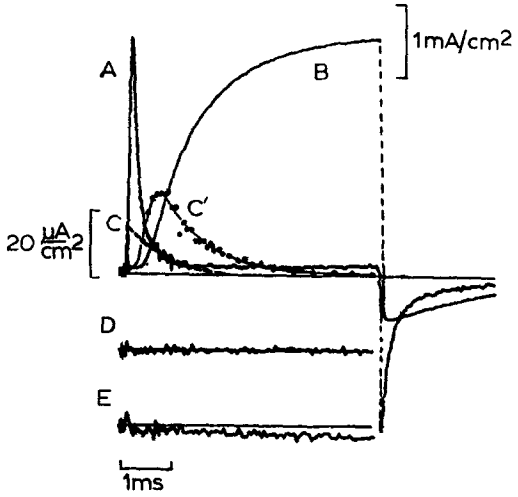

FIGURE 7. Membrane currents during and after pulses from −70 to +90 mV. Trace A: asymmetric displacement currents recorded in total absence of K (Tris-SW//200 TMA). Trace B: I_K recorded at beginning of experiment in 44 K-Tris-SW//SISA. Trace C and C′: hypothetical gating currents I_n and I_γ, respectively. For trace C′, $Q_{\gamma,max}$ = 2.5 nC/mS was assumed; this value is similar to that required to fit the secondary "hump" in displacement current records in frog skeletal muscle (Adrian and Peres, 1977). Traces D and E: membrane currents as in trace A but with $[K]_o$ = 3 mM (D) and 44 mM (E), both after subtraction of trace A. The right-hand scale applies to trace B, the left-hand scale to all other traces.

(e) per picosiemens of K^+ conductance (4e/pS), and those with time-course dg_K/dt carry <0.5 nC/mS or 3e/pS.

With external K^+ present, depolarizing pulses were followed by inward tails of the kind shown in Fig. 5. As discussed before, this indicates that K^+ channels opened and closed normally. During the pulse, however, currents were identical to those in trace A, and when trace A was subtracted from these records, no transient outward currents remained (traces D and E). Thus, we consider that the upper limits for K^+ gating charge movement apply also in the presence of external K^+.

Fig. 8 takes an alternative approach. Trace A shows K^+ outward current during a large depolarization to +90 mV; from another, similar record taken

immediately afterwards, we calculated a maximal K^+ conductance of $\bar{g}_K$ = 26.8 mS/cm^2. Removal of internal K^+ abolished all outward current except for the initial I_g transient (trace B). Removal of all external K^+ had no effect on the I_g transient, because subtracting from each other the transients recorded immediately before and after withdrawal of external K^+ caused nearly perfect cancellation (trace C). Between traces C and D, a 78-min soak took place with

