

# Activation-Inactivation of Potassium Channels and Development of the Potassium-Channel Spike in Internally Perfused Squid Giant Axons

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**ABSTRACT** A spike that is the result of calcium permeability through potassium channels was separated from the action potential in squid giant axons internally perfused with a 30 mM NaF solution and bathed in a 100 mM  $\text{CaCl}_2$  solution by blocking sodium channels with tetrodotoxin. Currents through potassium channels were studied under voltage clamp. The records showed a clear voltage-dependent inactivation of the currents. The inactivation was composed of at least two components; one relatively fast, having a time constant of 20–30 ms, and the other very slow, having a time constant of 5–10 s. Voltage clamp was carried out with a variety of salt compositions in both the internal and external solutions. A similar voltage-dependent inactivation, also composed of the two components, was recognized in all the currents through potassium channels. Although the direction and intensity of current strongly depended on the salt composition of the solutions, the time-courses of these currents at corresponding voltages were very similar. These results strongly suggest that the inactivation of the currents is attributable to an essential, dynamic property of potassium channels themselves. Thus, the generation of a potassium-channel spike can be understood as an event that occurs when the equilibrium potential across the potassium channel becomes positive.

## INTRODUCTION

Squid giant axons internally perfused with a dilute NaF solution can produce long-lasting action potentials (Watanabe et al., 1967; Meves and Vogel, 1973; Inoue et al., 1973; Inoue et al., 1974). I have demonstrated (Inoue, 1980) that the long-lasting action potential can be separated into two spike components by tetrodotoxin (TTX) and by tetraethylammonium ion (TEA). The sites responsible for production of the two components have also been identified; the sodium channel is responsible for development of one component of the action potential, which is selectively blocked by TTX, and the potassium channel for the slower phase, which is selectively suppressed by TEA but insensitive to TTX.

The main objectives of the present study were to gain an understanding of the ionic processes in the potassium channel that underly the TTX-insensitive action potential (the potassium-channel spike) and to investigate whether the potassium-channel spike reflects an essential time- and voltage-dependent property of the potassium channel or whether it results from abnormal behavior under nonphysiological conditions. Voltage clamp was used to study the characteristics of current through potassium channels, and the effects on the current of changing the environmental salt composition were studied. The experimental results reveal that there is an inactivation of current through potassium channels subsequent to activation and that the activation-inactivation is attributable to an essential time- and voltage-dependent characteristic of the potassium channel of the squid axon membrane.

#### METHODS

Experiments were performed on giant axons from two species of squid. One, *Doryteuthis bleekeli*, was available in the Sea of Japan, and the other, *Sepioteuthis lessoniana*, was caught in the Inland Sea of Japan. The axons from *Doryteuthis bleekeli* were 400–700  $\mu\text{m}$  in diameter, slightly larger than those from *Sepioteuthis lessoniana*, which were 350–500  $\mu\text{m}$  in diameter. No significant difference in electrical activity was recognized in the axons from the two kinds of squid. The procedures for internal perfusion with two glass cannulae and for voltage clamp have been reported (Inoue, 1980). The solutions employed in the present experiments are given in Table I. The external solution contained 1  $\mu\text{M}$  TTX to eliminate current contributed by sodium channels, unless otherwise indicated. The slash notation for solutions, x//y, which represents an external solution x and an internal solution y, is used in this paper, following the precedent of Almers and Armstrong (1980). All experiments were performed at room temperature,  $11.0 \pm 2.5^\circ\text{C}$ .

#### *Leakage Compensation*

A potassium-channel spike evoked from an axon with 100 Ca//30 Na is accompanied by a small rise in the  $\text{Ca}^{2+}$  permeability. The conductance at the peak of the spike is not much greater than the leakage conductance. Therefore, to study the properties of the potassium channel, correction for leakage current was necessary. Since the potassium-channel spike is selectively blocked by internally applied TEA, it was possible to study the voltage-current properties of the leakage component over a wide range of voltages, as shown in Fig. 2. Fig. 2 a (solid triangles) shows an example of the voltage-current relation of the leakage component. In this case, the leakage current is a linear function of voltage in the region below +30 mV. In this region, the sum of two currents produced by a step depolarization and a step hyperpolarization of the same amplitude from the holding potential was zero (for example, see the records on the right side of Fig. 5 a). In the region above +30 mV, the leakage current tends to deviate from linearity as voltage increases. In the region below  $-36$  mV (the resting potential), there is no significant difference in the voltage-current relation between the leakage component and the resting membrane before treatment with TEA.

The leakage current was subtracted in the following manner: In a voltage region where the leakage component was linear, two currents produced by two voltage pulses of the same amplitude but of opposite polarity were added with an averaging computer (Nihon Kohden, Japan, model ATAC 150). In the other voltage region, a leakage current at each corresponding voltage was subtracted from an original current measurement recorded before TEA treatment.

## RESULTS

*Time-Course of a Potassium-Channel Spike*

In axons with 100 Ca//30 Na, a potassium-channel spike can be separated from the original action potential by blocking sodium channels with TTX. Fig. 1 shows action potentials before (*left*) and after (*right*) blocking of sodium channels with 1  $\mu$ M TTX. The record on the right demonstrates a potassium-

TABLE I  
SOLUTION COMPOSITION

Internal solution	Na <sup>+</sup>	K <sup>+</sup>	TEA <sup>+</sup>	F <sup>-</sup>	Phosphate <sup>-</sup>	Glycerol
			<i>mM</i>			<i>% by volume</i>
30 Na	30	—	—	20	10	11.4
30 Na-3 K	30	3	—	23	10	11.3
30 Na-10 K	30	10	—	30	10	11.2
30 Na-30 K	30	30	—	50	10	10.8
30 Na-10 TEA	30	—	10	30	10	11.2
450 K	—	450	—	300	150	3.0

pH, 7.3  $\pm$  0.1 with phosphate buffer.

External solution	Ca <sup>++</sup>	K <sup>+</sup>	Na <sup>+</sup>	Cl <sup>-</sup>	Tris-HCl	Other	TTX	Glycerol
				<i>mM</i>			<i><math>\mu</math>M</i>	<i>% by volume</i>
100 Ca	100	—	—	200	5	—	1	8.9
10 Ca	20	—	—	200	5	180 choline <sup>+</sup>	1	8.1
100 Ca-30 K	100	30	—	230	5	—	1	8.3
100 Ca-60 K	100	60	—	260	5	—	1	7.7
ASW	10	9	423	498	6	48 Mg <sup>2+</sup> 25 SO <sub>4</sub> <sup>2-</sup>	1	—

pH, 7.9  $\pm$  0.1 with Tris-HCl buffer.

