The Influence of External Potassium on the Inactivation of Sodium Currents in the Giant Axon of the Squid, *Loligo pealei*

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ABSTRACT Isolated giant axons were voltage-clamped in seawater solutions having constant sodium concentrations of 230 mM and variable potassium concentrations of from zero to 210 mM. The inactivation of the initial transient membrane current normally carried by Na^+ was studied by measuring the Hodgkin-Huxley h parameter as a function of time. It was found that h reaches a steady-state value within 30 msec in all solutions. The values of h_∞, τ_h, α_h, and β_h as functions of membrane potential were determined for various $[K_o]$. The steady-state values of the h parameter were found to be inversely related, while the time constant, τ_h, was directly related to external K^+ concentration. While the absolute magnitude as well as the slopes of the h_∞ vs. membrane potential curves were altered by varying external K^+, only the magnitude and not the shape of the corresponding τ_h curves was altered. Values of the two rate constants, α_h and β_h, were calculated from h_∞ and τ_h values. α_h is inversely related to $[K_o]$ while β_h is directly related to $[K_o]$ for hyperpolarizing membrane potentials and is independent of $[K_o]$ for depolarizing membrane potentials. Hodgkin-Huxley equations relating α_h and β_h to E_m were rewritten so as to account for the observed effects of $[K_o]$. It is concluded that external potassium ions have an inactivating effect on the initial transient membrane conductance which cannot be explained solely on the basis of potassium membrane depolarization.

INTRODUCTION

Hodgkin and Huxley (1952) described in quantitative detail the time and voltage dependencies of the inactivation of the initial transient conductance which normally enables sodium ions to rapidly permeate the voltage-clamped membrane of the giant axon of the squid. Inactivation was represented by a

parameter, (1-h), determined by two voltage-dependent rate constants, α_h and β_h. The h process was shown to have a voltage-dependent time constant, τ_h, which varied from less than 1.0 to almost 10.0 msec with the membrane potential, E_m. The curve of τ_h vs. E_m has a peak close to the resting potential, E_{RP}.

The steady-state values, h_∞, of the slow facilitator factor, h, were shown to approach zero when the membrane was depolarized 30 mv and to approach 1 when the membrane was hyperpolarized 30 mv from the resting potential. When first formulated, α_h and β_h were assumed to be only voltage-dependent. However, Frankenhaeuser and Hodgkin (1957) showed that the absolute magnitude of h_∞ and the position of the h_∞ vs. E_m curve relative to voltage were functions of the external [Ca^{++}]. In this respect, it was considered that a fivefold increase in [Ca_o^{++}] was the equivalent of a hyperpolarization of the membrane of from 10 to 15 mv. The calcium ion effect has been discussed in detail by Huxley (1959) and has been extended to the lobster axon by Blaustein and Goldman (1966, 1968).

Internal perfusion experiments on the squid giant axon have revealed a dependency of the inactivation mechanism on the internal [K^+] (Narahashi, 1963; Baker, Hodgkin, and Meves, 1964; Adelman, Dyro, and Senft, 1965 *a*) and on external [K^+] (Adelman, et al., 1965 *b*). In intact axons, Adelman and Senft (1968) have shown that the amplitude of the sodium current is inversely related to [K_o] even when 3 sec hyperpolarizing conditioning pulses precede test depolarizations.

Various suggestions have been made as to the possibility of sodium and potassium ion-ion interactions in the early transient membrane currents produced in the voltage clamp (Mullins, 1960; Goldman, 1964; Adelman and Senft, 1968). Therefore, it was the purpose of this investigation to examine the effects of external potassium ions on the sodium ion inactivation system with respect to both voltage and time dependencies. In this respect, this work is concerned with inactivation phenomena as described by Hodgkin and Huxley (1952 *b*). These phenomena are in the millisecond time domain and are related to those processes which contribute to the normal action potential.

METHODS

Experiments were performed upon single giant axons obtained from *Loligo pealei* during the summer of 1968 at the Marine Biological Laboratory, Woods Hole, Massachusetts. The methods of preparation have been described previously (Adelman and Senft, 1968). Fig. 1 illustrates the axon cell and the voltage clamp system.

The experiments involved the measurement of membrane currents in the voltage clamp as a function of various concentrations of external potassium ion. In order to keep the external sodium concentration constant and vary potassium it was necessary to use external artificial seawater solutions in which the concentration of sodium was