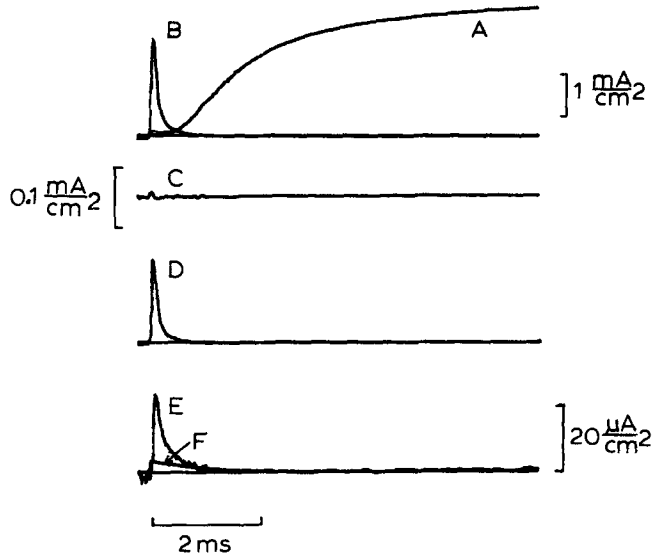

FIGURE 8. Asymmetric displacement currents during steps from −70 to 90 mV before and after removal of K^+-conductance. (A) I_K recorded in 44 K-Tris-SW//SISA at the beginning of the experiment. (B) Asymmetric displacement current recorded after removing internal K^+ (solutions 44 K-Tris-SW//200 TMA). (C) Difference between membrane currents recorded just before and just after replacement of 44 K-Tris-SW by Tris-SW; this trace shows that there was almost no change in asymmetric displacement current when all external K was removed. The record in Tris-SW//200 TMA was taken only 2 min after trace A, and we expect that virtually all K^+ channels were still functional. (D) Asymmetric displacement current recorded after ~78 min soak in Tris-SW when all K^+ channels have become inoperative. (E) Difference between traces B and D, digitally filtered by replacing the data point obtained at time n, namely y_n, by the weighted average of adjacent data points, namely by $0.1(y_{n-2} + 2y_{n-1} + 4y_n + 2y_{n+1} + y_{n+2})$. (F) Trace is given by $I_{n,o} \exp(-t/\tau_n)$, where $I_{n,o}$ = 3.16 μA/cm^2 and τ_n = 0.9 ms. Calibration bar on the left refers to traces B, C, D; that on the upper right refers to trace A.

[K] = 0 inside and out; during that time, K^+ conductance was lost entirely.

The gating current transient changed much less, consistent with the view that most of it is related to Na^+—rather than K^+—channels. A small loss in gating current can be shown, however, by subtracting D from a similar record taken immediately before the soak in $[K]_i = [K]_o = 0$. The resulting transient,

trace E, carries a charge of 5 nC/cm^2. If the soak in K^+-free solutions preferentially abolished K^+ gating current along with K^+ conductance, one might expect to see a slower component, namely I_{gK}, which is more pronounced in trace E than in traces B and D. Indeed, trace E does relax noticeably more slowly than traces B and D, and much of the slower component could be K^+ gating current. The fast component is probably due to spontaneous loss (rundown) of sodium gating current. Trace E shows no evidence of a component with time-course dg_K/dt, but it could contain a dn/dt contribution. Trace F is an exponential wih time constant $\tau_n = 0.91$ ms, obtained by fitting trace A with Hodgkin-Huxley kinetics, and carries a charge of 2.57 nC/cm^2 (161 e/μm^2). Calculated from the simultaneous loss of 26.7 mS/cm^2 of K^+ conductance, this amounts to 0.6 elementary charges per picosiemens of conductance. In another, similar experiment, the corresponding value was 2.2 e per picosiemens. These values would translate into 7.2–26 elementary charges per channel if the single-channel conductance is 12 pS (Conti et al., 1975). In the next section, we investigate whether these values are consistent with the steep voltage-dependence of K^+ conductance.

Voltage Sensitivity of the K^+ Channel

The well-known relationship between voltage V and the steady-state K^+ conductance g_K is sigmoid. At sufficiently negative potentials where g_K is only a small fraction of the maximum, $\bar{g}_K$, the "foot" of the g_K/V curve, is well described by an exponential

$$\frac{g_K}{\bar{g}_K} = A \exp(qV/kT), \tag{3}$$

where A and q are constants and $kT = -25$ meV. Since the behavior of a single open K^+ channel is probably ohmic over the limited potential range of 10–15 mV, the fraction of open K^+ channels over such range is also given by the above equation. The experimental result described by Eq. 3 is expected from a wide variety of models, Hodgkin and Huxley's (1952 *a*) among them, and can be used to set a lower limit on the maximal K^+ gating charge movement. For a multistate system such as a K^+ channel, theory shows (Almers, 1978) that the coefficient q/kT, called the logarithmic potential sensitivity, should grow to a limiting value as the analysis is pursued to more and more negative potentials:

$$q/kT \to q_g/kT, \qquad V \to -\infty, \tag{4}$$

where q_g (in units of elementary charges) is the gating charge movement necessary to open a single K^+ channel. It therefore seemed worthwhile to examine $g_K/\bar{g}_K$ as a function of V over as negative a potential range as possible.