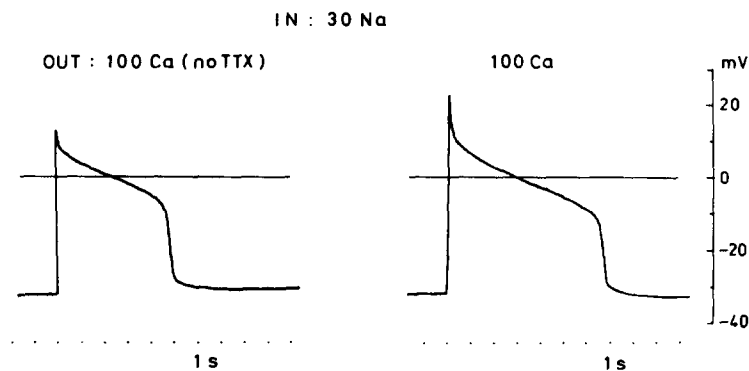


FIGURE 1. Action potentials before (*left*) and after (*right*) the addition of 1  $\mu$ M TTX to the external solution. The axon was internally perfused with a 30 mM NaF solution and bathed in a 100-mM CaCl<sub>2</sub> solution. The record on the right demonstrates a potassium-channel spike.

channel spike. The two spikes are similar in shape but different in size. The marked increase in the spike height after TTX treatment is due to the fact that the equilibrium potential across the potassium channel is much more positive than that of the sodium channel under these salt conditions (Inoue, 1980). As can be seen from the record on the right in Fig. 1, the membrane potential associated with the potassium-channel spike reaches its peak within 10 ms. Voltage decay is relatively fast at first, followed by a very slowly decaying plateau. Finally, voltage flips back to the resting level. Since the spike is accompanied by a rise in the ionic conductance of only potassium channels, it must be that, subsequent to their activation, there is an inactivation of potassium channels. Furthermore, the characteristics of the potential decay of the potassium-channel spike suggest that at least two processes may be involved in the potassium-channel inactivation.

#### *Voltage-Current Relation of the Potassium Channel*

Fig. 2 *a* illustrates three voltage current curves obtained from an axon under the three conditions given in the figure. The curves give the maximum current, which could be either a peak or a steady current. The addition of 1  $\mu\text{M}$  TTX to the external  $\text{CaCl}_2$  solution increased the separation of the two zero-current intercepts by 12 mV, but it decreased the peak conductance from 1.90 to 0.37  $\text{mmho}/\text{cm}^2$  (curves ① and ②). The conductance of the resting membrane was unchanged. These results are quite in agreement with those reported previously (Inoue, 1980). As described in Methods, curve ③ is the leakage component. The reversal potential of the leakage component is  $-36$  mV, which is equal to the resting potential. The leakage conductance in the linear region is 0.13  $\text{mmho}/\text{cm}^2$ .

Curves *a* and *b* in Fig. 2 *b* were obtained after subtraction of leakage current from curves ① and ②, respectively. Curve *a* represents current through both sodium and potassium channels. Curve *b* displays the voltage-current relation of the potassium channel under these conditions. From curve *b* it is seen that the current through the potassium channel, i.e.,  $I_{\text{Ca}}$ , does not reverse. This means that the  $\text{Na}^+$  permeability of the potassium channel becomes either very low or zero under these salt conditions (Bezanilla and Armstrong, 1972). (Similar voltage-current relations can be recognized for  $I_{\text{Ca}}$  in other excitable tissues (cf. Hagiwara et al. [1975] and Brown et al. [1978]). A reference voltage,  $V_{\text{ref}}$ , which is obtained by linear extrapolation of the curve at the maximum slope to the voltage axis, is  $+83$  mV in this case.  $V_{\text{ref}}$  is 53 mV more positive than the peak potential of the potassium-channel spike in this axon. The maximum slope conductance calculated from curve *b* is 0.19  $\text{mmho}/\text{cm}^2$ , which is almost the same as the leakage conductance.

#### *Voltage-Current Relation of the Open Potassium Channel*

In the voltage-current relation of the potassium channel under the condition 100 Ca//30 Na, the maximum slope is usually  $>+30$  mV. Since it is thought that most of potassium channels open at this voltage (cf. Hodgkin and Huxley [1952] and Hille [1973]), the following experiments were carried out to study the voltage-current characteristics of the open potassium channel.

After examination of the voltage-current relation for the peak current, voltage was first depolarized to the point of maximum slope. When the current reached its peak, voltage was changed stepwise, and current displacements associated with the voltage steps were measured. Leakage current, which was measured after treatment of the axon with TEA, was subtracted from an original current measurement at corresponding voltage changes.

Fig. 3 shows an example of the results. The solid circles represent the voltage-current relation for the peak value, and the solid squares display that of the open potassium channel. Voltage at the maximum slope is +40 mV in