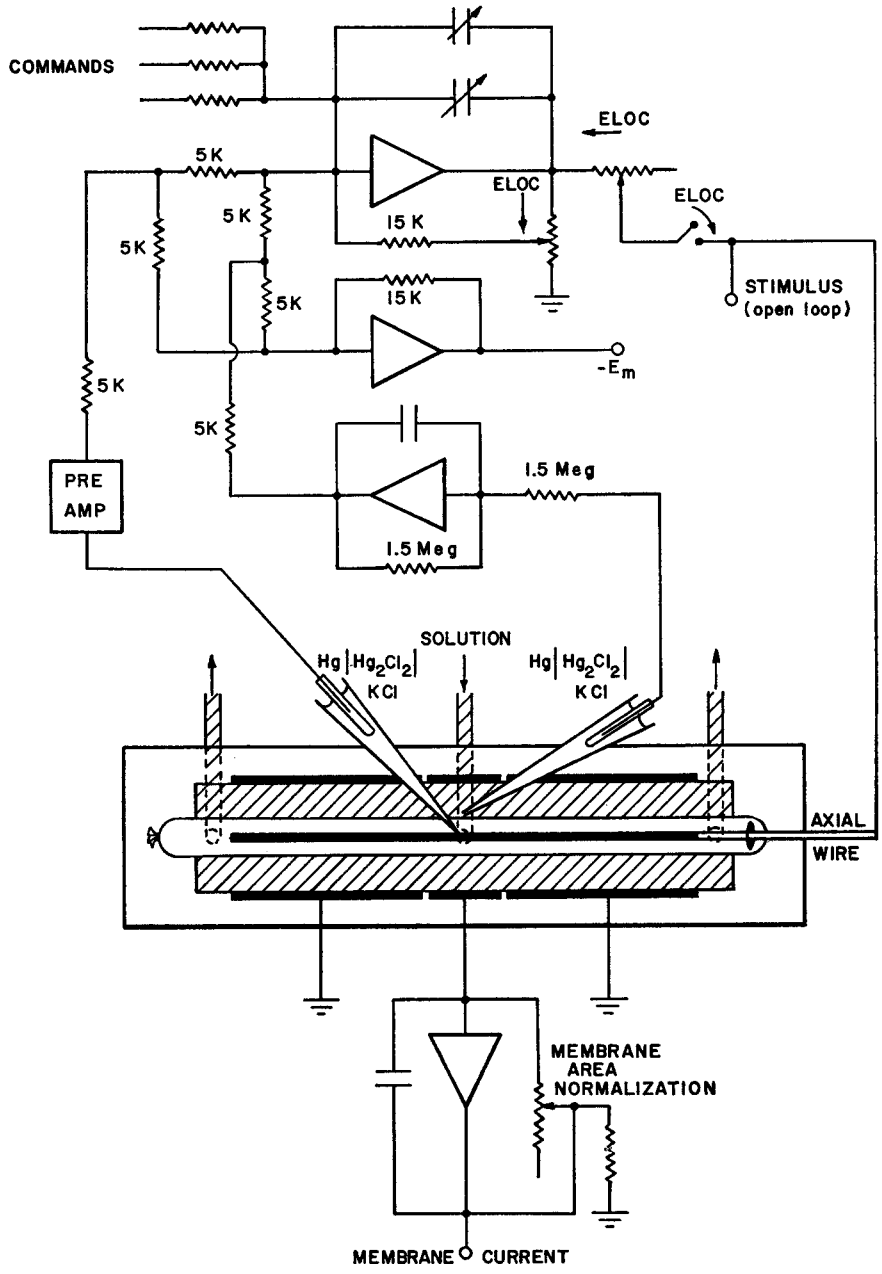

FIGURE 1. Schematic diagram of the point-control voltage clamp measurement and control circuit illustrating the relation between the electronic system and the axon. Triangles indicate operational amplifiers, ELOC and arrows indicate the closing of the feedback loop to achieve potential control over the axon membrane, and the blackened areas of the cell electrodes indicate platinized surfaces. Salt bridges are 3 M KCl; the external salt bridge contains 1% agar. $-E_m$ is the inverted value of the membrane potential.

reduced from 430 mM to 230 mM. Tris chloride was added to the solutions to maintain the osmolarity approximately equal to that of the 430 mM sodium seawater. External potassium ion substitutions were made for given fractions of the $Tris^+$ concentration. Table I lists those solutions that were used in the experiments. As experiments using all $Tris^+$ SW solutions failed to show any indication of inward initial transient currents in the voltage clamp, it was assumed that $Tris^+$ does not contribute to the early transient current.

The Tris Cl used in these experiments was prepared by titrating Tris base (Trisma) with HCl to a pH of 7.4 at 3.5°C ± 0.5°C, the temperature at which the experiments were carried out. At this pH and temperature the osmolarity of 0.1 M Tris Cl was about 95% of the osmolarity of an equivalent concentration of NaCl as estimated from freezing point depression measurements assuming that the osmolarity does not

TABLE I

ARTIFICIAL SEAWATER SOLUTIONS*

	$[Na^+]$	$[K^+]$	$[Tris^+]$
	mM	mM	mM
K-free, 230 Na ASW	230	0	210
5 mM K, 230 Na ASW	230	5	205
10 mM K, 230 Na ASW	230	10	200
25 mM K, 230 Na ASW	230	25	185
50 mM K, 230 Na ASW	230	50	160
100 mM K, 230 Na ASW	230	100	110
210 mM K, 230 Na ASW	230	210	0.5

* All ASW solutions have $[Ca^{++}]$ = 10 mM, $[Mg^{++}]$ = 50 mM, $[Cl^-]$ = 560 mM, and pH = 7.4.

change significantly upon elevating the temperature from the freezing point to 3.5°C. The specific conductivity of a 0.1 M Tris Cl solution was found to be 25% lower than that of an equivalent NaCl solution. This measurement indicates that the conductivity of 200 mM $Tris^+$, 230 mM Na^+ ASW solution was within 10% of that of 430 mM Na^+ ASW.

RESULTS

Fig. 2 illustrates the relationship between the peak amplitude, I_p, of the initial transient current (initiated by a test pulse) and the duration, t_{pp}, and amplitude, E_{pp}, of a conditioning prepulse as a function of the external potassium ion concentration, $[K_o]$. The procedure adopted for measurement of I_p and for analysis of these results was similar to that used by Hodgkin and Huxley (1952 *b*, compare Fig. 3). The values of I_p as in the Hodgkin and Huxley method (1952 *b*) were determined after correction for any current flowing during the prepulse and for the leakage current associated with the test pulse. All potentials were taken internally with respect to an external reference ground. Fig. 2 A is a plot of I_p as a function t_{pp} obtained upon step-

