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Effect of Calcium upon Sodium Inactivation in the Giant Axon of *Loligo pealei*

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Summary. Giant axons of *Loligo pealei* were voltage clamped in artificial seawater solutions containing varying concentrations of calcium from 10 to 100 mM, and the sodium conductance inactivation was measured with a series of two-pulse experiments. The h_∞ vs. voltage curve showed a shift of about 10 mV in the depolarizing direction on the voltage axis for a tenfold increase in external calcium without substantial alteration in the slope of the voltage dependence. The kinetics of the inactivation process were found to be exponential for hyperpolarizing prepulses, but showed some indication of a sigmoidal decay for depolarizing prepulses in all calcium concentrations employed. Increasing calcium increased the delay in the sigmoidal response. The inactivation time constant τ_h increased as a function of calcium concentration over the potential range studied, -10 to -90 mV. The values of the rate constants α_h and β_h are decreased with an increase in calcium and these effects are not consistent with parallel shifts of the rate constant vs. voltage curves along the voltage axis for changes in calcium concentration.

Magnesium does not behave as an equimolar substitute for calcium. The effect of a solution containing 10 mM calcium and 50 mM magnesium is intermediate to that of solutions containing 10 and 30 mM calcium alone.

Predictions of a recent model for the sodium conductance (Moore, J.W., Cox, E.B., 1976 *Biophys. J.* **16**:171) which employs calcium binding were compared with the experimental data.

The surface charge screening hypothesis has been successfully employed to explain the effects of external calcium ions upon the conductances of a variety of excitable membranes. This theory was originally presented by Frankenhaeuser and Hodgkin (1957) as a possible explanation for the wide range of data that they obtained from squid axon with changes in the external calcium concentration. Since this work, a number of studies have been made upon both biological and artificial membranes that appear to support this interpretation of the calcium effect. Gilbert and Ehrenstein (1969) presented a formal model for cation

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binding to surface charges and used the Grahame (1947) equation to estimate the charge density of squid axon from their data on the effect of calcium upon the potassium conductance. McLaughlin, Szabo and Eisenman (1971) showed that artificial membranes composed of negatively charged lipids possess a surface potential whose magnitude is accurately described by the Gouy equations and also showed that divalent cations altered the surface potential by charge screening alone. A similar effect was proposed for biological membranes. Schauf (1975) has extended the use of this theory to explain the effect of calcium upon the conductances in *Myxicola* axons and has also been able to estimate the surface density from curve fitting to the Grahame equation. In addition, he showed that calcium and magnesium behave as equimolar substitutes. The equivalence of these ions in their effect upon the potassium conductance/voltage relationship lends strong support for the charge screening theory. Begenisich (1975) made an analysis of data from frog node and squid axon, as well as from *Myxicola,* and showed that the amount of specific binding to surface charges was very low.

However, Frankenhaeuser and Hodgkin (1957) found that Mg^{++} was roughly one half as potent as Ca^{++} in its effects on squid axon. In addition, Frankenhaeuser and Hodgkin (1957) gave an overall summary of their results by saying that fivefold reduction in external calcium ions was the equivalent of a membrane depolarization of 10 to 15 mV. However, if one looks more closely at their results it is apparent that various curves relating some membrane conductance parameter to voltage show different amounts of shift along the voltage axis for the same change in calcium concentration. For example, in *Loligo* axons, like *Myxicola* (Schauf & Davis, 1975), the steady-state inactivation vs. voltage curve shows a shift along the voltage axis that is about one half that seen for the peak g_{Na} vs. voltage curve. The change in the rate of the decay of g_{Na} with repolarization for an altered external calcium concentration is much larger than would be expected from the peak g_{Na} vs. voltage curve shift.

Inactivation of the sodium conductance for different calcium concentrations shows several anomalies with respect to the charge screening theory. It is the purpose of this paper to study systematically the effects of external calcium upon both the steady state and transient characteristics of sodium inactivation in squid axon. These effects are compared with predictions made on the basis of the charge screening theory and also with predictions of a recent model for the Na conductance developed by Moore and Cox (1976).

Materials and Methods

Voltage clamp experiments were performed upon giant axons of *Loligo pealei* at the Marine Biological Laboratory, Woods Hole, Mass., during the summer and fall of 1976.

The voltage clamp consisted of a conventional feedback circuit which incorporated series resistance compensation (Binstock, Adelman, Senft & Lecar, 1975) and employed a separate voltage electrode and axial wire. Pulses were generated and data acquisition controlled by a SPC-12 minicomputer (General Automation) with provisions for the digitizing of the current record and storage of the digitized record upon magnetic tape for subsequent analysis[1].

All experiments followed a two-pulse protocol for determining the effect of conditioning potential upon the activation capability of the sodium system as evidenced by a standard depolarizing test pulse. The test pulse potential was 0 mV (all potentials are absolute internal voltage referenced to the bath potential) and was well on the ascending limb of the peak sodium current-voltage curve. The prepulse potentials and durations ranged from −90 to −10 mV from 1 to 30 msec, respectively. Peak values of the inward current during the test pulse were measured by the method of Hodgkin and Huxley (1952 *b*) to correct for any variation in the potassium conductance during the prepulse. Correction for leakage current was made by adding the current records for equal and opposite polarity test pulses. In all axons from which data in this study were obtained the leak current was less than 0.4 mA/cm^2 for a 60 mV pulse.

Artificial seawater solutions contained calcium concentrations in the range of 10 to 100 mM. All solutions except the standard ASW were magnesium free and contained a reduced amount of sodium. The total composition of each seawater is given in Table 1. All solutions containing only calcium as the divalent cation will be referred to in the text by their calcium concentration. Tris was used as a substitute for calcium to maintain the osmolarity and ionic strength of each seawater approximately equal to the reference ASW. The chloride ratio is the ratio of the chloride concentration in each solution relative to the standard ASW chloride concentration. Tris was tritrated with HCl for each solution to a pH of 7.4 at 5.5 °C, the temperature at which the experiments were conducted. For any one set of pulse schedules, the temperature did not vary by more than 0.4 °C.

For all experiments the holding potential was set at the initial resting potential in ASW and this value was −60 mV for most experiments.

Table 1. Composition of artificial seawaters

	Na^+	K^+	Ca^{++}	Mg^{++}	$Tris^+$	Cl^-	Cl^- ratio
ASW	430	10	10	50	10	570	1.0
10 Ca^{++}	370	10	10	–	140	540	0.96
30 Ca^{++}	370	10	30	–	110	550	0.97
70 Ca^{++}	370	10	70	–	50	570	1.01
100 Ca^{++}	370	10	100	–	5	585	1.04

Concentrations of ions are millimolar. The definition and significance of the chloride ratio is given in the text.