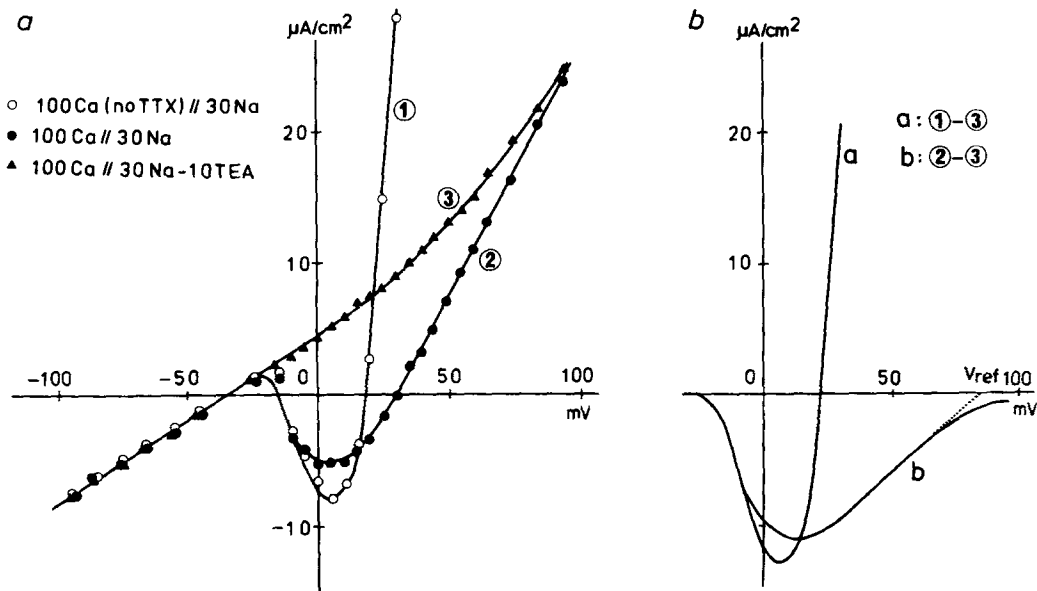


FIGURE 2. (a) Voltage-current relations obtained from an axon under the three conditions given in the figure. The curves give the maximum current, which could be either a peak or a steady current. Curve ③ is the leakage component. (b) Voltage-current relations after subtraction of leakage current. Curve *b* displays the voltage-current relation of the potassium channel. For further details, see the text.

this case. The data indicate that the open potassium channel voltage-current relation is linear in a voltage range between  $-30$  and  $+40$  mV and that the slope is almost coincident with the maximum slope of the original voltage-current curve.

For convenience,  $V_{\text{ref}}$  was used for calculating chord conductances, since the reversal potential could not be determined from the voltage-current curve. According to the results shown in Fig. 3, the chord conductances relative to their maximum slope conductance correspond to the fraction of potassium channels in the open state.

*Effect of a Change in the  $\text{Ca}^{2+}$  Concentration on the Voltage-Current Relation of the Potassium Channel*

Fig. 4 illustrates an example of voltage-current curves for the peak values, showing the effect of a change in the external  $\text{Ca}^{2+}$  concentration on the potassium channel. In this experiment, an axon internally perfused with 30 Na was initially immersed in 10 Ca. The bathing solution was then replaced with 100 Ca. The curves represented by the half-solid squares and by the open squares were obtained before and after the elevation of the  $\text{Ca}^{2+}$  level,

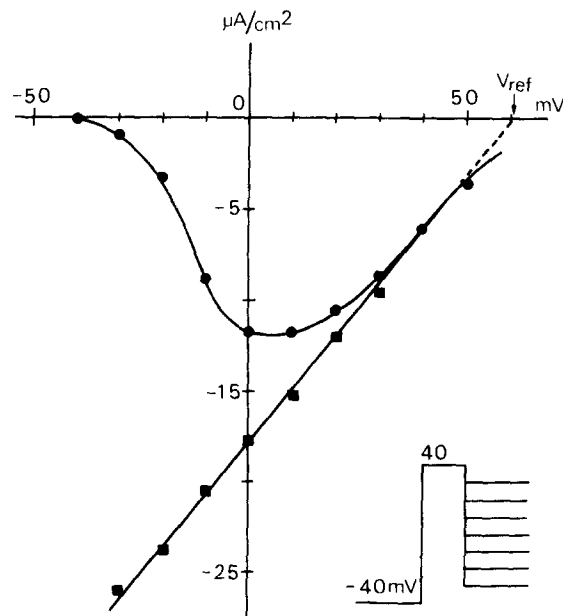


FIGURE 3. Voltage-current relations for the peak values (●) and for the open potassium channel (■) obtained from an axon with 100 Ca/30 Na. The holding potential was  $-40$  mV, and the voltage at the maximum slope was  $+40$  mV. For further details, see the text.

respectively. Although 10-fold elevation of the  $\text{Ca}^{2+}$  concentration shifted  $V_{ref}$  by 23 mV in the positive direction, there was no significant change in the maximum slope conductance. This means that the  $\text{Ca}^{2+}$  conductance of the potassium channel had reached its upper limit at a  $\text{Ca}^{2+}$  concentration below 10 mM; thus, the shift in the equilibrium potential of the potassium channel was not accompanied by a rise in the  $\text{Ca}^{2+}$  conductance.

*Effect of  $\text{K}^+$*

The addition of  $\text{K}^+$  to either the external or the internal solution, or to both solutions, greatly changed the voltage-current characteristics of the potassium channel. Fig. 5 exhibits typical examples of such K effects on the voltage-

current relations for the peak values observed in two experiments. In each experiment the holding potential was kept at a constant level ( $-40$  mV). The curves represented by the open symbols in Fig. 5 *a* and *b* display the control data obtained from two axons with 100 Ca//30 Na.

The curve represented by the half-solid triangles in Fig. 5 *a* was obtained after the bathing solution was changed to 100 Ca-30 K. Externally applied  $K^+$  increased values of the maximum peak current, the maximum slope

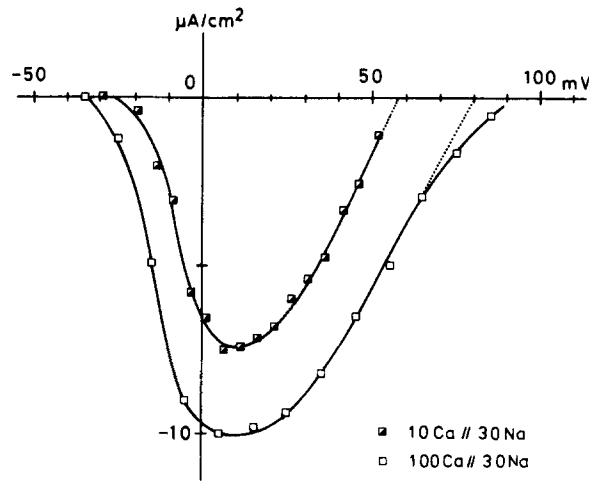


FIGURE 4. Effect of a change in the external  $Ca^{2+}$  concentration on the voltage-current relation for the peak value of the potassium channel.