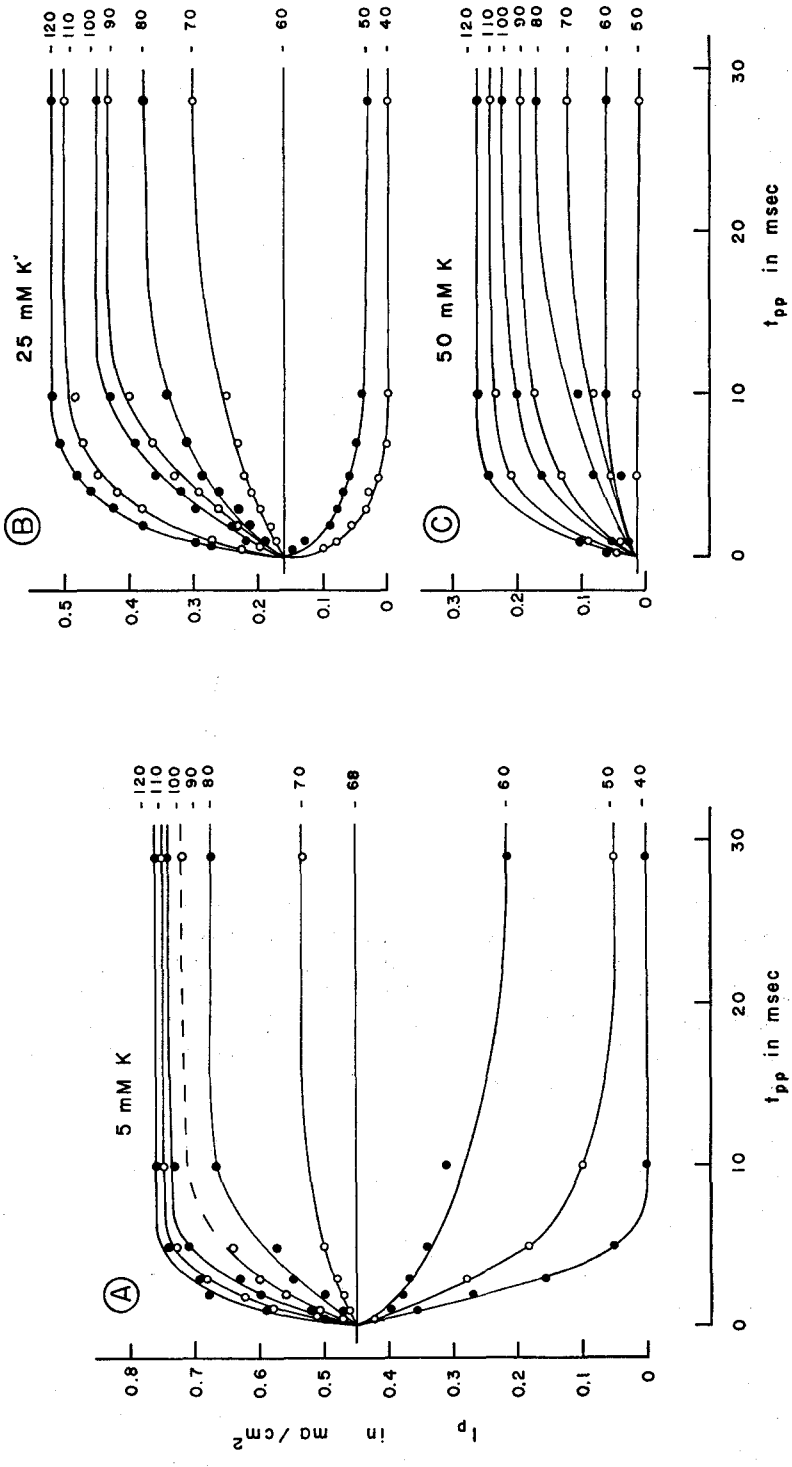

FIGURE 2. Time course of the variation in the peak value of the initial transient membrane current, I_p, as a function of the duration, t_{pp}, of a conditioning prepulse potential step. The values of the prepulse step potential are given to the right of each curve. I_p was always measured upon stepping from the prepulse potential to the test potential of zero millivolts. Axon 68-50. A, axon in 5 mM K, 230 mM Na ASW, $E_{HP} = -68$ mv. B, axon in 25 mM K, 230 mM Na ASW, $E_{HP} = -60$ mv. C, axon in 50 mM K, 230 mM Na ASW, $E_{HP} = -44$ mv. E_{HP} is the value of the holding potential.

ping the membrane potential to zero from various values of E_{pp} when the axon was exposed to 230 mM Na^+, 5 mM K^+ ASW. Figs. 2 B and 2 C are similar plots for exposure to 230 mM Na^+, 25 mM K^+ ASW, and 230 mM Na^+, 50 mM K^+ ASW, respectively. Notice that the development or removal of inactivation in time is approximately exponential, and that the curves in Fig. 2

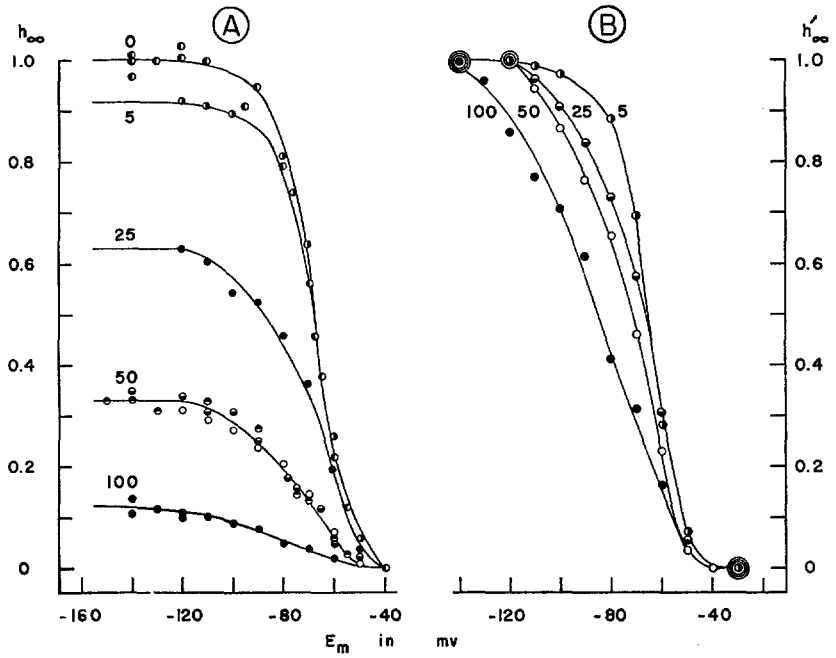

FIGURE 3. Steady-state relations between h and E_m as a function of $[K_o]$. Numbers on each curve refer to the $[K_o]$ in 230 mM Na ASW. Zero mM [K]: axons 68-17 and 68-33, 5 mM K: axon 68-50, 25 mM K: axon 68-50, 50 mM K: axons 68-50, 68-17, 68-34, 100 mM K: axon 68-34. (A) h_∞ = steady-state values of h normalized with respect to the maximum value of h obtained in K-free, 230 Na ASW. (B) h'_∞ = steady-state values of h normalized for each solution with respect to the maximum value of h obtained in the given solution. Curve marked 5 also fits points for zero mM $[K_o]$.

tend toward a steady state at conditioning prepulse durations of up to 28 msec. The slopes of the I_p vs. t_{pp} curves were found to be approximately zero over the range from t_{pp} = 20 msec to t_{pp} = 100 msec for E_{pp} values from -140 to -40 mv in three axons exposed to a variety of $[K_o]$.

Within the Hodgkin-Huxley (1952 *c*) framework, $I_{Na} = G_{Na}\,\Delta E$. G_{Na}, which has the dimensions of conductance, is composed of a constant, $\bar{g}_{Na}$, and two voltage- and time-dependent parameters, m and h. It will be shown that the effect of $[K_o]$ on I_p is both voltage- (Fig. 3 B) and time-dependent (Fig. 4). Therefore, it seems logical to assign the change in I_p induced by $[K_o]$ to the voltage- and time-dependent parameters rather than to $\bar{g}_{Na}$. Because we

have no evidence for any effect of $[K_o]$ on the rising phase of the initial transient current we shall confine our analysis of the $[K_o]$ effect to the h parameter.