1 Adelman, W.J., Jr., French, R. 1977. Blocking of the squid axon potassium channel by external cesium ions (*submitted for publication*).

Results

Kinetics

The effect of the conditioning prepulse is expressed as the ratio of the peak current obtained from the subsequent test pulse to the current obtained in the absence of any prepulse (I_{Na}/I_{Na0}). This ratio will be termed h' to distinguish it from the Hodgkin-Huxley h parameter, from which h' differs by a normalization constant. These ratios are plotted in Fig. 1 as a function of prepulse amplitude and duration for ASW and each calcium concentration seawater for a typical set of experiments. For prepulses more negative than the holding potential (hyperpolarizing) the current ratio in all solutions shows an exponential increase to some steady-state level at 30 msec for all calcium concentrations. For prepulses more positive than the holding potential (depolarization) h' also reaches a steady-state value within 30 msec for all calcium concentrations.

However, there is an indication that for depolarizing prepulses the time course of h' is sigmoidal in higher calcium concentrations. This

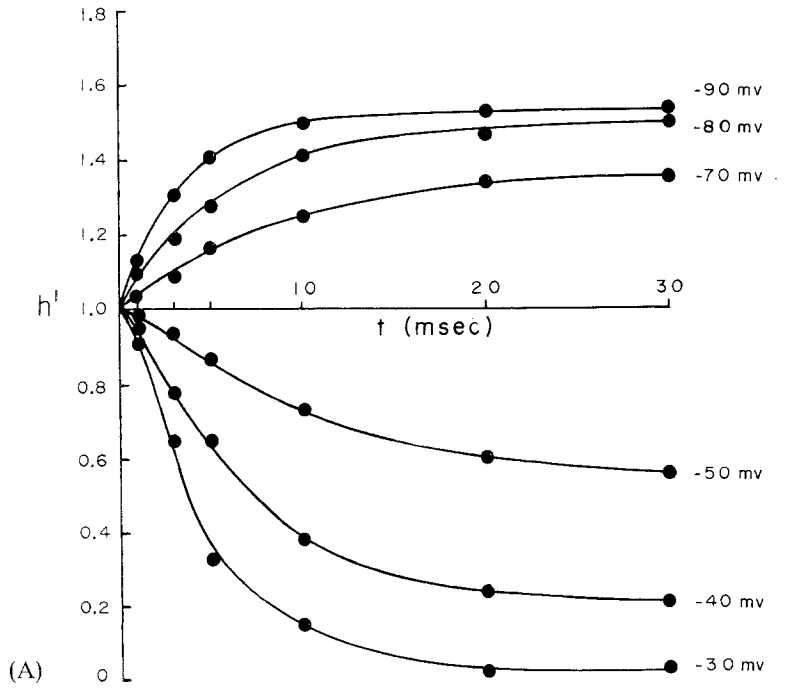

Fig. 1. Current ratio h' as a function of prepulse duration for different prepulse amplitudes (number to the right of each curve). Families of curves correspond to the following seawater solutions: (*A*): ASW; (*B*): 10 mM Ca^{++}; (*C*): 30 mM Ca^{++}; (*D*): 70 mM Ca^{++}; (*E*): 100 mM Ca^{++}. Smooth curves are drawn by eye

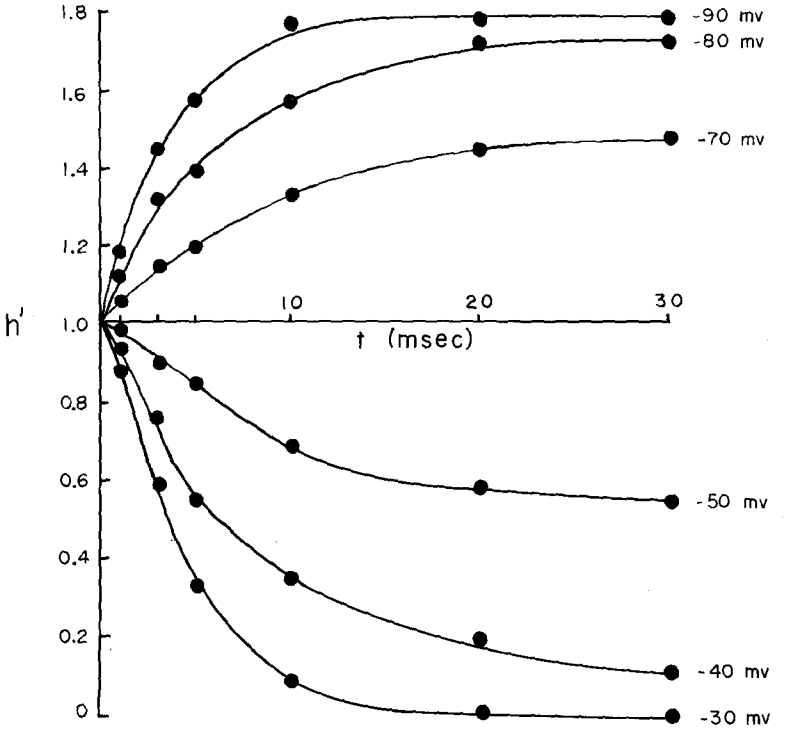
h'
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
10
20
30
t (msec)
-90 mv
-80 mv
-70 mv
-50 mv
-40 mv
-30 mv

Fig. 1B

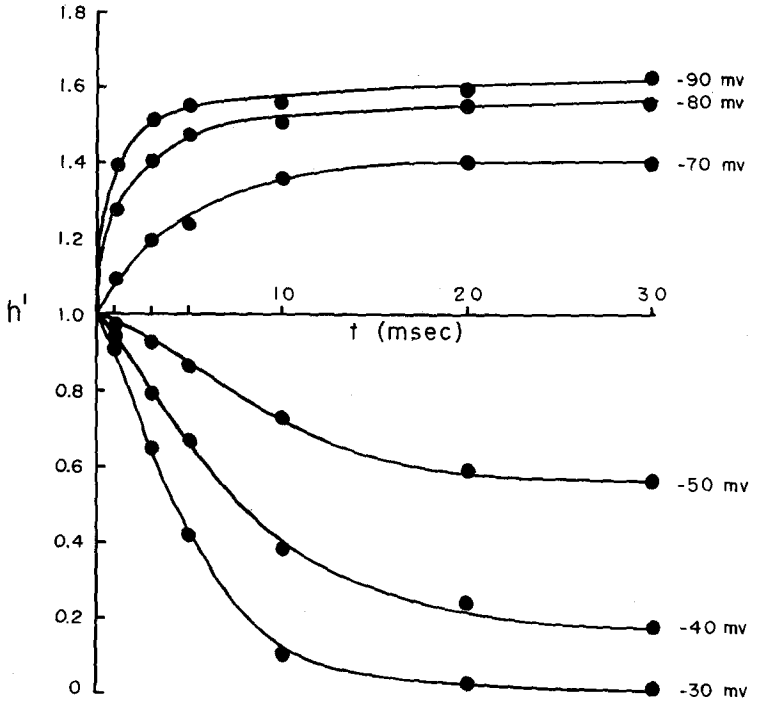
h'
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
10
20
30
t (msec)
-90 mv
-80 mv
-70 mv
-50 mv
-40 mv
-30 mv