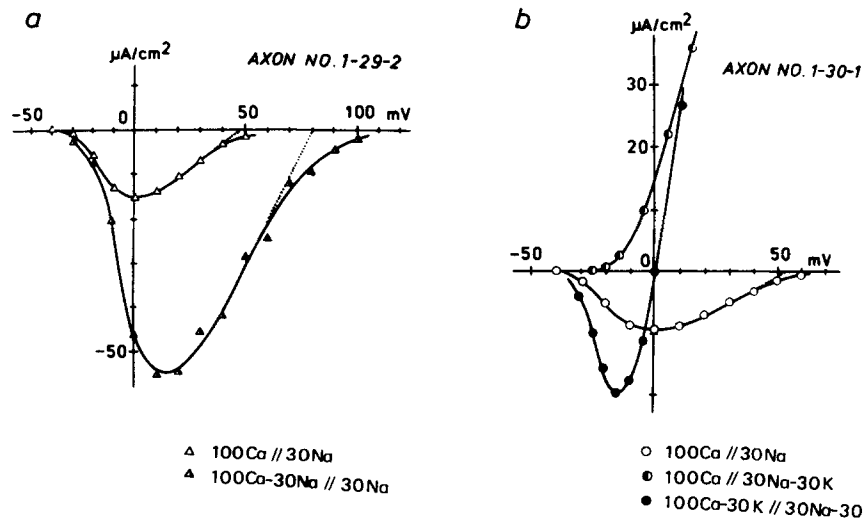


FIGURE 5. Effects of  $K^+$  on the voltage-current relation of the potassium channel for the peak value. For further details see the text.

conductance, and  $V_{ref}$ . However, outward current through the potassium channels was still undetectable.

Inward current immediately disappeared when  $K^+$  at the same concentration was applied intracellularly. As shown by the half-solid circles in Fig. 5 *b*, step depolarization produced only outward current instead of inward current, which shows a large amount of rectification that is similar to so-called delayed rectification.

As shown by the solid circles in Fig. 5 *b*, the reversal potential of the potassium channel became almost zero when the same concentration of  $K^+$  (30 mM) was applied to the solutions on both sides of the axon membrane. Under these conditions, step depolarization between the holding potential and the reversal potential demonstrated inward current through the potassium channel, and step polarization above the reversal potential brought about outward current. The slope conductance at the zero-current intercept obtained from the curve is 2.80 mmho/cm<sup>2</sup>, which is ~15 times as large as that of the maximum slope conductance of the control.

These  $K^+$  effects are interpreted as indicating that the  $K^+$  permeability of the potassium channel is much greater than the  $Ca^{2+}$  permeability or the  $Na^+$  permeability of the potassium channel, and, consequently, that  $K^+$  plays a leading role in generating the equilibrium potential and the conductance of the potassium channel as long as  $K^+$  is present in the internal and/or external fluid medium. In fact as given in Table II, the maximum  $K^+$  conductance

TABLE II  
MAXIMUM IONIC CONDUCTANCES OF POTASSIUM CHANNEL

Solution		<i>n</i>	<i>g</i> <sub>max</sub> (mean ± SD)
Internal	External		
30 Na	100 Ca	14	0.25±0.07
450 K	ASW	5	22.9±0.5

obtained with artificial seawater (ASW)//450 K is approximately 100 times as large as the maximum  $Ca^{2+}$  conductance of the potassium channel.

It is also noted in Fig. 5 *b* that the slope conductance is larger when  $K^+$  is on both sides. The data support the model proposed by Hille and Schwarz (1978) that a potassium channel is a multi-ion, single-file pore.

#### *Time-Courses of Currents through Potassium Channels*

Fig. 6 *a* and *b* shows current records obtained in the two experiments of Fig. 5 *a* and Fig. 5 *b*, respectively. Leakage current has been subtracted; thus, the records demonstrate fairly well the time-courses of currents through potassium channels. The salt conditions for each set of records are given on the top and the voltage of step depolarization on the right of each record. The duration of the depolarization was 100 ms.