Obviously, it is also possible to assume that $[K_o]$ affects both the h and $\bar{g}_{Na}$ parameters. In this case the voltage dependency of the rate constants, α_h and

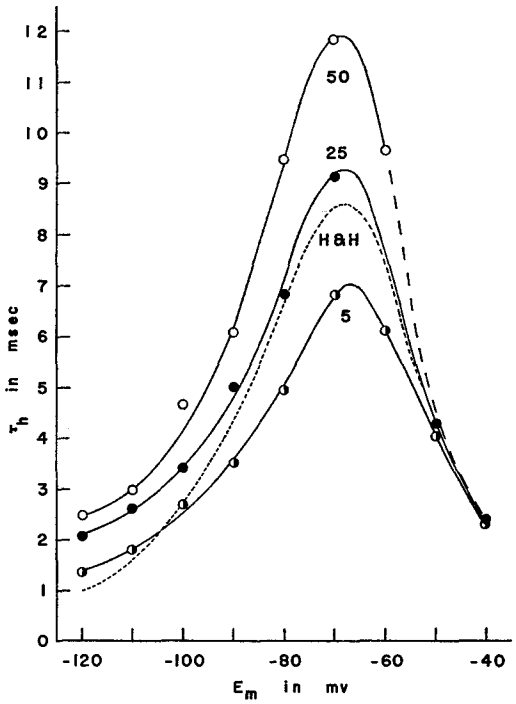

FIGURE 4. Values of the time constant of the h process, τ_h, plotted against E_m as a function of $[K_o]$. Experimental points obtained from the exponential portions of the curves shown in Fig. 2. Open circles, experimental points in 50 mM K, 230 mM Na ASW. Solid circles, experimental points in 25 mM K, 230 mM Na ASW. Half-filled circles, experimental points in 5 mM K, 230 mM Na ASW. Axon 68-50. Dashed line, normalized digital computer solutions of the Hodgkin-Huxley equations (1952 *c*) for ASW containing 10 mM K plotted to indicate only the shape of the H and H τ_h vs. E_m relation. The amplitude of the H and H curve for τ_h was arbitrarily scaled so as to fall between our τ_h curves for 25 and 5 mM $[K_o]$.

β_h, will be somewhat different from that presented here. Such an approach was adopted, for example, by Taylor (1959). Inasmuch as an effect on the h parameter will be shown to be sufficient to account for all the experimental results, and as we have no direct evidence for combined $\bar{g}_{Na}$ and h involvement, we choose to analyze the results with respect to the h parameter.

From Fig. 2 it is apparent that the peak values of the transient sodium current reach a steady state with increasing values of t_{pp}. These plateau

values are inversely related to the external potassium concentration, even though the sodium concentration remains constant in each case. The ratio of these values of I_p for any E_{pp} to the maximal value of I_p obtained with $E_{pp_{max}}$ and zero $[K_o]$ will be defined as h_∞. This definition of h_∞ follows the logic behind the original Hodgkin-Huxley (1952 *b*, p. 501) definition, namely that "h is the fraction of the sodium-carrying system which is not inactivated, and is therefore rapidly available for carrying sodium ions when the membrane is depolarized." Thus, for any one axon the value of $h_{\infty_{max}}$ is a constant irrespective of the membrane potential, composition of the media, etc. Typical plots of h_∞ vs. membrane potential for various $[K_o]$ are shown in Fig. 3 A.[1] Note that for each external potassium concentration a plateau value of h_∞ is obtained with hyperpolarizing prepulses, $h_{\infty plt}$. Each curve has a general shape typified by the K-free curve, but is depressed with increasing $[K_o]$ so that $h_{\infty plt}$ is inversely related to $[K_o]$. This relation is described by stating that $h_{\infty plt}$ is a linear function of the log $[K_o]$.

The ratio of the steady-state values of I_p for any E_{pp} and $[K_o]$ to the maximal value of I_p obtained with the same $[K_o]$, will be defined as h'_∞. Thus h'_∞ is taken as 1 for hyperpolarizing E_{pp} values greater than -140 mv for any $[K_o]$. This process arbitrarily normalizes the curves disregarding the fact that 28 msec hyperpolarizing prepulses may not completely overcome resting inactivation when the external $[K_o]$ is equal to or greater than 25 mM. Typical plots of h'_∞ vs. membrane potential for various $[K_o]$ are shown in Fig. 3 B. The E_{pp} values for $h'_\infty = \frac{1}{2}$ are -67 mv for K-free, 5 mM K^+ and 25 mM K^+, 230 mM Na^+ ASWs, -75 mv for 50 mM K^+, 230 mM Na ASW, and -82 mv for 100 mM K^+, 230 mM Na^+ ASW. The results shown in Fig. 3 are typical of those obtained in nine axons.

Inspection of Figs 3 A and 3 B indicates that external [K] changes the relation between h_∞ and membrane potential in two significant ways. The first is a general decrease in the value of h_∞ at any voltage as a function of $[K_o]$, such that 30 msec hyperpolarizing prepulses of up to -140 mv cannot bring I_p to the equivalent value of I_p found in K-free, 230 mM Na^+ ASW. The second is a change in the slopes of both the h_∞ and h'_∞ vs. membrane potential relations as functions of $[K_o]$. This second change implies that the magnitude of the potassium effect on the h process is voltage-dependent.

[1] The points plotted in Fig. 3 A are seen to plateau as a function of E_m at values which are attenuated by about 87% for 100 mM $[K_o]$ as compared to values obtained in zero $[K_o]$. This change which was obtained from *Ip* values can be due to a change in g_{Na} or to a change in the reversal potential for the initial transient current. The change in reversal potential seen in this work for changing $[K_o]$ from zero to 100 mM when [Na] was kept constant at 230 mM was about 4 mv. Such a change in reversal potential would result in less than 5% reduction in the *Ip* values relevant to the h_∞ values discussed above. In solutions with $[K_o]$ less than 100 mM these reductions would be correspondingly less. Therefore, only a maximum of 5% of the 87% attenuation of these h_∞ values (which are used in our analysis) can be attributed to a change in reversal potential, per se.

