Inactivation of the Sodium Current

in *Myxicola* Giant Axons

Evidence for coupling
to the activation process

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ABSTRACT   Experiments were conducted on *Myxicola* giant axons to determine if the sodium activation and inactivation processes are coupled or independent. The main experimental approach was to examine the effects of changing test pulses on steady-state inactivation curves. Arguments were presented to show that in the presence of a residual uncompensated series resistance the interpretation of the results depends critically on the manner of conducting the experiment. Analytical and numerical calculations were presented to show that as long as test pulses are confined to an approximately linear negative conductance region of the sodium current-voltage characteristic, unambiguous interpretations can be made. When examined in the manner of Hodgkin and Huxley, inactivation in *Myxicola* is quantitatively similar to that described by the *h* variable in squid axons. However, when test pulses were increased along the linear negative region of the sodium current-voltage characteristic, steady-state inactivation curves translate to the right along the voltage axis. The shift in the inactivation curve is a linear function of the ratio of the sodium conductance of the test pulses, showing a 5.8 mv shift for a twofold increase in conductance. An independent line of evidence indicated that the early rate of development of inactivation is a function of the rise of the sodium conductance.

INTRODUCTION

Recently, Hoyt (1963, 1968) has proposed a formalism, alternative to that of Hodgkin and Huxley (1952 *b*), for describing the potential and time dependency of the sodium currents which may be observed under voltage clamp in squid giant axons. This alternative formalism is interesting in that it arises from a different mechanistic model than that of Hodgkin and Huxley. Specifically, while Hodgkin and Huxley regarded activation and inactivation as strictly independent processes, in the Hoyt model they are coupled. And,

Hoyt (1968) has claimed that there is a differential prediction. For the Hodgkin-Huxley formalism, Hoyt reckoned that the relation between the steady-state level of the inactivation variable, h_∞, and conditioning (pre-)potential is almost independent of the test pulse selected, while in the Hoyt model h_∞ (V) will shift to the right with larger (more depolarizing) test pulses, i.e., with a larger sodium conductance during the test pulse. Indeed, Hoyt and Adelman (1970) did see such h_∞ (V) shifts in data from squid axons.

A major criticism of the experiments of Hoyt and Adelman, however, is that the series resistance (Hodgkin et al., 1952) was not compensated for. Moreover, the effects of series resistance on a system obeying Hodgkin-Huxley kinetics were not included in Hoyt's calculations. As is shown below, with an uncompensated series resistance it is not possible to decide to what extent the shifts reported by Hoyt and Adelman were generated by the series resistance, and to what, if any, extent they indicate coupling of the activation and inactivation processes.

The question of whether the activation and inactivation of the sodium current generate from independent underlying events in separate structures or rather represent different states of the same molecular structure is an interesting one to resolve. It seemed worthwhile therefore to see if the criterion developed by Hoyt, i.e. shifts in the h_∞ (V) relation with changing test pulses, could actually be shown to be an unambiguous test of coupling, and if so to look experimentally for shifts in *Myxicola* axons. An independent test for coupling as proposed by Chandler et al. (1965) has also been made.

METHODS

All methods for preparing and voltage clamping *Myxicola* axons are as described by Binstock and Goldman (1969). The data reported here were obtained using compensated feedback (Hodgkin et al., 1952) to reduce the error due to the series resistance, R_s . For most preparations, including all the axons used for the Hoyt shift measurements, R_s was measured at the start of the experiment from the jump in the potential trace produced by brief constant current pulses. The mean value of R_s measured in this way was 13.4 ohm cm² with a range of 9.1–21.4 ohm cm². These values agree well with a mean of 16.5 ohm cm² and a range of 12.1–20.8 ohm cm² determined on an earlier series of preparations from the time constant of decay of the capacitative surge and the measured membrane capacity (mean of 0.76 μF/cm²) (Goldman and Binstock, unpublished observations).