Fig. 1C

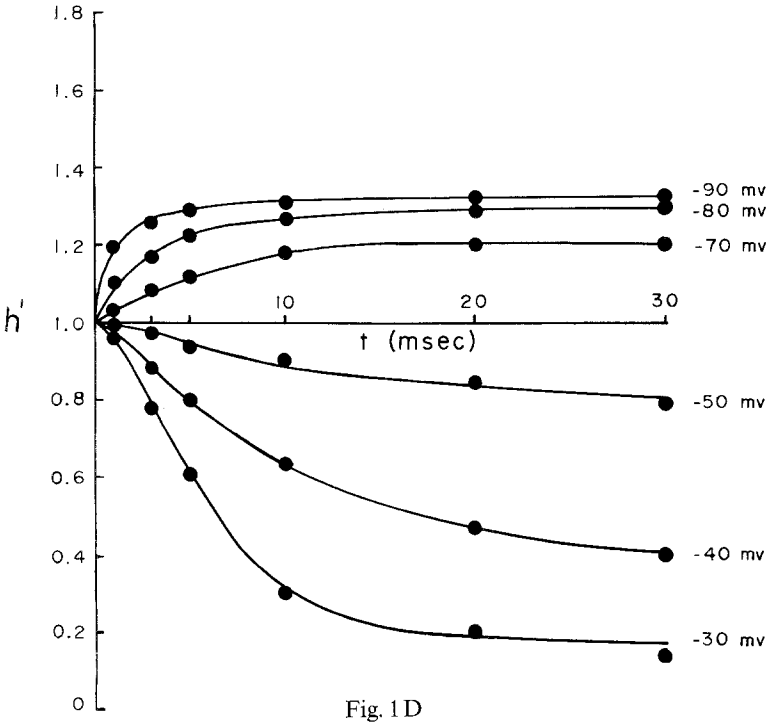
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
h'
10
20
30
t (msec)
-90 mv
-80 mv
-70 mv
-50 mv
-40 mv
-30 mv

Fig. 1D

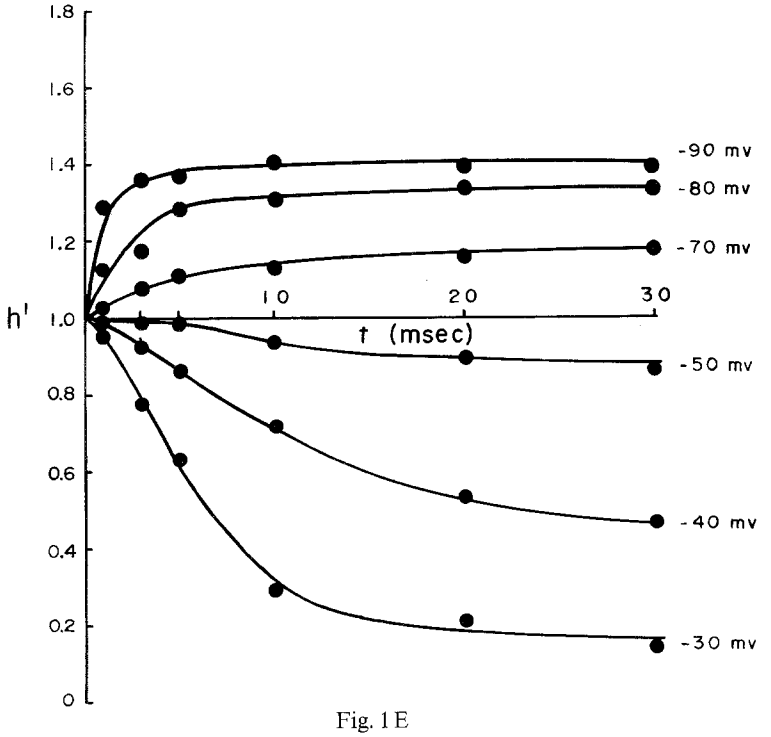
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
h'
10
20
30
t (msec)
-90 mv
-80 mv
-70 mv
-50 mv
-40 mv
-30 mv

Fig. 1E

effect is most noticeable for a prepulse potential to -50 mV in 100 mM Ca^{++}. For the purpose of comparison with earlier work the data were fit by the equation that describes the time course of inactivation as a first order process:

$$\ln(h-h_\infty)=\ln(h_0-h_\infty)-t/\tau_h. \quad (1)$$

From the least squares fits the first order exponential time constant τ_h was determined for each prepulse amplitude. A small but consistent deviation from an exponential time course in high Ca suggests a model predicting a sigmoidal time course would provide an even better fit to the data. This phenomenon is also evident in Goldman and Schauf's (1972, Fig. 1) study on inactivation in *Myxicola,* although they also chose to describe inactivation as a first order process. The mean values of τ_h and the SEM for each seawater solution are shown in Table 2. In Fig. 2*a* the mean values of τ_h are plotted as a function of prepulse potential. The smooth curves for each solution are computed from best fits to the expression of the form originally given by Hodgkin and Huxley (1952*b*).

Table 2. Mean values of τ_h and standard error of the mean (SEM) for each prepulse potential and external solution

mV	pp	ASW	10 Ca^{++}	30 Ca^{++}	70 Ca^{++}	100 Ca^{++}
−10	τ_h	1.90	1.71	1.62	1.72	1.67
	SEM	0.14 (3)	0.08 (7)	1.37 (4)	0.11 (4)	0.13 (4)
−20	τ_h	4.06	2.15	2.24	3.78	4.72
	SEM	1.21 (4)	0.35 (7)	0.48 (5)	0.12 (5)	0.45 (4)
−30	τ_h	4.13	3.54	5.08	6.41	6.35
	SEM	0.80 (5)	0.42 (11)	0.41 (5)	0.53 (7)	0.58 (6)
−40	τ_h	5.85	5.80	6.25	8.25	8.13
	SEM	0.38 (5)	0.47 (11)	0.36 (5)	0.58 (7)	1.02 (6)
−50	τ_h	8.18	8.18	8.78	12.68	13.93
	SEM	1.64 (5)	0.96 (11)	1.11 (6)	2.77 (7)	1.01 (6)
−70	τ_h	7.06	6.71	8.15	9.68	10.47
	SEM	0.79 (4)	1.24 (5)	1.11 (5)	1.63 (3)	1.44 (4)
−80	τ_h	5.82	5.10	5.59	5.99	8.38
	SEM	0.69 (9)	0.14 (2)	1.32 (4)	1.12 (8)	1.65 (8)
−90	τ_h	3.17	3.90	4.05	3.63	5.56
	SEM	0.86 (6)	0.36 (3)	0.61 (4)	174 (3)	1.47 (4)

For each solution and voltage, number of observations are given in parentheses.