Values of the time constant, τ_h, obtained from the exponential curves in Fig. 2 are illustrated in Fig. 4. Three bell-shaped curves of τ_h vs. prepulse potential are shown for three external concentrations of potassium. While the three curves which were obtained from the same axon have the same

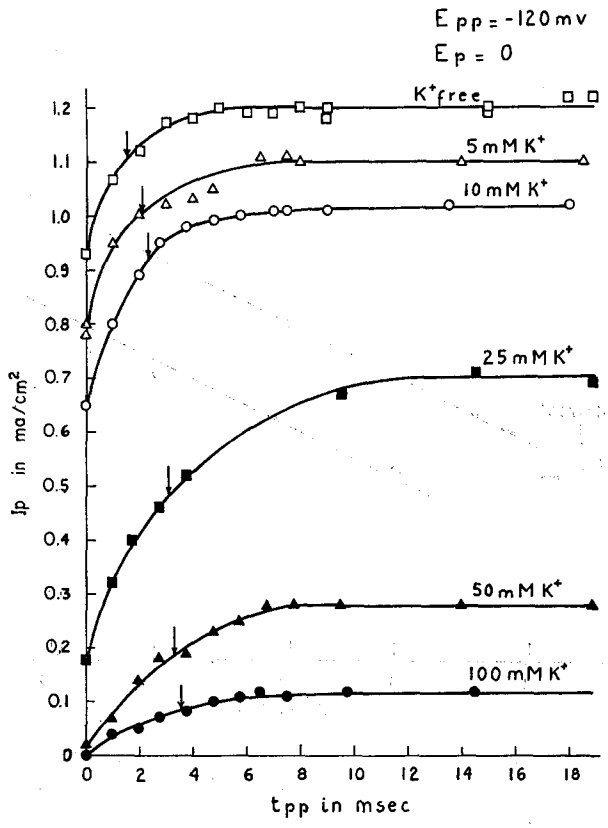

FIGURE 5. Time course of the variation in the peak value of the initial transient membrane current, I_p, plotted against the duration, t_{pp}, of a constant conditioning prepulse potential as a function of $[K_o]$. I_p was always measured upon stepping from the prepulse potential, E_{pp}, of -120 mv to the test potential, E_p, of zero millivolts. Values of $[K_o]$ in 230 mM Na ASW given above curves. Arrows indicate values of the time constant of the exponential portions of the curves. See text. Axon 68–27.

general shape, it may be seen that the presence of external potassium in-increases the values of τ_h at all prepulse potentials. The results shown in Fig. 4 are typical of those obtained in three axons.

Fig. 5 illustrates the effects of six different external potassium ion concentrations on the time course for removal of inactivation by a particular conditioning prepulse, $E_{pp} = -120$ mv. It is apparent that the inactivating effects of external potassium ion in the resting state are not overcome within

the range of prepulse amplitudes and durations generally considered by Hodgkin and Huxley (1952 *b*).

The values of τ_h obtained from the curves in Fig. 5 are plotted as a function of the logarithm of $[K_o]$ in Fig. 6 together with a similar set of values obtained from another axon. It is apparent that for a prepulse potential of -120 mv there is a linear relation between τ_h and log $[K_o]$. τ_h was determined in axon 68-27 when $[K_o]$ = zero, at $E_{pp} = -120$ mv. In this case, the experimental value was 1.35 msec. Obviously, this value cannot fall on the

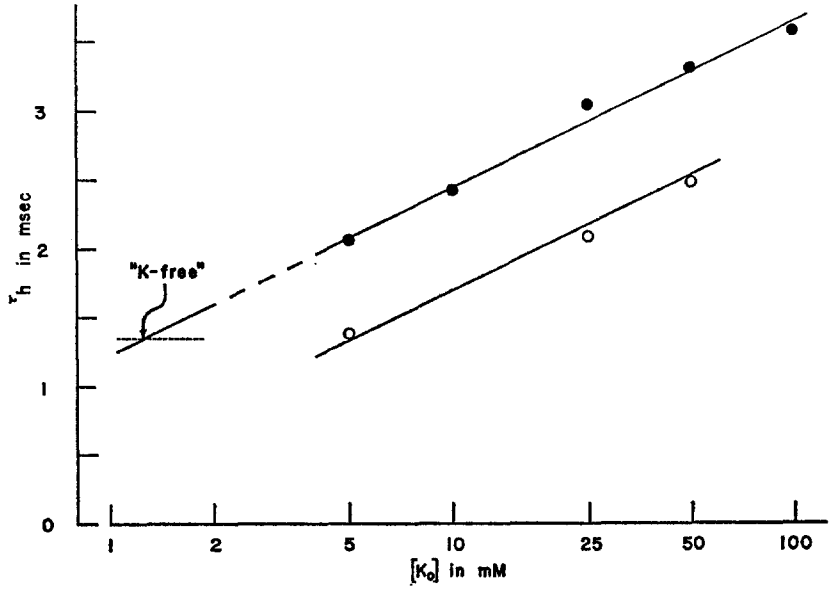

FIGURE 6. Values of the time constant of the *h* process, τ_h, plotted against the log $[K_o]$. $E_{pp} = -120$ mv; E_p = zero millivolts. Solid circles, axon 68-27; open circles, axon 68-50. See text.

linear curve illustrated in Fig. 6. However, one might consider that the external potassium concentration in close proximity to the outer membrane surface is greater than zero even though the bulk external $[K^+]$ is zero because of a continual leakage of K^+ from the axon and possibly from the Schwann cells. If this were true then one might plot the τ_h value, for $[K_o]$ = zero, on the extrapolated linear curve shown in Fig. 6. When this is done, the point locates at an external potassium concentration of 1.25 mM. Such a procedure is indicated in Fig. 6 by drawing a dashed horizontal line for the τ_h value obtained in the K-free external solution. The justification and significance of such a procedure will be discussed later.

It is also possible to obtain relations between τ_h and the log $[K_o]$ for other values of the prepulse potential. These relations are only roughly linear.

Deviations from linearity increase in the depolarizing direction. The maximum dependence of τ_h on $[K_o]$ occurs at the peak of the relation for τ_h vs. E_{pp} (see Fig. 4).

From values of h_∞ and τ_h it is possible to calculate values for the rate constants, α_h and β_h (Hodgkin and Huxley, 1952 *c*), for the membrane potential

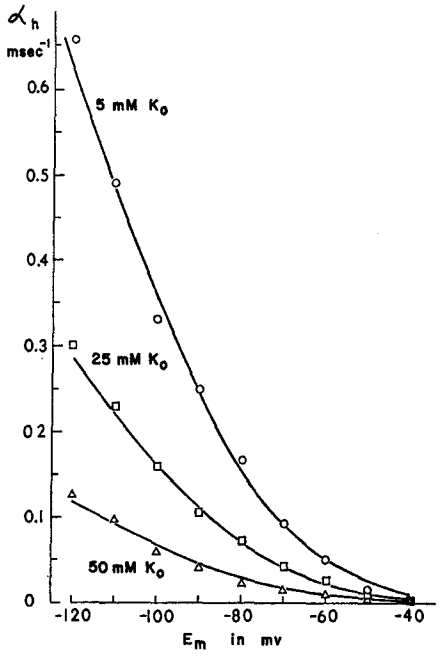

FIGURE 7. Values of the rate constant, α_h, of the h process plotted against E_m as a function of $[K_o]$. Values of $[K_o]$ in 230 mM Na ASW are given above each curve. Points obtained from experimental values of h_∞ and τ_h. Curves plotted from equation 5. Axon 68–50.