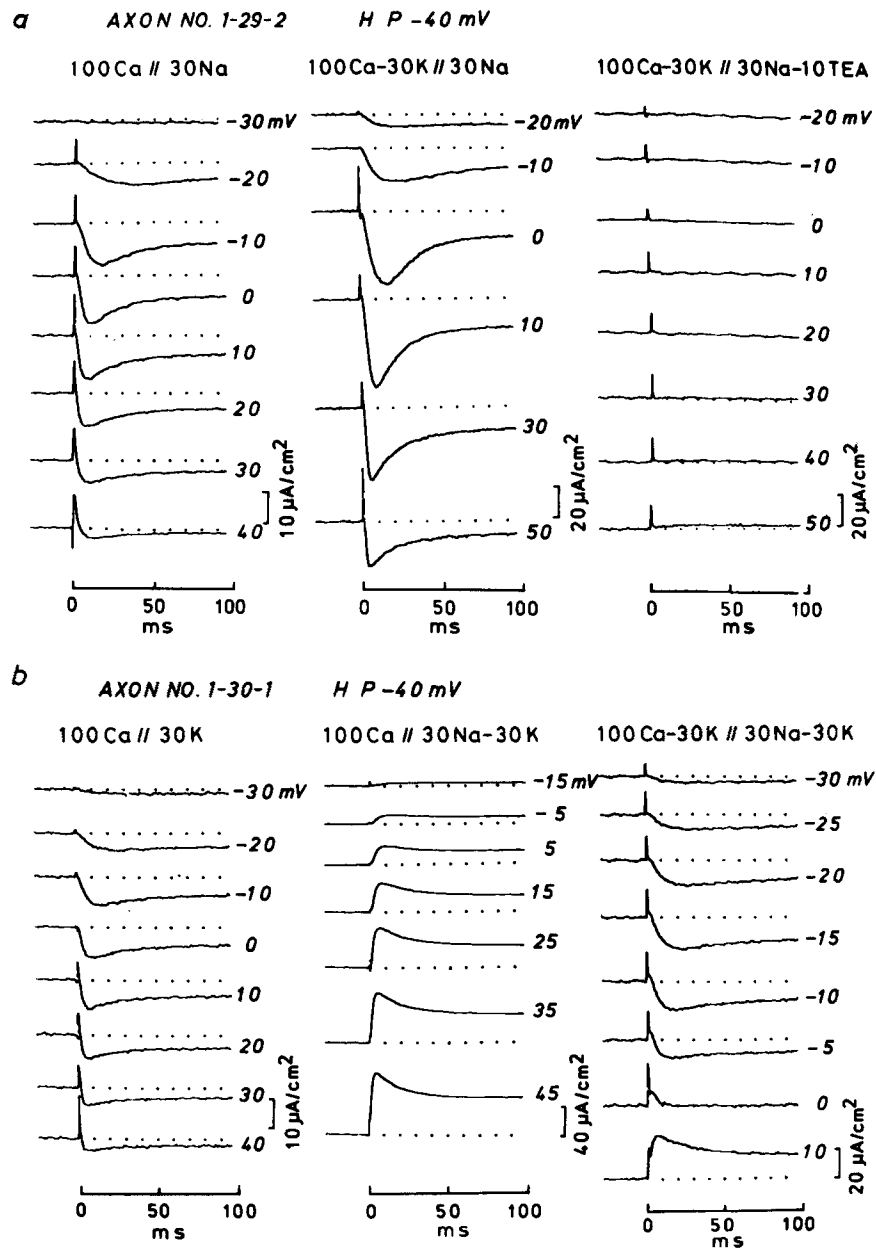


FIGURE 6. Currents through potassium channels obtained in the two experiments of Fig. 5. *HP*, holding potentials.

The two sets of records on the left side of Fig. 6 *a* and *b* were taken from the respective axons under the condition of 100 Ca//30 Na. These demonstrate  $I_{Ca}$  through potassium channels. A clear inactivation of  $I_{Ca}$  subsequent to activation is recognized in all the records. Both the activation and the

inactivation are related to voltage. This voltage-dependent characteristic of  $I_{Ca}$  is somewhat similar to that of  $I_{Na}$  through sodium channels, although there is a great difference in the time-course of the two currents; the rate of activation of  $I_{Ca}$  is close to that of  $I_K$  observed under physiological conditions.

Fig. 6 *a* (middle) and *b* (middle and right) show the effects of  $K^+$ . As described above, the direction and the intensity of current through potassium channels strongly depend on  $K^+$  applied to either side or both sides of the axon membrane. Nevertheless, an inactivation after activation is seen in all the current records. Furthermore, it seems that there is a correlation among the time-courses of the currents at corresponding voltages.

The records on the right of Fig. 6 *a* demonstrate that current through potassium channels is completely suppressed by 10 mM TEA added to the internal perfusion fluid. This TEA effect is stronger than that observed in TEA-injected squid axons (Armstrong and Binstock, 1965). A possible reason for such as intensification of the TEA effect has been discussed (Inoue, 1980).

#### *Inactivation of Potassium Channels*

To compare the time-courses of currents through potassium channels at each voltage under different salt conditions, current amplitudes after step depolarization were divided by their peak value. Fig. 7 shows an example of such corrected values,  $I(t)/I_P$ , plotted against time after the depolarization, obtained from the data shown in Fig. 6 *b*.  $I(t)/I_P$  has a similar time-course at each voltage, irrespective of the direction and intensity of the currents. A fast decay of outward  $I_K$  in squid axons similar to that represented by the half-solid circles has been explained in terms of a consecutive change in the equilibrium potential across the potassium channel due to an accumulation of  $K^+$  in a periaxonal space (cf. Frankenhaeuser and Hodgkin [1957] and Adelman et al. [1973]). However, the present results strongly suggest that the major portion of such a decay of outward  $I_K$  is attributable to the same event that creates the inactivation of inward currents through potassium channels. Since there is no structure like a periaxonal space at the inner membrane surface under internal perfusion (Takenaka et al., 1968), it is possible that the innactivation of currents through potassium channels is a result of an essential dynamic process of the potassium channels themselves. Inactivation of potassium channels has been reported by Adrian et al. (1970) for muscle fibers and by Schwarz and Vogel (1971) for nodes of Ranvier.

Fig. 8 *a* shows currents through potassium channels obtained under the condition of 100 Ca-60 K//30 Na-10 K. Since both the external and internal solutions contained  $K^+$ , step depolarization between the holding potential (-60 mV) and the reversal potential (+34 mV, in this case) produced inward current through potassium channels, and that above the reversal potential created outward current. Both the inward and outward currents inactivated in a manner similar to that observed in the experiments of Fig. 6. Because the  $K^+$  concentration of the external solution is six times higher than that of the internal solution, it is unlikely that the inactivation of the outward current can be attributed to a  $K^+$  accumulation in the periaxonal space.

Similar inward and outward currents through potassium channels were demonstrated in axons bathed in ASW. Since ASW contained 9 mM  $K^+$ , a lowering of the internal  $K^+$  concentration to below 9 mM brought the reversal potential of the potassium channel to above 0 mV. Thus, step depolarization from a holding potential of  $-40$  to  $-60$  mV to a level below the reversal

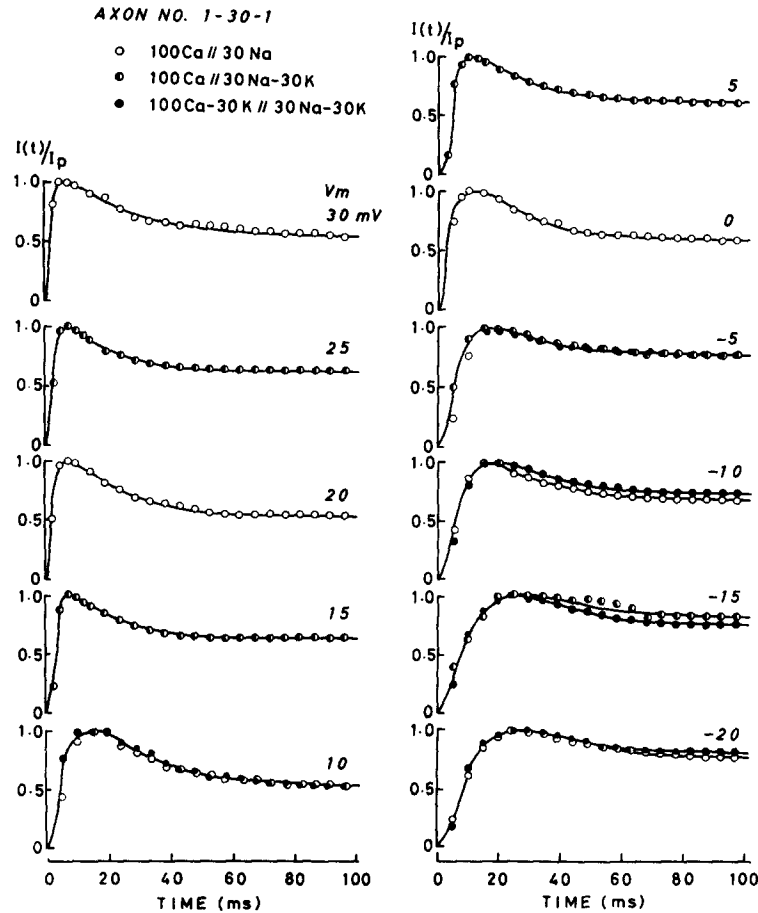


FIGURE 7. Time-courses of corrected currents,  $I(t)/I_P$ , at various voltages of step depolarization for the data shown in Fig. 6 *b*.  $I_P$  is the peak value of each record. Different symbols represent the data obtained under the different salt conditions given in the Figure.

potential produced inward current, and that above the reversal potential caused outward current. Fig. 8 *b* shows an example of such currents. The characteristics of the currents are similar to these shown in the previous figures.