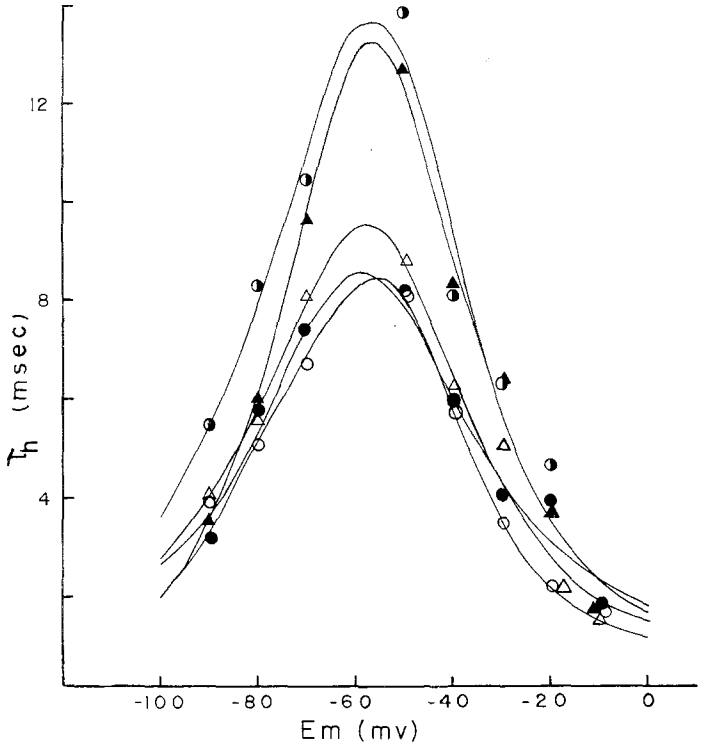
12
8
4
τ_h (msec)
-100
-80
-60
-40
-20
0
Em (mv)

Fig. 2A

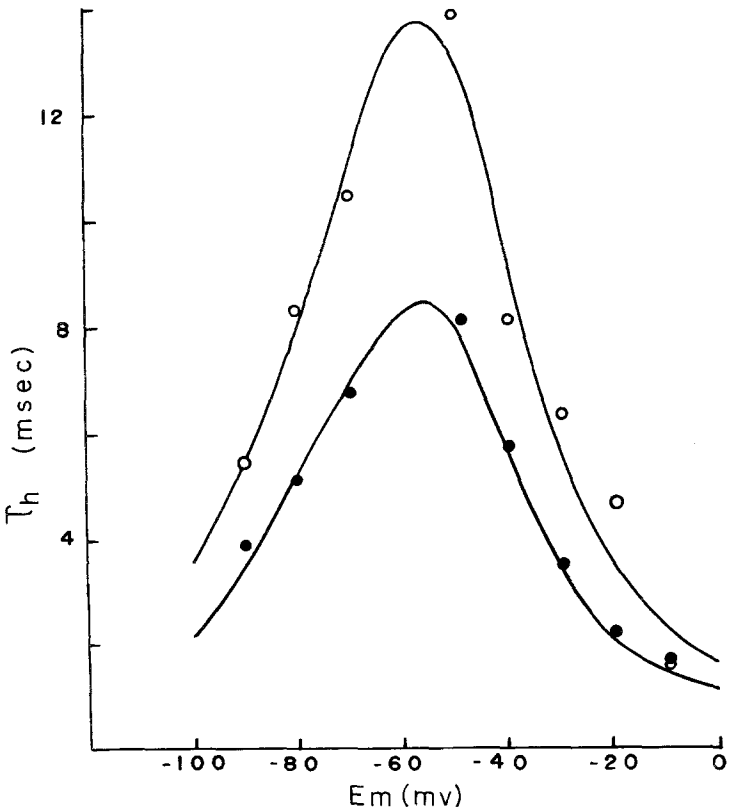
12
8
4
τ_h (msec)
-100
-80
-60
-40
-20
0
Em (mv)

Fig. 2B

Fig. 2*a* shows that over the entire potential range studied, an increase in calcium concentration causes an increase in τ_h. The data for 10 and 100 mM calcium only are plotted in Fig. 2*b* to show more clearly the effect of a tenfold change in concentration. Two points concerning this result should be emphasized. First, the positions of the τ_h vs. voltage curves with increasing calcium do not appear to show a shift on the voltage axis. All curves are roughly bell-shaped with a calcium dependent maximum centered in the region of the holding potential. Surprizingly, this relationship between τ_h and calcium is similar to that shown by Adelman and Palti (1969*a*) for τ_h and external potassium concentration. Second, the curve for ASW which contains both 10 mM Ca^{++} and 50 mM Mg^{++} lies intermediate between the curves for 10 and 30 mM Ca^{++} alone.

In general the experimental curves obtained in ASW fall between the curves for 10 and 30 mM Ca^{++} solutions. Fig. 3 shows a typical set of data for a prepulse to −90 mV. If Ca^{++} and Mg^{++} ions showed identical effects upon squid axon one would expect that the ASW curve should lie between the curves for 30 and 70 mM Ca^{++}. This was never observed experimentally.