range from -120 to -50 mv. The rate constants, α_h and β_h, are determined as follows:

$$\alpha_h = \frac{h_\infty}{\tau_h}, \tag{1}$$

and

$$\beta_h = \frac{1}{\tau_h} - \alpha_h = \frac{1 - h_\infty}{\tau_h}. \tag{2}$$

Fig. 7 is a plot of α_h vs. E_m for 5, 25, and 50 mM K_o, 230 mM Na ASW's. Notice that the values of α_h are a smooth function of E_m and that α_h is inversely related to $[K_o]$. The points for 5 mM K_o follow a locus somewhat similar to that published by Hodgkin and Huxley (1952 *b*) for normal seawater (when a temperature correction is made assuming a temperature coefficient, Q_{10}, of 3). The curve published by Hodgkin and Huxley would fall slightly below our 5 mM K_o^+ curve. As the Hodgkin-Huxley data were ob-

tained from axons bathed in 10 mM K ASW this slight deviation is to be expected on the basis of the $[K_o]$ effect illustrated in Fig. 7.

Over the membrane potential range from −90 to −120 mv the relations for α_h as a function of E_m appear to be linear in Fig. 7. The relation derived by Hodgkin and Huxley (1952 *c*) for α_h as a function of V_m is exponential over this range, assuming that their zero membrane voltage is equivalent to our −60 mv. However, Hodgkin and Huxley's (1952 *b*) experimental determination of α_h was not carried beyond $V_m = +30$ ($E_m = -90$ mv). Their values of α_h and β_h were fitted for $[K_o] = 10$ mM by the following equations

$$\alpha_h = 0.07 \exp \frac{-(E + 60)}{20}, \tag{3}$$

$$\beta_h = 1 \Big/ \left(\exp \frac{-(E + 30)}{10} + 1\right). \tag{4}$$

The experimental points plotted in Fig. 7 are fitted by curves which were obtained from

$$\alpha_h = A_{(K)}(E - 40) \Big/ \left(\exp \frac{E - 40}{10} - 1\right) \tag{5}$$

where

$$A_{(K)} = 0.0185 \exp(-0.037 [K_o]), \tag{6}$$

Notice that equation 5 is a good fit for potentials up to $E_m = -120$ mv and for the range of $[K_o]$ from 5 to 50 mM. From data such as those shown in Fig. 7, it is possible to derive the quantitative relation between α_h and $[K_o]$ upon which equation 6 was based. This is done in Fig. 8 which plots values of log α_h vs. $[K_o]$. Fig. 8 illustrates that the log α_h vs. $[K_o]$ relation is linear over a wide range of membrane potentials and that the slope of this relation is independent of membrane potential.

The form of equation 5 was chosen because of its similarity to the Hodgkin and Huxley equations for α_m and α_n. The Hodgkin-Huxley relation for α_h as a function of E_m can be regarded as a simplified approximation chosen for curve-fitting purposes (compare Hodgkin and Huxley, 1952 *c*, p. 510). The points in Fig. 7 can be fitted quite well with an equivalent alternative simplified form:

$$\alpha_h = (0.126 - 0.065 \log [K_o]) \exp \frac{-(E + 60)}{27.4}. \tag{7}$$

Equation 7 may be reduced to

$$\alpha_h = 0.06 \exp \frac{-(E + 60)}{27.4} \tag{8}$$

where $[K_o]$ = 10 mM. Equation 8 is very similar to Hodgkin and Huxley's (1952 *c*) relation (equation 3, above) for α_h as a function of E. While Fig. 8 illustrates that the experimental values of log α_h are linearly related to $[K_o]$, equation 7 implies that α_h should be linearly related to the log $[K_o]$. Fig. 9 is a plot of the relation between α_h and log $[K_o]$. The good fit which the experimental points yield for both relationships (Figs. 8 and 9) makes it difficult to choose between equations 5 and 7. Further discussion of the forms of these α_h equations will be presented later.

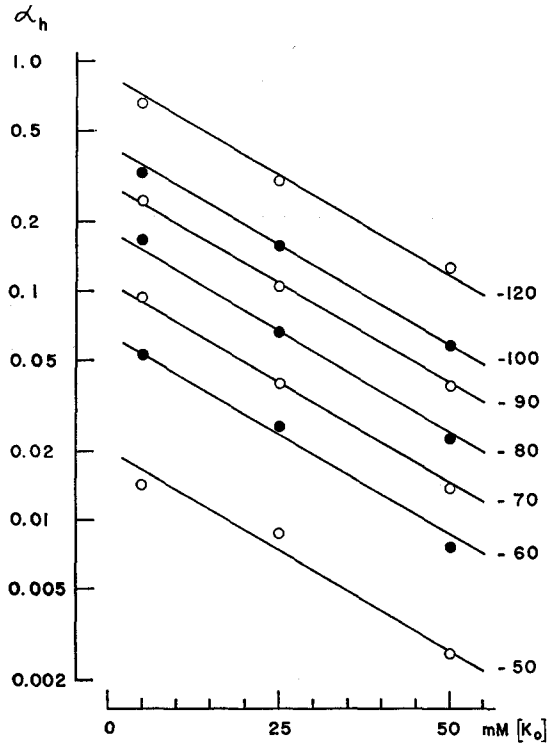

FIGURE 8. Values of the log α_h plotted against $[K_o]$ as a function of E_{pp}. E_p = zero millivolts; values of E_{pp} are given in millivolts to the right of each curve. Axon 68-50.