Since the g_K/V relationship is very steep, it is necessary to test for and minimize potential nonuniformity. One difficulty arises at the platinum/electrolyte interface of the axial wire. For short periods (a few milliseconds or less), Pt-black allows large currents without difficulty, and we have full confidence that voltage steps are transmitted uniformly and faithfully to the

membrane. However, the standing current necessary to establish the holding potential eventually creates a counter-electromotive force (emf) that acts as a battery between axial wire and axolemma. If the holding current is nonuniform, then so is the emf and therefore the holding potential. In practice it appears that the emf is largest where extra currents leave the cut ends of the axon, especially at the end kept moist by the perfusion fluid. Exploring the internal potential of axons with a roving micropipette showed that the holding potential at one end of the supposedly voltage-clamped region was up to 5 mV less negative than the central region. To improve uniformity of holding potential, a constant current was sent into one end of the axon via an electrode unconnected to the voltage-clamp circuitry. It was hoped that this electrode would deliver the extra current flowing through the cut ends of the axon, thereby improving the uniformity of the emf. The extra current was adjusted manually so as to minimize the potential variations recorded by the roving internal electrode. Once the optimal current was set, the experiment could start. Fig. 9 (top) shows the internal potential along the axon in a typical experiment. Holding potential nonuniformity in the central "measuring region" was now <3 mV.

Fig. 9 (bottom) shows K^+ current during weak depolarizations. They are small throughout, but increase steeply with potential. Final g_K was calculated as $\Delta I/\Delta V$, where ΔV is the amplitude of depolarization and ΔI the change in current upon repolarization. In this axon, the value of $\bar{g}_K = 51$ mS/cm^2 was measured at the end of a large depolarization to 50 mV; the average (± SEM) in 10 axons internally perfused with SISA was 39.8 ± 3.1 mS/cm^2. In Fig. 10, $g_K/\bar{g}_K$ is plotted on a logarithmic ordinate against V. The line represents Eq. 3 with A and q/kT adjusted to provide the best least-squares fit to the data. kT/q was 4.3 mV in Fig. 9, the average from two runs on this axon being 4.6 mV. Another axon gave an average value of $kT/q = 5.2$ mV. Thus g_K grows e-fold in ~5 mV when $g_K/\bar{g}_K$ <0.02, indicating a gating charge movement of at least five elementary charges per channel upon opening. This figure is lower than in previous work on frog skeletal muscle (> eight charges/channel over a similar range of $g_K/\bar{g}_K$; Almers, 1976).

DISCUSSION

When studying gating currents, one must first abolish the large ionic currents which flow through the normal activated membrane. Currents through sodium channels are readily prevented by tetrodotoxin, and K^+ currents through leakage- and delayed K^+ channels are abolished by replacing all intra- and extracellular K^+ with impermeant cations like $Tris^+$ or TMA^+. After ionic currents are removed in this fashion, axons show large Na^+ gating currents but no component easily identifiable as K^+ channel gating current (Armstrong and Bezanilla, 1977). It seems natural to conclude that K^+ channel gating currents are too small to be recognized easily among the other and larger asymmetric displacement currents, but this conclusion does not follow immediately from such experiments. Our experiments show that in an environment totally lacking in permeant ions, K^+ channels revert irreversibly and

fairly rapidly (time constant of the order of 10 min at 8°C) into a nonfunctional state or are lost from the membrane altogether. Absence of large K^+ gating currents in previous recordings could thus have been due to absence of functional K^+ channels, among other factors.