The R_s compensation was adjusted, under clamp, by increasing the feedback compensation potentiometer to the point of ringing of the potential trace. Further compensation throws the system into oscillation. The R_s actually compensated for can be estimated from the dip in the potential trace and the membrane current at that time. This was generally about ⅔ of the measured R_s , leaving typically about 3–4 ohm cm² of uncompensated R_s . Since the effects of the R_s cannot usually be completely eliminated, it was necessary to compute the sort of effect a residual R_s might be expected

to have on h_∞ (V) relations determined with different test pulses. This is presented below (see Results).

For large depolarizing prepulses the measured value of the sodium current, I_{Na}, is very sensitive to the accuracy of the corrections for leak and potassium currents. For h_∞ curves the effect will be to shift the foot of the h_∞ (V) relation with changing test pulses (Jakobsson and Moore, 1971). We have corrected for this by repeating each measurement with an identical pulse schedule but in bathing media containing 10^{-6} M tetrodotoxin (TTX; Calbiochem, Los Angeles, Calif.). The currents in TTX were subtracted from the corresponding currents in artificial seawater to obtain relatively uncontaminated measurements of I_{Na}. All of the observations reported below are on such corrected I_{Na}'s.

We have found I_{Na} in *Myxicola* to be somewhat labile. Lability was reduced by waiting 15 sec between each voltage-clamp pulse. In addition, each individual clamp run was bracketed with observations made with identical pulses. These reference currents often drifted down by as much as 10–15% during a given run. A linear correction in time for this drift was routinely applied.

Potentials are reported as absolute membrane potential (inside minus outside), and are corrected for liquid junction potentials according to the values of Cole and Moore (1960; see also Binstock and Goldman, 1971). Artificial seawater had the following composition: 430 mM Na, 10 mM K, 10 mM Ca, 50 mM Mg, 560 mM Cl, 5 mM tris(hydroxymethyl)aminomethane, pH 8.0 ± 0.1. Temperature was 5 ± 0.5°C unless otherwise noted.

RESULTS

h-Type Inactivation in Myxicola

In this section we present observations on the effects of conditioning (pre-) pulses of various potentials and durations on the I_{Na} produced by a fixed test pulse. For these observations the test pulse potential was always well into the region of the positive limb of the Na current-voltage, I_{Na} (V), characteristic (i.e., the region of positive slope). An analysis of the data has been made by assuming that the effects of the prepulses can be described by a variable, h, defined according to

$$\frac{dh}{dt} = \alpha_h(1 - h) - \beta_h h, \tag{1}$$

where α_h and β_h are voltage-dependent rate constants and t is time. The aim of these experiments was to demonstrate that inactivation of the sodium current in *Myxicola* axons is quantitatively similar to that in squid (Hodgkin and Huxley, 1952 *b*).

In Fig. 1, I_{Na} relative to that with no prepulse (I_{Na}/I_{Na_0}) is shown as a function of prepulse duration for each of several prepulse potentials (numbers to the right of each curve) in a single axon. The solid lines are drawn ac-

cording to

$$I_{Na}/I_{Na_0} = (I_{Na}/I_{Na_0})_\infty - [(I_{Na}/I_{Na_0})_\infty - 1]e^{-t/\tau_h} \tag{2}$$

where $(I_{Na}/I_{Na_0})_\infty$ is the steady-state (i.e., 80 msec) value, t is prepulse duration, and τ_h is the time constant of inactivation. Equation 2 is a solution of equation 1 and is the expression used by Hodgkin and Huxley (1952 *a*) to describe similar observations in squid axons.