*Time- and Voltage-dependent Characteristics of Potassium Channels*

If the ion-conducting system of potassium channels mainly depends on time and voltage, the fraction of potassium channels in the open state should, in principle, be a function of time and voltage. As has been described above, the chord conductance normalized to its maximum value is a parameter that represents the fraction of open potassium channels. Fig. 9 *a* illustrates the

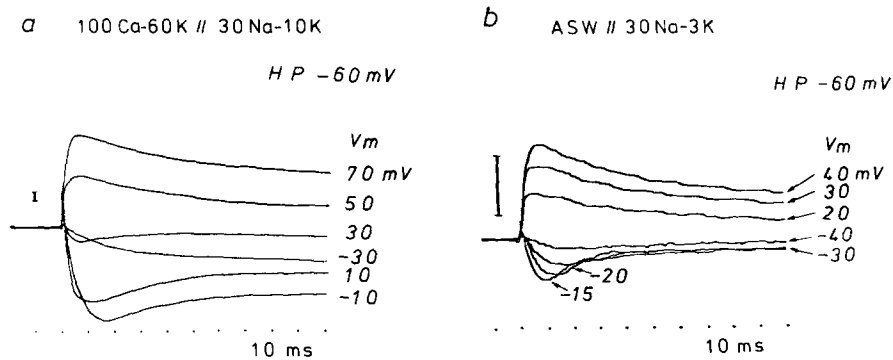


FIGURE 8. Traces showing currents through potassium channels obtained from an axon with 100 Ca-60 K//30 Na-10 K (*a*) and from another axon with ASW//30 Na-3 K (*b*). Voltages of the holding potential (*HP*) and step depolarization are given. The vertical bars indicate 10  $\mu\text{A}/\text{cm}^2$ .

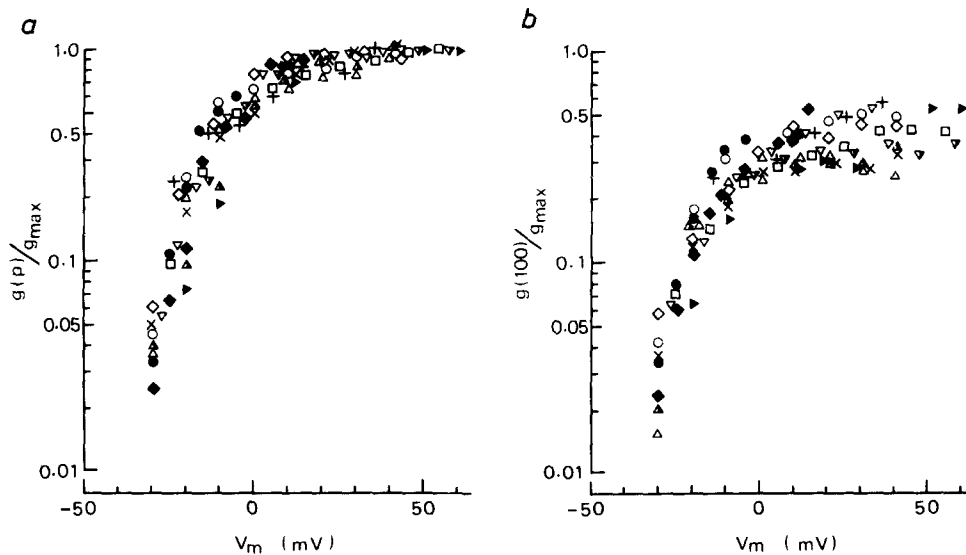


FIGURE 9. (*a*) Relation between voltage and the normalized peak conductances,  $g(p)/g_{\text{max}}$  obtained under the various salt conditions given in Table III. (*b*) Relation between the normalized conductances at 100 ms after step depolarization,  $g(100)/g_{\text{max}}$ . For further details, see the text.

relation between voltage and the normalized peak conductance obtained under the various salt conditions given in Table III. For outward current through potassium channels, the chord conductance at the peak at  $\sim +50$  mV was taken as unity. The data indicate that the fraction of open potassium channels at the peak is, in principle, a function of voltage only, whereas the ionic compositions surrounding the axon membrane strongly affect the direction and the intensity of currents, the reversal potential or the reference voltage, or the maximum conductance. The normalized peak conductance is a steep function of voltage in the region below  $-10$  mV, where it changes  $e$ -fold for an elevation of 5.2 mV. On the other hand, the values approach unity at  $>+20$  mV. The shape and other characteristics of the curve are similar to

TABLE III  
EXPLANATION OF SYMBOLS AND OTHER INFORMATION FOR FIGS. 9 AND 11

Symbol	Axon No.	Solution		Holding potential <i>mV</i>	$V_{ref}$ <i>mV</i>	$g_{max}$ <i>mmho/cm<sup>2</sup></i>
		Internal	External			
$\triangle$	1-29-2	30 Na	100 Ca	-40	48	0.19
$\Delta$	1-29-2	30 Na	100 Ca-30 K	-40	79	1.00
$\nabla$	1-29-1	30 Na	100 Ca	-40	65	0.41
$\nabla$	1-29-1	30 Na	100 Ca-30 K	-37	89	0.89
$\square$	1-29-3	30 Na	100 Ca	-35	81	0.21
$\circ$	1-30-1	30 Na	100 Ca	-40	54	0.37
$\odot$	1-30-1	30 Na-30 K	100 Ca	-40	—	—
$\bullet$	1-30-1	30 Na-30 K	100 Ca-30 K	-40	0*	3.75
$\diamond$	1-25-1	30 Na	100 Ca	-40	53	0.22
$\blacklozenge$	1-25-1	30 Na-30 K	100 Ca-30 K	-40	-2*	3.58
$\blacktriangle$	1-27-2	30 Na-30 K	100 Ca	-40	—	—
+	1-24-2	30 Na-30 K	ASW	-35	-30‡	1.20
$\blacktriangleright$	11-23-1	30 Na-3 K	100 Ca-30 K	-40	34*	2.48
$\times$	11-23-2	30 Na-3 K	ASW	-40	8*	0.64

\* Reversal potential.