Steady-State Effects

As mentioned above, for all prepulses and calcium concentrations the inactivation ratio h' appears to reach a steady-state value within

Fig. 2. (*A*): Mean values of τ_h plotted as a function of prepulse potential for each seawater solution. The following symbols are used for each solution: ●, ASW; ○, 10 mM Ca^{++}; △, 30 mM Ca^{++}; ▲, 70 mM Ca^{++}; ◑, 100 mM Ca^{++}. For each solution the smooth curve was drawn according to the following expressions:

ASW:

$$\tau_h = 1/[0.06\exp(-(E_m+60)/19.17)+1/(\exp(-(E_m+60)+57.06/20.64)+1] \quad (2.1)$$

10 mM Ca^{++}:

$$\tau_h = 1/[0.10\exp(-(E_m+60)/31.23)+1/(\exp(-(E_m+60)+42.84/11.26)+1] \quad (2.2)$$

30 mM Ca^{++}:

$$\tau_h = 1/[0.07\exp(-(E_m+60)/24.20)+1/(\exp(-(E_m+60)+49.04/14.72)+1] \quad (2.3)$$

70 mM Ca^{++}:

$$\tau_h = 1/[0.05\exp(-(E_m+60)/17.07)+1/(\exp(-(E_m+60)+55.13/15.71)+1] \quad (2.4)$$

100 mM Ca^{++}:

$$\tau_h = 1/[0.05\exp(-(E_m+60)/24.12)+1/(\exp(-(E_m+60)+54.65/14.37)+1] \quad (2.5)$$

(*B*): Mean values of τ_h for 10 mM Ca^{++} and 100 mM Ca^{++} solutions only plotted as a function of prepulse potential. ●, 10 mM Ca^{++}; ○, 100 mM Ca^{++}

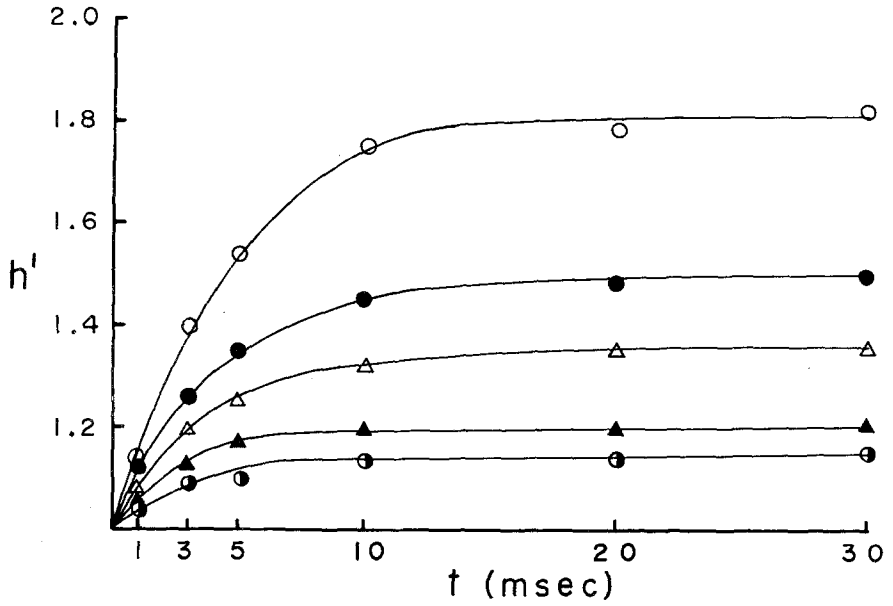

Fig. 3. h' as a function of prepulse duration for a prepulse potential to -90 mV. The symbols used for each solution are: ●, ASW; ○, 10 mM Ca^{++}; △, 30 mM Ca^{++}; ▲, 70 mM Ca^{++}; ◑, 100 mM Ca^{++}. Smooth curves are drawn by eye

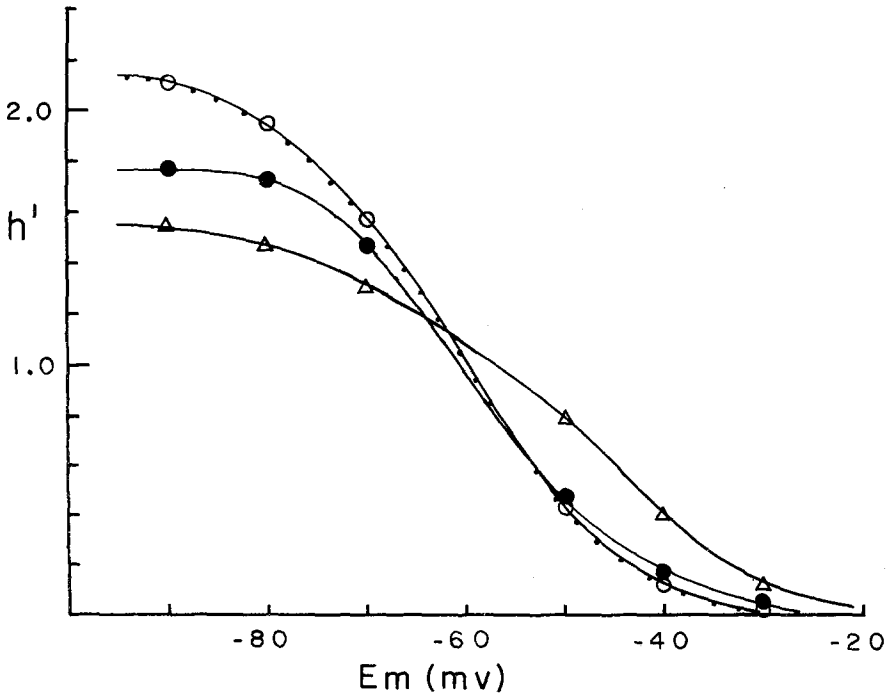

Fig. 4. Steady-state values of h' as a function of prepulse potential. ●, ASW; ○, 10 mM Ca^{++}; △, 100 mM Ca^{++}. Smooth curves are drawn by eye

30 msec. The mean steady-state values of h' are plotted in Fig. 4 as a function of voltage for ASW plus 10 and 100 mM Ca. For potentials more positive than -70 mV the h' vs. voltage curve for 10 mM Ca^{++} is slightly stepper than the ASW curve. For potentials more negative than -70 mV, little additional inactivation is removed in ASW while the curve for 10 mM Ca^{++} shows considerably more resting inactivation. In comparison the curve for 100 mM Ca shows a marked flattening over the entire potential range relative to the curve for 10 mM Ca. If the steady-state values of h' are normalized so that the maximum value is equal to 1, the equivalent Hodgkin-Huxley relationship for h_∞ as a function of voltage is obtained. This relationship is shown in Fig. 5. This figure allows direct comparison of the present data with previous results obtained from squid axons by Frankenhaeuser and Hodgkin (1957). At the point where $h_\infty = 0.5$, the slopes of the curves for each seawater solution are approximately the same. The 100-mM Ca^{++} curve lies about 10.2 mV in the depolarizing direction from the 10-mM Ca^{++} curve at the point where $h_\infty = 0.5$. The points for the 10-mM Ca^{++} curve can be fitted to the expression:

$$h_\infty = 1/(1+\exp((V-V_h/k)))$$

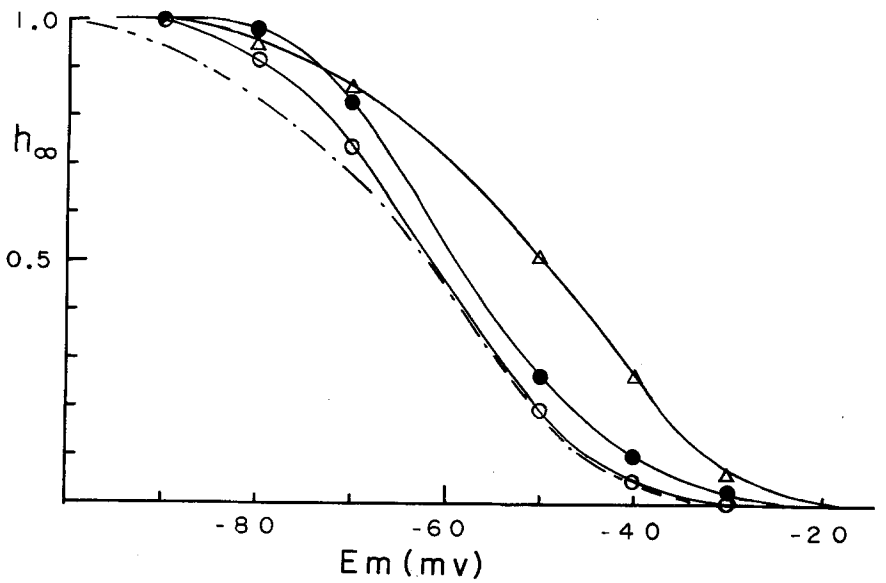

Fig. 5. h_∞ as a function of prepulse potential. ●, ASW; ○, 10 mM Ca^{++}; △, 100 mM Ca^{++}. The 100 mM Ca^{++} curve shifted 10.2 mV in hyperpolarized direction is shown by a broken curve. Smooth curves are drawn by eye

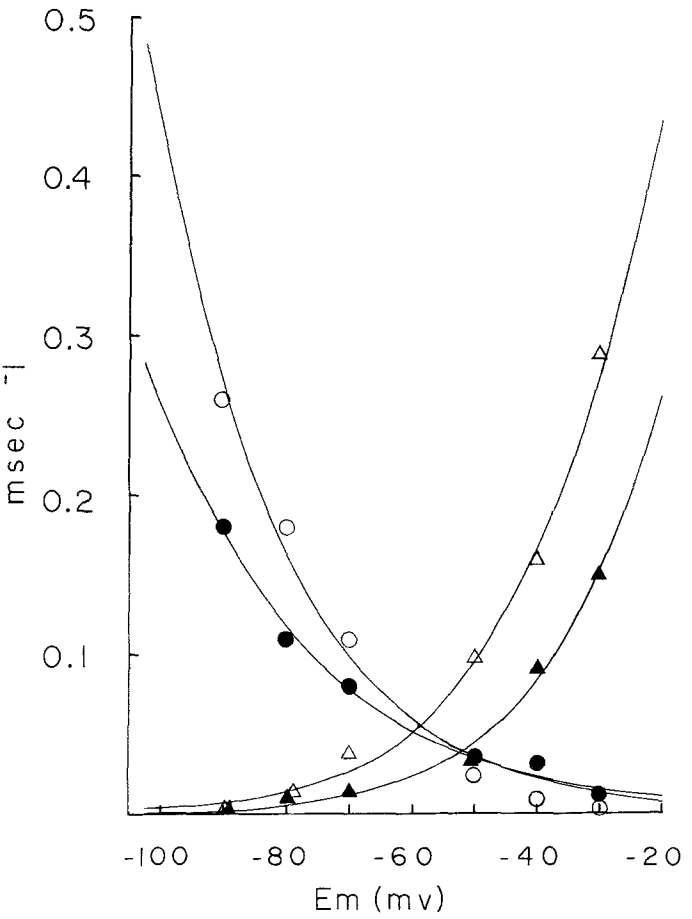

Fig. 6. Rate constants α_h and β_h as a function of prepulse potential. α_h: ○ 10 mM Ca^{++}, ● 100 mM Ca^{++}; β_h: △ 10 mM Ca^{++}, ▲ 100 mM Ca^{++}. The smooth curves for 10 mM Ca^{++} solution are drawn according to the expression:

$$\alpha_h = 0.06\exp(-(E_m+60)/20.14) \tag{3}$$

$$\beta_h = 1/\{\exp[(-E_m+60)+43.83/15.06]+1\} \tag{4}$$

while for 100 mM Ca^{++} solution the smooth curves are drawn according to the expressions:

$$\alpha_h = 0.05\exp(-(E_m+60)/25.02) \tag{5}$$

$$\beta_h = 1/\{\exp[(-E_m+60)+55.22/14.68]+1\} \tag{6}$$

for $V_h = 62$ mV and $k = 8$ mV. These values for V_h and k are intermediate between those given by Frankenhaeuser and Hodgkin's (1957) Table 6 for 22 and 4.4 mM Ca^{++} and are very close to the values of Hodgkin

and Huxley (1952*b*) (mean $V_h = 62.5$ mV and $k = 7$ mV) and Chandler, Hodgkin and Meves' (1965) values ($V_h = -63$ mV and $k = 7.5$ mV). The ASW curve shows a shift of about 3 mV in the depolarizing direction relative to 10 mM Ca^{++}. If the ASW curve is shifted 3 mV in the hyperpolarized direction, the 10 mM Ca^{++} and ASW curves superimpose. However, if the 100 mM Ca^{++} curve is shifted 10.2 mV in the hyperpolarized direction as indicated by the dotted curve in Fig. 5, the 10 and 100 mM Ca^{++} curves do not superimpose for potentials more negative than the holding potential, due to the lesser steepness of the 100 mM Ca^{++} curve in this range.

If one interprets this result by saying that the effect of a 10-fold increase in divalent cation is to shift the h_∞ curve 10 mV in the hyperpolarized direction, then the shift for the ASW curve is smaller than expected since it contains a total divalent cation concentration of 60 mM.

The values of the rate constants, α_h and β_h for the inactivation process can be determined from the experimental values for h_∞ and τ_h. These constants were calculated as a function of potential for 10 and 100 mM Ca and are shown in Fig. 6. Because the curve fits were done on experimental values of α_h and β_h derived from both the τ_h and h_∞ data, the constants in Eqs. (3)–(6) differ slightly from the constants in the equations derived from curve fits to the τ_h data alone (*see* legend, Fig. 2). These equations also follow the form used by Hodgkin and Huxley. The β_h-voltage curve appears to show a shift of about 10 mV in the depolarizing direction for a tenfold increase in calcium concentration. However, the reverse effect is seen for the α_h curves. In higher external calcium the α_h curve lies further to the left or in the hyperpolarizing direction. In comparing the two α_h curves it is also evident that in higher calcium the steepness or voltage dependence of the curve is reduced. A modification of the voltage dependence of the rate constants by shifting both rate constant vs. voltage curves in the depolarizing direction is not consistent with these findings.