In Fig. 2, τ_h is shown as a function of prepulse potential. These data are pooled from five axons. The solid line is computed from equations 4, 6, and 7 below. The relationship between τ_h and V is generally similar to that seen in squid axons (see, for example, Chandler et al., 1965).
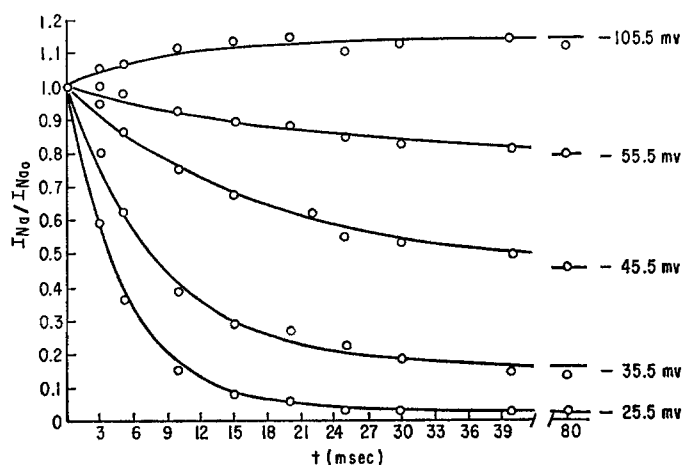

FIGURE 1. Sodium current, relative to that with no prepulse (I_{Na}/I_{Na_0}) as a function of prepulse duration, for each of several prepulse potentials (numbers to the right of each curve). The solid lines are drawn according to

$$I_{Na}/I_{Na_0} = (I_{Na}/I_{Na_0})_\infty - [(I_{Na}/I_{Na_0})_\infty - 1]e^{-t/\tau_h}.$$

Fig. 3 shows the steady-state level of h (i.e., I_{Na} following a long prepulse relative to the maximum I_{Na} obtainable with that test pulse $I_{Na}/I_{Na_{max}}$) as a function of prepulse potential in a single *Myxicola* axon. The solid line is drawn according to

$$h_\infty = \frac{1}{1 + e^{\frac{V_h - V}{k_h}}}, \tag{3}$$

where V_h is the potential at which h_∞ is 0.5 and k_h is a shape parameter. Equation 3 is, again, identical to the expression used by Hodgkin and Huxley (1952 *a*) to describe their h_∞ data in squid. V_h ranged from -46 to -53 mv with a mean of -48.5 mv (mean of seven axons). k_h had a mean value of
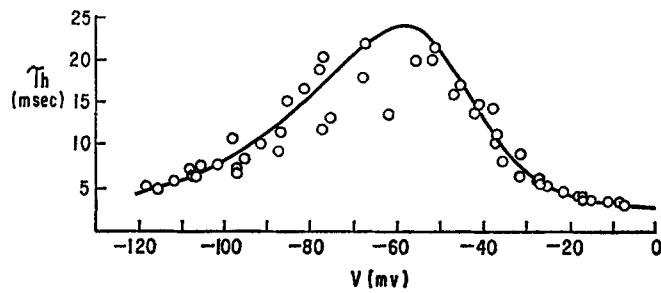
FIGURE 2.   Time constant of inactivation (τ_h) as a function of prepulse potential. Data were pooled from five axons. The solid line is computed from equations 4, 6, and 7 in the text.
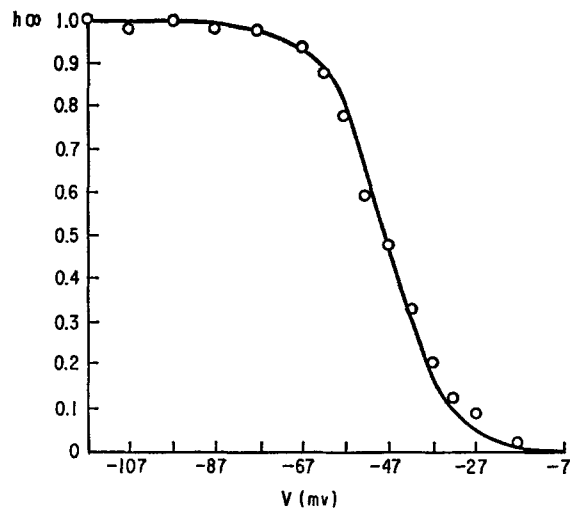

FIGURE 3.   Steady-state inactivation (h_∞) as a function of prepulse potential. The solid line was drawn according to

$$h_\infty = [e^{(-48-V)/-6.9} + 1]^{-1}.$$

−7.9 mv with a range of ±1.3 mv. With these mean values of V_h and k_h, resting inactivation is about 0.9 at a resting potential of −65 mv.