Fig. 10 is a plot of β_h vs. E_m for 5, 25, and 50 mM K, 230 mM Na ASW solutions. Unlike the α_h curves, the β_h curves show minimal values between $E_m = -70$ and $E_m = -90$ mv. For depolarizations, the values of β_h are similar to those reported by Hodgkin and Huxley (1952 *c*). The increasing values of β_h observed with high $[K_o]$ in the hypolarized membrane are the result of the normalization of h_∞ values for different $[K_o]$ with respect to a single $[K_o]$ (compare Fig. 3 A). The equation used to fit the curves shown in Fig. 10 is

$$\beta_h = 1 \Big/ \left[\exp \frac{-(E + 36)}{9} + 1\right] + 0.01 \exp\left[-EB_{(K)}\right] \qquad (9)$$

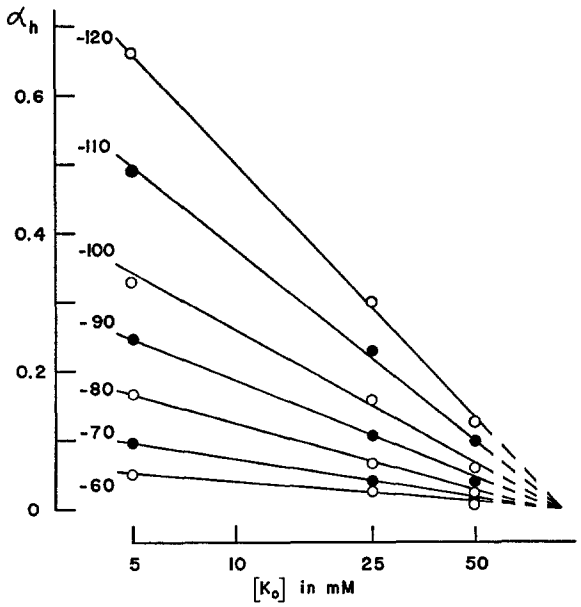

FIGURE 9. Values of the rate constant, α_h, plotted against the log [K$_o$] as a function o E_{pp}. E_p = zero millivolts; values of E_{pp} are given in millivolts to the left of each curve Axon 68-50.

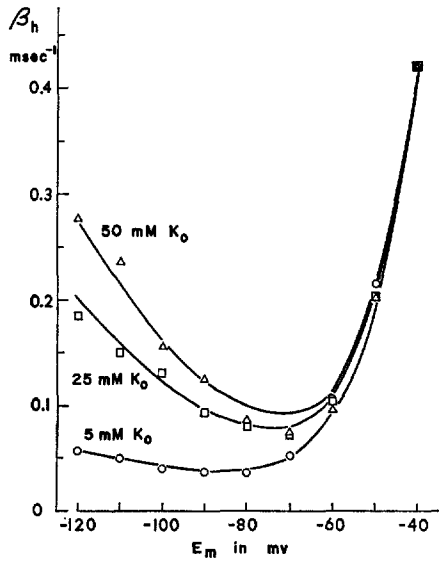

FIGURE 10. Values of the rate constant, β_h, of the h process plotted against E_m as a function of [K$_o$]. Values of [K$_o$] in 230 mM Na ASW are given above each curve. Points obtained from experimental values of h_∞ and τ_h. Curves plotted from equation 9. Axon 68-50.

where

$$B_{(K)} = [K_o]/(32.5[K_o] + 185). \quad (10)$$

The first term in equation 9 is independent of [K$_o$]. This term is almost identi-

cal to the relation for β_h (equation 4, above) given by Hodgkin and Huxley (1952 *c*).

The quantitative relationship between β_h and $[K_o]$ is given in Fig. 11. While α_h is always inversely related to $[K_o]$, β_h is directly related to $[K_o]$ for hyperpolarizations, and is virtually independent of $[K_o]$ for depolarizations. The β_h relations imply that the potassium effect on β_h is membrane potential–dependent. Thus it is apparent that the two rate constants, α_h and β_h, have different dependencies on potassium ions and probably represent dissimilar processes.

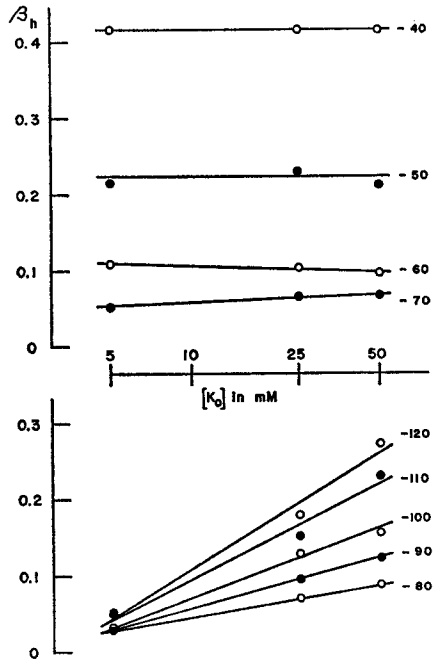

FIGURE 11. Values of the rate constant, β_h, plotted against the log $[K_o]$ as a function of E_{pp}. Upper curves are for E_{pp} values of from −40 millivolts to −70 mv; lower curves are for E_{pp} values of from −80 to −120 mv. E_p = zero millivolts. Axon 68-50.

DISCUSSION

This paper demonstrates that there is an effect of external potassium ions on the initial transient conductance to sodium ions in the squid axon membrane. This effect is primarily manifest by an increase in the values of τ_h and a corresponding decrease in the values of h_∞ with increasing $[K_o]$. The paper does not concern itself with possible K^+ effects on the m process because the data indicated that $[K_o]$ had no significant effect on the rising phase of the initial transient current.

From our data it is apparent that both h_∞ and τ_h are proportional to log $[K_o]$. For $[K_o]$ equal to or greater than 25 mM the change in resting potential is proportional to log $[K_o]$ as was demonstrated by Curtis and Cole (1942). Thus, it would appear that h_∞ and τ_h values might be related to the

value of the resting potential in each solution. However, normalization of the h_∞ vs. E_{pp} curves, h'_∞ (Fig. 3 B), indicates that the effect of $[K_o]$ is both to shift the h_∞ curves on the voltage axis and to change significantly the slope of such curves. The change in slope implies that external potassium ions affect the inactivation process in other ways than those strictly mediated through a change in resting potential.

As the values of I_p in Figs. 2 and 5 reach a steady state or plateau within 30 msec, the duration of prepulses used, any additional change in the values of I_p brought about by very long prepulses, such as that demonstrated by Narahashi (1964), should be regarded as a new process taking effect after the classical inactivation process has plateaued.

Examination of Fig. 4 reveals that there is little or no shift in the position of the τ_h vs. E_{pp} curve on the potential axis with variation in $[K_o]$. Indeed, the curves shown in Fig. 4 are quite similar to each other when multiplied by appropriate scaling constants. One possible means whereby such an effect upon τ_h and h_∞ could come about involves a general removal of conductance carriers or channels by potassium ions rather than by a modification of the kinetics of such carriers. However, one should be aware that the value of a time constant such as τ_h is determined by the values of its corresponding rate constants (α_h and β_h). The difference in the effect of $[K_o]$ on τ_h vs. E_{pp} and h_∞ vs. E_{pp} curves can be attributed to the observed difference in the effects of $[K_o]$ upon α_h and β_h (see Figs. 7 and 10).