Discussion

The results of this paper suggest that the action of calcium upon inactivation in squid axon cannot be explained solely by surface charge effects. Frankenhaeuser and Hodgkin (1957) rejected the idea of a voltage-dependent calcium flux from sites within the membrane, since such a model would result in too large an effect upon membrane conductance parameters for an *e*-fold change in external calcium. As an alternative

they postulated that calcium could affect the membrane electrical field by screening of fixed, negative surface charges. Absolute alterations in the field due to changes in the amount of screening would not be detectable by potential measuring electrodes. In the Hodgkin-Huxley model the sole dependence of the rate constants upon the membrane field enables the incorporation of this type of effect of external calcium without any alterations in the theory describing the behavior of the axon.

If the outer surface of the membrane contains uniform, fixed, negative charges, the Grahame (1947) treatment of this component of the total membrane field and the interaction of cations with the charges should yield equivalent shifts for all voltage-dependent parameters. This has not been the case for the voltage dependence of steady-state inactivation in either *Myxicola* or squid axons. Further, the question has been raised (Moore & Jakobsson, 1971; Moore & Cox, 1976) as to whether the slope change in the h_∞ vs. voltage curve should not be regarded as incompatible with the charge-screening hypothesis.

The results of this study do not show an appreciable slope change for the steady state h vs. voltage relationship and the shift in the steady-state curve is consistent with both the magnitude of the effect previously shown and also, at least in part, consistent with a charge-screening explanation. However, the effect of calcium upon the kinetics of inactivation, as shown here, is at variance with a charge-screening model. The lack of any real shift in the τ_h vs. voltage relationship and the general increase in τ_h as a function of external calcium is a strong indication of the need for an alternative kinetic model that incorporates the interaction of calcium with the inactivation process in a way that does not solely invoke a voltage shift. For depolarizing voltages the effect of calcium upon τ_h found in this work agrees with the summary given by Frankenhaeuser and Hodgkin. In contrast to the data presented by Frankenhaeuser and Hodgkin for a hyperpolarizing voltage of approximately -116 mV, the present data shows over the range of -70 to -90 mV that a reduction in external calcium increases the rate of the removal of inactivation.

One possible difficulty in the interpretation of these results arises from the phenomenon of slow inactivation (Adelman & Palti, 1969*b*; Chandler & Meves, 1970). In higher Ca^{++} the slow inactivation may have been removed by the holding potential of -60 mV. This effect would tend to depress the h' vs. voltage curve for hyperpolarizing prepulses, as has been seen experimentally. However, in all solutions the

axon was clamped at long durations to the holding potential relative to the pre- and test pulse durations before the pulse regime began. Thus slow inactivation should have only a small effect on the measurements. The very slow removal of inactivation seen by Adelman and Palti (1969) has a time constant of minutes and would not be expected to influence time constant measurements in the msec domain.

It became apparent from the effects of calcium upon sodium inactivation seen in the present data that it would be profitable to investigate alternative models of the sodium conductance in which the specific binding of calcium was incorporated.

One such model has recently been proposed by Moore and Cox (1976) which not only duplicates the predictions of the Hodgkin-Huxley model, but also predicts some of the data for calcium interactions with sodium conductance. Predictions of the model were compared with the present data. Rate equations describing reaction 7 (Moore & Cox, 1976) were derived and these equations were then solved numerically with a fourth order Runge Kutta algorithm (Carnahan, Luther & Wilkes, 1969). I obtained solutions corresponding to the experimental procedure for 10 and 100 mM Ca^{++}. Fig. 7 gives the results of computations for steady state h' as a function of prepulse potential while Fig. 8 shows the equivalent h_∞ vs. voltage relationship.

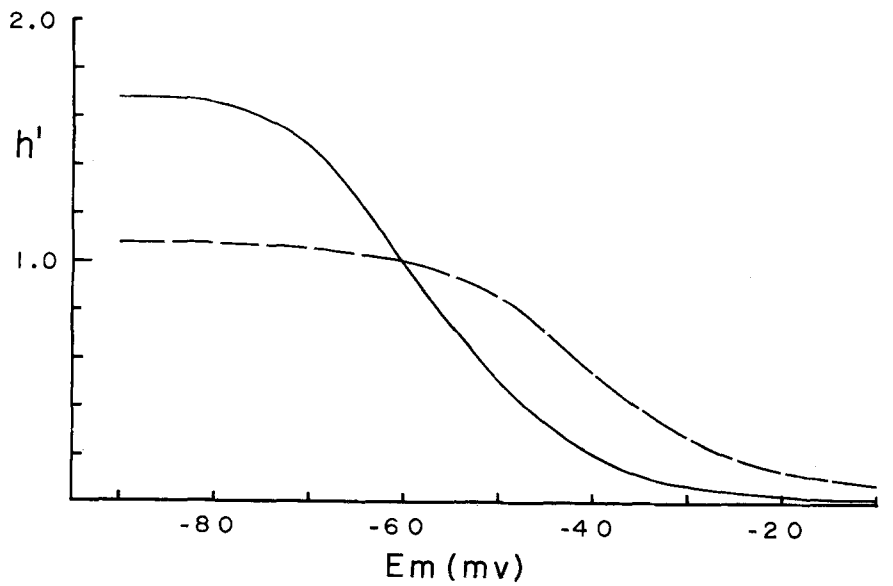

Fig. 7. Steady-state values of h' as a function of prepulse potential calculated from the Moore and Cox (1976) sodium conductance model. Solid curve: 10 mM external Ca^{++}; Broken curve: 100 mM external Ca^{++}