From equation 1,

$$\tau_h = \frac{1}{\alpha_h + \beta_h} \tag{4}$$

and

$$h_\infty = \frac{\alpha_h}{\alpha_h + \beta_h}. \tag{5}$$

Fig. 4 shows α_h (open circles) and β_h (filled circles) computed from equations

4 and 5 as a function of potential. These data are pooled from the same five axons of Fig. 2. The solid lines are drawn according to

$$\alpha_h = 0.0051 \ e^{-V/31.4} \tag{6}$$

and

$$\beta_h = \cfrac{1}{3(e^{\frac{V+25.5}{-9.2}} + 1)}. \tag{7}$$

These expressions, which have been used to compute the solid line of Fig. 2,
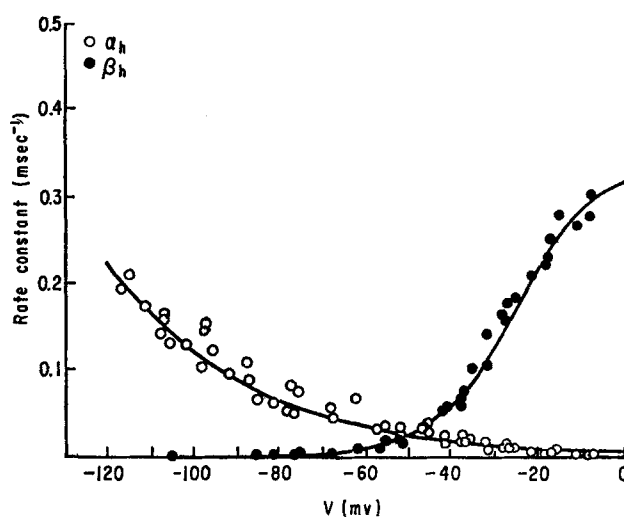

FIGURE 4.   Rate constants of inactivation, α_h (open circles) and β_h (filled circles) as functions of potential. Data were pooled from five axons. The solid lines are drawn according to

$$\alpha_h = 0.0051 \ e^{-V/31.4}$$

and

$$\beta_h = [3(e^{(V+25.5)/-9.2} + 1)]^{-1}.$$

are also identical in form to those used by Hodgkin and Huxley (1952 b) to describe α_h and β_h functions in squid axons.

Equations 6 and 7 together with equation 1 provide a complete description of h as a function of potential and time over that potential range for which they have been tested, and indicate that inactivation of the I_{Na} in *Myxicola* is quantitatively very similar to that in squid axons.

The h_∞ (V) Relation as a Function of Test Pulse Potential

INTERPRETATION OF SHIFTS IN THE h_∞ (V) RELATION   The Hoyt criterion for determining coupling of the activation and inactivation processes is that h_∞ (V) curves shift to the right with more depolarizing test pulses (larger sodium conductance during the test pulse). To evaluate experimental results it is first necessary to determine what sort of behavior would be expected from a system obeying Hodgkin-Huxley (independent) kinetics. Problems arise primarily because the experimental h_∞ (V) relation obtained as described in the previous section is, strictly, an approximation to the true h_∞ (V) function. The measurement is of peak $I_{Na}/I_{Na_{max}}$ during the test pulse. In general, at the time of peak I_{Na}, h will have declined from the value it had at the end of the prepulse, while the Na activation variable, m, will have not yet reached its steady-state value.

In the absence of an R_s, Hoyt (1968) has already pointed out that as long as h_∞ during all *test* pulses remains close to zero, the Hodgkin-Huxley equations predict that h_∞ (V) will shift slightly to the left with more depolarizing test pulses, i.e., in the direction opposite to that predicted by the Hoyt model. Moreover, the effect will be pronounced only for small values of h_∞ as it is due to the increase in m_0 of the test pulse with strong depolarizing prepulses and the reduction in significance of this effect with larger test pulses (i.e., with larger m_∞ values). However, complete compensation for R_s is not usually possible (see Methods), and it is necessary, then, to determine the effect of an R_s on these measurements.