‡ Equilibrium potential for  $K^+$ .

—, not determined.

those for the potassium conductance of intact squid axons obtained by Hodgkin and Huxley (1952). (The peak conductance was to a certain extent influenced by prepotential. Since the holding potential in most of the present experiments was  $-40$  mV, this brought about a small difference in position between the curve in Fig. 9 *a* and that of Hodgkin and Huxley in the negative voltage range.)

A similar curve was obtained for the normalized conductances at 100 ms after depolarization. In this case, steady values of the normalized conductances do not reach unity, but approach a level between 0.3 and 0.6, showing a large amount of scatter. It is thought that the steady level of  $<1$  is due to the inactivation of potassium channels. The large amount of scatter of the data

results from irregularity in the inactivation process among experiments. As can be seen from the data, alteration of the salt composition of the solutions does not seem to be the main source of the scatter. In fact, the inactivation of currents through potassium channels was rather strongly influenced by the conditions of internal perfusion, e.g., a period of internal perfusion, fluctuation of temperature, etc.

*Time Constant of Fast Inactivation of Potassium Channels*

As shown by the data in Figs. 6–8, the current through potassium channels almost reaches its steady level at 100 ms after depolarization. The relatively fast inactivation may correspond to the process underlying the initial fast decay in potential of the potassium-channel spike shown in Fig. 1. To study the time-dependent characteristics of fast inactivation, a value of current at 100 ms after step depolarization,  $I(100)$ , was subtracted from the original current value. Fig. 10 exhibits an example of such corrected values obtained from the data in Fig. 7. Since the ordinates are plotted on a logarithmic scale, the data indicate that the initial fast process of inactivation of potassium

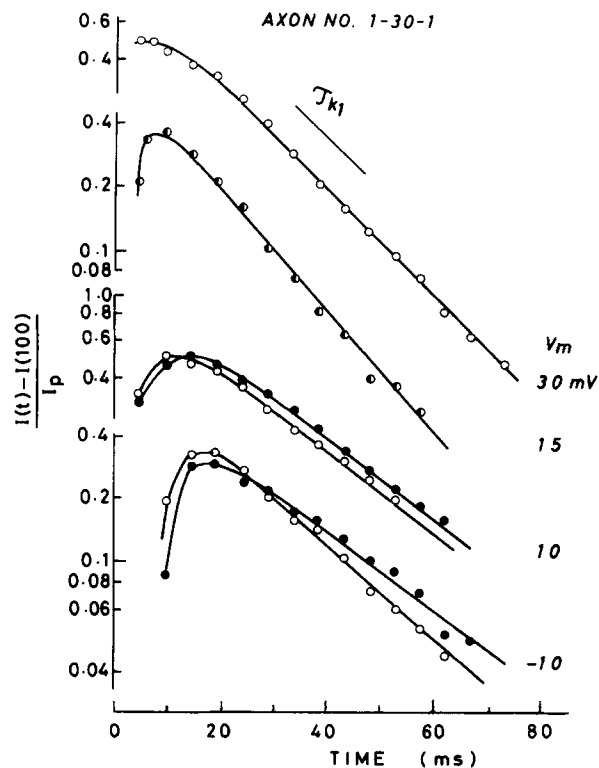


FIGURE 10. Time-courses of values of  $\log \{ [I(t) - I(100)] / I_p \}$ , showing that the fast inactivation of the potassium channel is an exponential function of voltage. The values were calculated from the data shown in Fig. 7.

channels is an exponential function of time. The time constants of these data,  $\tau_{K_1}$ , lie between 16 and 22 ms in this case.

Fig. 11 shows the relation between voltage and  $\tau_{K_1}$  obtained with a variety of salt compositions, as given in Table III.  $\tau_{K_1}$  is almost a constant value (20–30 ms), at least in the voltage region between  $-15$  and  $+40$  mV. The scatter of the data seems to arise mainly from the conditions of internal perfusion rather than from the alteration of the salt compositions of the solutions.

#### *A Slow Inactivation of Potassium Channels*

An inactivation of potassium permeability having a very large time-constant (on the order of 10 s) has been observed for intact squid axons (Ehrenstein and Gilbert, 1966), for nodes of Ranvier (cf. Lüttgau [1960] and Schwarz and Vogel [1971]), for muscle fibers (Adrian et al., 1970), and for other excitable tissues (cf. Reuben and Gainer [1962], Hall et al. [1963], and Nakamura et al.

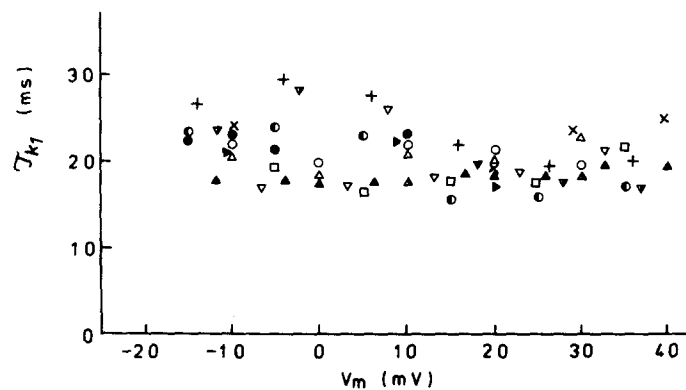


FIGURE 11. Relation between voltage and time-constants of the fast inactivation of the potassium channel under the various salt conditions given in Table III.

[1965]). The existence of a very slow inactivation of potassium channels of the squid axon membrane has also been postulated from the characteristics of the potassium-channel spike. Slowly inactivating  $I_{Ca}$  through potassium channels was in fact recorded in the present experiments.