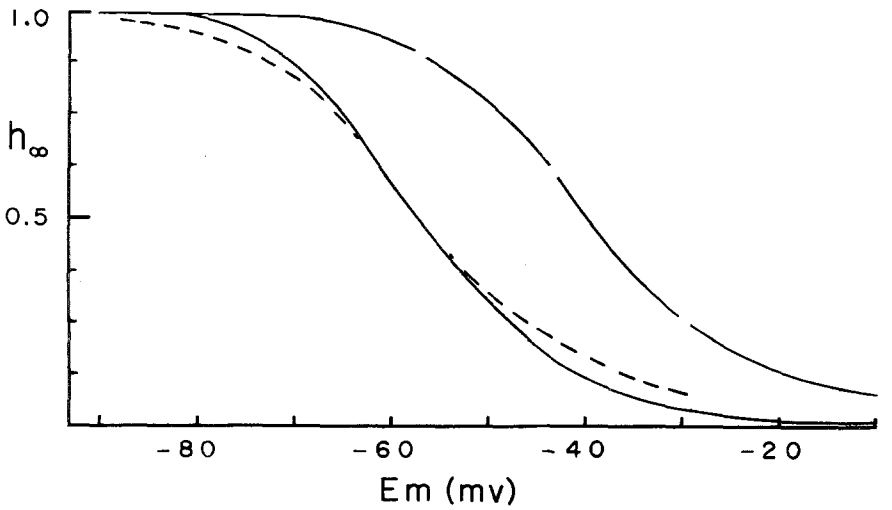

Fig. 8. h_∞ as a function of prepulse potential. Moore and Cox (1976) sodium conductance model. Solid curve: 10 mM external Ca^{++}; Broken curve: 100 mM external Ca^{++}; Dashed curve: 100 mM external Ca^{++} curve shifted 17 mV in hyperpolarizing direction on the voltage axis

The h' vs. voltage curve is similar to the experimental results (Fig. 4). However, the difference in amplitude between the computed 10 and 100 mM Ca^{++} curves for pulse more hyperpolarized than the holding potential (−60 mV) is about 15% larger than seen experimentally. The h_∞ vs. voltage curve for 100 mM Ca^{++} shows a shift in the depolarizing directions of about 17 mV at the point where $h_\infty = 0.5$ relative to the 10 mM Ca^{++} curve. The dotted curve is obtained by shifting the 100 mM Ca^{++} curve 17 mV in the hyperpolarized direction. The model predicts a slight flattening of the 100 mM Ca^{++} curve relative to the 10 mM^{++} curve, but the difference at $E_m = -80$ mV is about 4% for the model and 15% experimentally.

Fig. 9 shows the model calculations for h' as a function of prepulse durations for 10 and 100 mM Ca^{++} concentrations. Curves for both calcium concentrations display apparent exponential time courses for the two prepulses shown here, a prepulse to −80 mV and to −40 mV. The 1-1/e fraction of the steady state for each curve is shown by an arrow. The experimental data replotted on this figure for a prepulse to −40 mV in 100 mM Ca^{++} differs from the model in two respects. First, the development of inactivation is slower in high calcium, and, second, the time course of inactivation is markedly sigmoidal in comparison to the model prediction. A possibility exists that, since this model

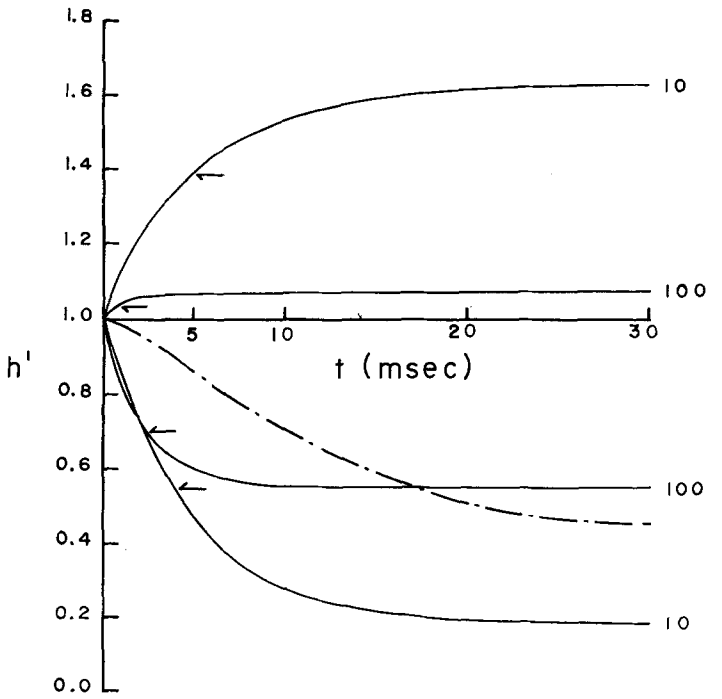

Fig. 9. h' as a function of prepulse duration for Moore and Cox (1976) model. Prepulse potential equals -80 mV for upper curves; -40 mV for lower curves. Arrows indicate 1-1/e of final h' value for each calculated curve. The number to the rigth of each curve gives the Ca^{++} concentration. The broken curve is the experimental curve for a prepulse potential to -40 mV in the 100 mM Ca^{++} solution

was simulated with the Hodgkin-Huxley voltage dependence for the rate constants, a better fit could be achieved if a different set of rate constants were used. At present this possibility has not been explored.

Several authors (Hoyt, 1963; Hoyt & Adelman, 1970; Goldman & Schauf, 1972; Goldman, 1975; Moore & Cox, 1976) have suggested that the development of inactivation may be linked to the rise of the sodium conductance. It is interesting to speculate that the increase in the delay for the development of inactivation may be related to the slower rise of the sodium conductance seen in higher concentrations of calcium. A rough estimate of this effect can be seen in the experimental time course of inactivation during depolarization. If the delay as a function of prepulse voltage is compared for a tenfold increase in calcium, a shift of roughly 10 mV in the depolarizing direction is observed.

Previous work (Frankenhaeuser & Hodgkin, 1957; Schauf, 1975) has shown that the h_∞ vs. voltage curve shows a shift of about one half

that seen for the peak sodium conductance vs. voltage curve for the same calcium concentration change. Thus the shift in the delay of the development of inactivation would be about 2 times smaller than expected if the delay increase is in fact a reflection of the calcium influence upon the rise of conductance. This possibility will be studied more thoroughly in the future.

Although the effect of varying magnesium upon inactivation has not been studied systematically here, it is apparent from comparisons of the ASW data with solutions containing only calcium that, for squid axon, magnesium is not an equimolar substitute for calcium. This observation confirms that of Frankenhaeuser and Hodgkin and suggests the interesting possibility of some fundamental differences in the action of divalent cations upon the conductances of the two species, *Loligo* and *Myxicola*. Thus, charge screening may be of greater importance in its effect upon the sodium conductance in *Myxicola* axon while for *Loligo* axon it may possibly have a secondary effect. Certainly, the demonstration of substitution equivalence for Mg^{++} and Ca^{++} in *Myxicola* lends strong support for a charge-screening action of these ions while in squid and lobster (Blaustein & Goldman, 1968) this equivalence does not appear to hold.

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