Another interesting characteristic of τ_h vs. E_{pp} curves is the tendency for τ_h to approach a constant value at high hyperpolarized membrane potentials (Fig. 4). The Hodgkin-Huxley equations (1952 *c*) predict that τ_h approaches zero at such potentials. The difference between the curves shown in Fig. 4 and the τ_h curves predicted from the Hodgkin and Huxley formalism is due to the fact that β_h does not tend toward zero but tends to increase with increasing hyperpolarization (Fig. 10). The fact that τ_h does not tend toward zero at either end makes it easier to envision the h process as some physicochemical reaction. The increase in β_h with hyperpolarization provides an explanation for the observation that some test sodium currents are smaller following very large hyperpolarizing prepulses ($E_m > |-150|$ mv) than they are following moderate hyperpolarizations ($E_m = -100$ to -140 mv).

The explicit functions of α_h vs. E_m as given by Hodgkin and Huxley (1952 *c*) were derived on the basis of a specific potassium concentration $[K_o] = 10$ mM and for $|E_m| \leq 90$ mv. They can be regarded as special cases, for $[K_o] = 10$, of equations 5 or 7 and 9 arrived at in this work which incorporate the effect of external potassium in a general way. The α and β equations were formulated by Hodgkin and Huxley so as to match in form the constant field equations for the rate of transfer of charged particles across the membrane. Equations 5 and 9 fall into the same category.

The α_h and β_h functions as formulated by Hodgkin and Huxley are mono-

tonic functions of membrane potential. However, they increase in opposite directions. The two relations that we are proposing for α_h and β_h as a function of E_m are even more dissimilar, with respect both to their dependency upon E_m and upon $[K_o]$. The relation describing β_h is not monotonic and has a distinct minimum at a given E_m for each $[K_o]$. Hodgkin and Huxley envisioned the α_h and β_h rate constants as representing inward and outward movements of inactivating factors. It appears from their formulation as well as from the evidence presented here that these two processes are asymmetrical in behavior. Other evidence favoring dynamic membrane asymmetry has been presented by Adelman and Senft (1968). One might speculate that other ions such as Ca^{++} may show interactions with the sodium conductance mechanism in a manner somewhat similar to that shown for K^+ in this work.

Slowing of inactivation of the sodium conductance when internal [K] is decreased is now well-known (Narahashi, 1963; Baker et al., 1964; and Adelman et al., 1965 *b*). However, in these cases, loss of sodium inactivation occurred only when the external [K] was reduced toward zero (Adelman et al., 1965 *b*). Perhaps the most interesting example is seen when axons are perfused internally with NaF and externally with K-free ASW (Adelman and Senft, 1966; and Chandler and Meves, 1966). Under these conditions, outward sodium currents are greatly prolonged and show plateaus which indicate only slight inactivation. Both Adelman and Senft (1966) and Chandler and Meves (1966) indicated that in such cases inactivation was voltage-dependent with a very long time constant (seconds). Inasmuch as these internal perfusion studies resulted in a virtually K-free system, it would seem that potassium ions are a necessary part of the inactivation mechanism.

K. S. Cole (1968) has recently discussed the separation of the total membrane current into sodium and potassium currents by means of a reduction in the external sodium concentration. Cole (see 1968, Fig. 3: 28d) was able to predict the shapes of membrane current records obtained by Hodgkin and Huxley (1952 *c*) in sodium-free ASW when he assumed that the seawater in direct contact with the axolemma actually contained 3 % of the normal sodium concentration. Cole has suggested to us that his finding bears on the results obtained in our Fig. 6 in which the τ_h value obtained in K-free, 230 Na ASW seems to be related to a concentration of potassium in contact with the plasmalemma, $[K_s]$, of 1.25 mM rather than zero. Similar extrapolation of the h_∞ vs. log $[K_o]$ linear relationship produces values of $[K_s]$ between 2 and 4 mM. Intuitively, one would expect that bathing an axon externally with a zero concentration of either sodium or potassium should not result in a zero concentration of either ion in the space between axolemma and the Schwann cell layer or in any unstirred layer close to the membrane. In addition, under normal conditions outward going potassium currents may temporally influence the immediate concentration of potassium in contact with the axolemma (Frankenhaeuser and Hodgkin, 1956). It is also interesting to note the very long delay following the reduction in external [K] before the appearance

of the loss in sodium inactivation seen in axons internally perfused with low [K] solutions (Adelman et al., 1965 *b*, Fig. 2). This delay is many times longer than expected for equilibrating the bulk external phase to the new concentration. A slow washout of the immediate extracellular space close to the axon proper is one possible explanation for this effect. From these rather indirect considerations, one might expect that sodium inactivation should be reduced to zero if the axon membrane were ever exposed to a true zero potassium environment.

It is possible to conceive of several mechanisms whereby potassium ions might influence the sodium inactivation system. First, the prolonged change in E_m resulting from changes in $[K_o]$ may result in strictly voltage-dependent alterations in membrane structure. Second, a sensitivity of any fraction of the sodium conductance system to $[K^+]$ may result in a potassium-dependent noncompetitive inhibition. Third, potassium may inhibit sodium conductance through competitive inhibition of sodium carriers. The first possibility considers that prolonged membrane structural alterations can come about as a result of persistent changes in membrane potential. For example, the change of membrane potential or field brought about by increasing K_o may result in a change of the configuration of membrane polar groups which are directly or indirectly associated with the initial transient conductance mechanism. The probability that this mechanism is solely responsible for the potassium effect seems unlikely as there is ample evidence from this work and the internal perfusion studies for some direct potassium involvement in inactivation irrespective of membrane potential.

The second possibility considers the properties of potassium when it acts as an inhibitory agent. An example would be the simple plugging of some sodium conductance channels by potassium ions. Such plugging need not specify site or carrier affinities. The third possibility demands that there be some site associated with the sodium conductance for which both sodium and potassium ions can compete. The actual magnitude of the sodium inactivation in this case would depend upon the relative affinities of sodium and potassium for the site as well as upon their concentrations.

Until further information is obtained as to the physical nature of the initial transient conductance mechanism, it remains extremely difficult to choose among the possible mechanisms for the role played by potassium in the inactivation process.

The effect of potassium on the inactivation process obviously leads to the postulation that the inactivation mechanism for the initial transient conductance is at least in part a potassium ion phenomenon, involving as sources of potassium at the external membrane surface both the external bulk phase potassium and the potassium made available from outward current flow through the delayed conductance mechanism in response to depolarization. This postulation might account for the similarity between τ_h and τ_n values at

various membrane potentials. In this regard, the *h* parameter may be considered in terms of irreversible thermodynamics as the cross-correlation coefficient between sodium and potassium currents.

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