Here we report first attempts to explore the conditions for K^+ channel survival, and the following preliminary picture seems to emerge: (*a*) K^+

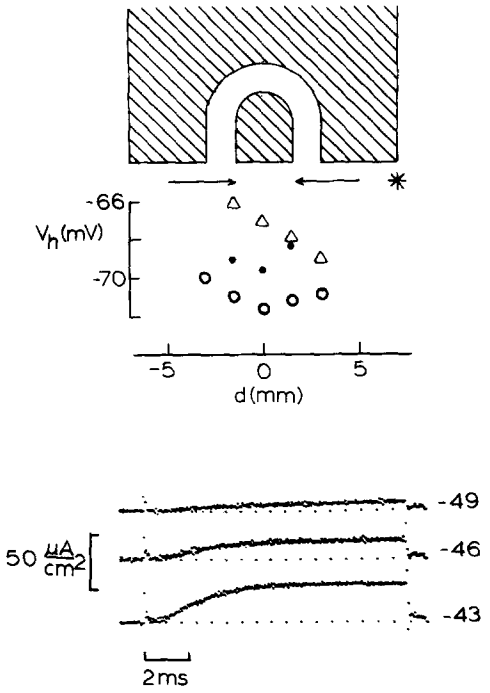

FIGURE 9. Dependence of g_K on voltage with test of internal isopotentiality. Top: ground electrodes of axon chamber (hatched) showing guard and central measuring electrode. Measuring region indicated by arrows. Middle: internal potential vs. distance recorded with a pipette inserted longitudinally into the axon while under current clamp. Holding current was applied through the usual platinized axial wire, as well as through a separate electrode positioned at (*). Different symbols represent series of measurements taken at different times during the experiment. Bottom: K^+ currents recorded under weak depolarizations from −70 mV to the potential next to each trace (in millivolts). In the middle drawing, circles were obtained before, and triangles after this series of records was taken. Tris-SW//SISA. Axon JL107A.

channels survive for many hours in K^+-free internal fluids if external K^+ is present. Even 10 mM $[K]_o$ affords appreciable (but noticeably incomplete) protection. The permeant ions NH_4 and Rb^+, but also the supposedly impermeant Cs^+, can substitute for K^+. There must therefore be a protecting site accessible to external cations. (*b*) K^+ channels survive well in nominally K^+-free external fluids if internal K^+ is present. Internal K^+ could leak from a quiescent axon, accumulate beneath the Schwann cell layer and thus act

extracellularly, but it seems difficult to see how such a leak of K^+ (20–30 pmol/cm^{2}s in intact axons of *Sepia*; Hodgkin and Keynes, 1955 *a*) could raise the [K] in the Schwann cell space by more than a few millimolar (Baker et al., 1969) which, judging from our one experiment at $[K]_o$ = 10 mM, should not give complete protection. Thus there must also be a protecting site accessible to intracellular cations. Cs^+ (Chandler and Meves, 1970) and probably also Rb^+ (Almers and Armstrong[2]) can substitute for internal K^+.

It is natural to suggest that the protecting site(s) lie, in fact, inside the pore. Then a given site could receive both internal and external cations. Multiple

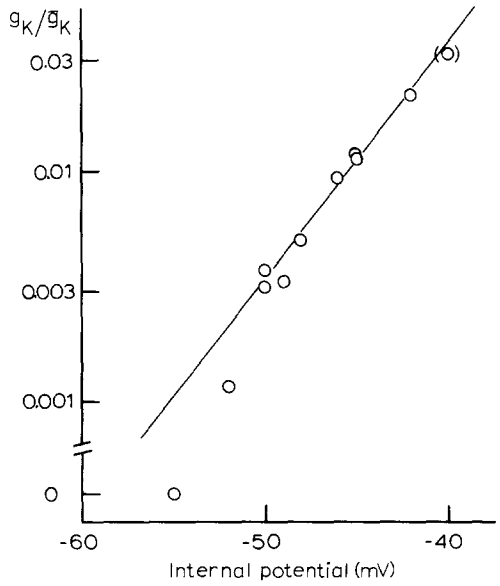

FIGURE 10. Steady-state K^+ conductance (g_K) as a fraction of the maximum ($\bar{g}_K$) plotted against potential. $\bar{g}_K$ = 50 mS/cm^2 was obtained at the end of a 5-ms depolarization to +50 mV. The line indicates an exponential which rises *e*-fold in 4.2 mV. It provides the best least-squares fit to the data. Point in parentheses not included during fitting procedure. Same experiment as Fig. 9.