The records at the top of Fig. 12 demonstrate slowly inactivating  $I_{Ca}$  obtained from an axon with 100 Ca//30 Na. The duration of step depolarization from the holding potential of  $-45$  mV was 9 s. Because of the difficulty of reproducibility, only three records were successfully taken from the axon. As can be seen from the diagram in the figure, the chord conductances, which are normalized to the maximum slope conductance, decrease exponentially with time. The time constants of these slow conductance changes are 5.3 s at  $+5$  mV, 5.1 s at  $-5$  mV, and 7.4 s at  $-15$  mV. These values are in agreement not only with that obtained by Ehrenstein and Gilbert (1966), but also with

that expected from the potential decay of the potassium-channel spike in Fig. 1.

#### DISCUSSION

It has been shown that the dynamic processes of the potassium channel of the squid axon membrane depend mainly on voltage, at least with the salt compositions examined, while currents through potassium channels are strongly influenced by the composition of the solutions. Development of a

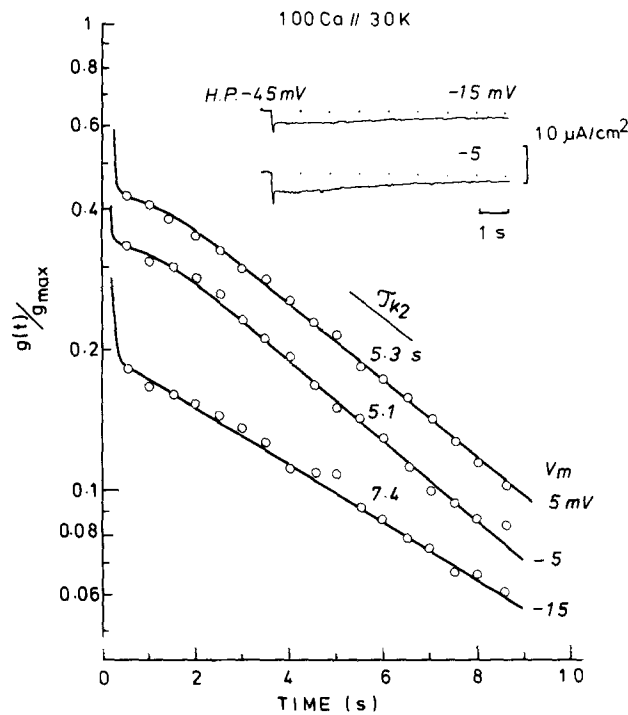


FIGURE 12. Records demonstrating slowly inactivating  $I_{K_1}$  through potassium channels obtained from an axon with 100 Ca//30 Na. (*Bottom*) Time-courses of the logarithm of the normalized conductances, showing that the slow inactivation of the potassium channel is an exponential function of time. *HP*, holding potential.

potassium-channel spike, thus, can be understood as one of those cases in which the equilibrium potential of the potassium channel becomes positive.

The processes of the potassium channel may be explained in terms of the following equation, which is in the same form as that proposed by Ehrenstein and Gilbert (1966), considering a slow inactivation

$$g_K = \bar{g}_K n^4 k [k = k(k_1, k_2, \dots)].$$

Here,  $g_K$  and  $\bar{g}_K$  are the ionic conductance of the potassium channel and its



maximum value, respectively. The parameter  $k$  represents the inactivation of potassium channels, which is composed of at least two components,  $k_1$  and  $k_2$ . One component represents the initial fast inactivation, having a time constant of 20–30 ms, and the other shows the slow inactivation, having a time constant of 5–10 s. The slow inactivation corresponds to the one characterized by Ehrenstein and Gilbert (1966) for a slow change in  $I_K$  in intact squid axons. The fast component, however, has not yet been well discussed; but a similar fast inactivation can be recognized in the records of  $I_K$  presented in published papers (cf. Yeh et al. [1976] and Meves and Pichon [1977]).

One of the reasons that the fast inactivation in squid axons has not been well documented may be that the time constant of the fast inactivation is close to that of a change in the equilibrium potential of the potassium channel due to an accumulation of  $K^+$  in the periaxonal space (cf. Frankenhaeuser and Hodgkin [1957] and Adelman et al. [1973]). Because of the similarity of the time constants of the two processes and because of the difficulty of separating the two processes in intact axons, the effect of  $K^+$  accumulation on the decay of  $I_K$  may have been overestimated. In the present experiments, which were performed on internally perfused squid axons, the  $K^+$  accumulation effect was ruled out by the examination of inward currents through potassium channels. The experiments have revealed that there is a fast inactivation of potassium channels.

Schwarz and Vogel (1971) observed two inactivation processes for  $I_K$  in myelinated nerve fibers. According to their observations, the time constants of the two processes are 0.5 s for the fast component and 3.6–20 s for the slow one. The slow component may correspond to that of the squid axon membrane. But the fast component is obviously different from the fast process that I have seen in this work, because of the great difference in time constant between the two processes. Since the present experiments were performed with either short (50–200 ms) or very long (5–10 s) voltage pulses, it is not certain whether such an intermediate processes, having a time constant of 0.1–1 s, is involved in the inactivation of potassium channels of the squid axon membrane.

#### *Role of the Leakage Component*

To complete a potassium-channel spike, a factor besides the activation-inactivation of potassium channels is necessary. This factor plays an indispensable role not only in keeping the resting potential at a negative level but also in forcing the potassium-channel spike back to the resting potential. This factor corresponds to what I have referred to as the leakage component. Although the leakage component has not yet been identified, it may be separate from the potassium channel. The reason is as follows. From the results shown in Fig. 9 *a*, it can be seen that most of the potassium channels open at a voltage near the peak of a potassium-channel spike ( $\sim +30$  mV). The calcium conductance of the potassium channel at the spike peak is 0.19 mmho/cm<sup>2</sup> according to the data of Fig. 2 *b*. On the other hand, the fraction of open potassium channels decreases to a level below 1% at a voltage near the resting

potential ( $\sim -35$  mV). The calcium conductance of the potassium channel of the resting membrane is, thus, as low as  $0.0019$  mmho/cm<sup>2</sup>. This value is approximately 1/70 of the resting membrane conductance. Therefore, it is unlikely that the potassium channel contributes significantly to the conductance and the potential of the resting membrane. Further study to identify the leakage component is required to lead us to a deeper understanding of the generation mechanisms of the membrane potential of excitable tissues as well as that of nonexcitable tissues.

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