cation-binding sites inside the channel must exist, since there is good evidence (Hodgkin and Keynes, 1955 *b*) that a K^+ channel is filled at all times by one or more small cations. It is therefore a "multi-ion pore" (Hille and Schwarz, 1978). In Hodgkin and Keynes' (1955 *b*) experiments, two to three sites inside the channel must have been occupied simultaneously by K^+, since the flux ratio in their experiments depended on the 2.5th power of the electrochemical gradient. Although simultaneous occupation of a channel by one cation at either end need not be prohibitive energetically, triple occupancy might be (Levitt, 1978), and Hille and Schwarz (1978) have suggested that the pore walls may be lined with negative charges or properly oriented dipoles to counteract electrostatic repulsion between neighboring cations. If so, such charges or dipoles on their own may generate electrostatic forces which are large enough to destroy the channel if the resident cations are removed. The

observed instability of K^+ channels in the absence of permeant ions may thus be a consequence of K^+ channels being multi-ion pores.

Stabilization of K^+ channels by resident cations is reminiscent of the finding that tetrodotoxin stabilizes the extracted tetrodotoxin receptor (Agnew et al., 1978). Both findings may be compared to the well-known effects of substrates and competitive inhibitors in protecting some enzymes against denaturation (e.g, Burton, 1951, or Lumry, 1959).

In attempts to record K^+ channel gating current, loss of K^+ channels can be minimized by keeping periods of complete K^+ deprivation brief, or by retaining small amounts (3–44 mM) of external K^+. Our failure to find large K^+ gating currents under these conditions reinforces previous conclusions (Almers, 1976, 1978; Chandler et al., 1976 *b*) that K channel gating current is small and does not constitute a major portion of asymmetric displacement currents in skeletal muscle. If the early exponential component of displacement current in frog muscle were due to K^+ channel gating, and if the conductance of single open K^+ channels in squid and frog are similar, then component C in Fig. 7 should be fives times larger. Clearly trace A of Fig. 7 contains no such large, slowly relaxing component.

In Fig. 8 we hoped that recording gating currents before and after deliberate destruction of K^+ conductance might help in identifying K^+ gating currents. Loss of K^+ conductance was indeed accompanied by loss of charge movement, which amounted to 1.6 e/pS (3.4 e/pS in another, similar experiment), and contained components relaxing noticeably more slowly than Na^+ gating current. However, it remained unclear what fraction of the missing charge movement corresponded to $I_{g,\,K^+}$. If $I_{g,\,K^+}$ follows Hodgkin and Huxley's (1952 *a*) kinetic description of K^+ conductance, the missing charge component could have contained a K^+ gating charge movement of 0.6–2.2 e/pS.

On the other hand, a lower limit of 5 e/K^+ channel can be obtained from the voltage sensitivity of g_K. In the literature values given for the conductance of an open K^+ channel vary from 2–3 pS (Armstrong, 1975) to 12 pS (Conti et al., 1975) and suggest that K^+ gating charge movement of only 0.42 to 2.5 e/pS would still be consistent with the observed voltage sensitivity. Such charge movements could easily be contained in the "missing component" of Fig. 8 (trace E) or similar experiments.

To summarize, there are two major difficulties in recording and recognizing K^+ channel gating currents: their expected small size and the instability of K^+ channels in the absence of permeant ions. The latter difficulty can be overcome but the former remains.

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