The time constants for activation and inactivation, τ_m and τ_h, as well as h and m, are all functions of potential. To predict correctly the effects of different test pulses on h_∞ (V) curves on a system obeying Hodgkin-Huxley kinetics, it is necessary to solve numerically the full Hodgkin-Huxley equations, in the presence of some R_s, for every test pulse of interest. However, we can fairly readily gain some insight into the effects of R_s on h_∞ curves if we make the approximation that τ_m is always $\ll\tau_h$. This is equivalent to assuming that the effects of the test pulse potential on m^3 will dominate over those on τ_m/τ_h.

With a residual uncompensated R_s the potential of the test pulse selected for the determination of the h_∞ (V) relation will not be the measured test pulse potential, V_t, but will be displaced from V_t by some amount ΔV. ΔV, moreover, will diminish as h_∞ decreases as it is determined entirely by the magnitude of the current during the test pulse and the fixed series resistance. The problem therefore is to determine the effect of the series resistance on the h_∞ curves for any given test pulse and to compare these effects on test pulses from various different regions of the sodium current-voltage

characteristic. The very small effect of R_s on the prepulse potential will not be considered as this effect will not shift h_∞ relations determined with different test pulse potentials relative to one another.

If we consider only inward I_{Na}, then for peak sodium current during the test pulse

$$I_{Na} = h_o g'_{Na}(V_t - \Delta V - V_{Na}) \qquad (8)$$

where V_{Na} is the sodium equilibrium potential, g'_{Na} is the sodium conductance (here exclusive of inactivation, i.e. $\overline{g_{Na}}m^3$ in the Hodgkin-Huxley notation), and h_o is the actual value of h_∞ at the end of the prepulse. Note that we subtract the negative displacement potential (ΔV) to allow for the effect under voltage clamp. For $I_{Na_{max}}$

$$I_{Na_{max}} = g'_{Na_{max}}(V_t - \Delta V_{max} - V_{Na}). \qquad (9)$$

Dividing equation 8 by equation 9, noting that

$$\Delta V = I_{Na}R_s,$$

we have

$$\frac{I_{Na}}{I_{Na_{max}}} = h_{\infty(observed)} = \frac{g'_{Na}}{g'_{Na_{max}}} \left(\frac{V_t - I_{Na}R_s - V_{Na}}{V_t - I_{Na_{max}}R_s - V_{Na}} \right) h_{\infty(true)}. \qquad (10)$$

Consider first the special case that g_{Na} is constant. This corresponds to confining test pulses to that region of the sodium current-voltage characteristic where g_{Na} has reached its maximum value, and is a reasonable approximation for test pulses entirely on the positive limb of the sodium characteristic. Equation 10 becomes

$$h_{\infty(observed)} \left(\frac{V_t - I_{Na_{max}}R_s - V_{Na}}{V_t - I_{Na}R_s - V_{Na}} \right) = h_{\infty(true)}. \qquad (11)$$

The coefficient of $h_{\infty(observed)}$ in equation 11 is always ≤ 1. Therefore $h_{\infty(observed)}$ is always $\geq h_{\infty(true)}$, and measured h_∞ (V) curves shift to the right as R_s increases. The magnitude of this effect is illustrated in Fig. 5 a. The solid line is an experimental h_∞ (V) curve. The dotted line is the true h_∞ (V) curve computed from the experimental assuming an $I_{Na_{max}}$ of 1 ma/cm^2, V_{Na} of 70 mv (Goldman and Binstock, 1969), and R_s of 10 ohm cm^2, which would be a very large value for a residual uncompensated R_s. The effect is clearly very small, and is independent of test pulse anywhere in the positive linear region of the I_{Na} (V), as may be readily seen by setting the coefficients of $h_{\infty(observed)}$ in equation 11 equal for two test pulses.
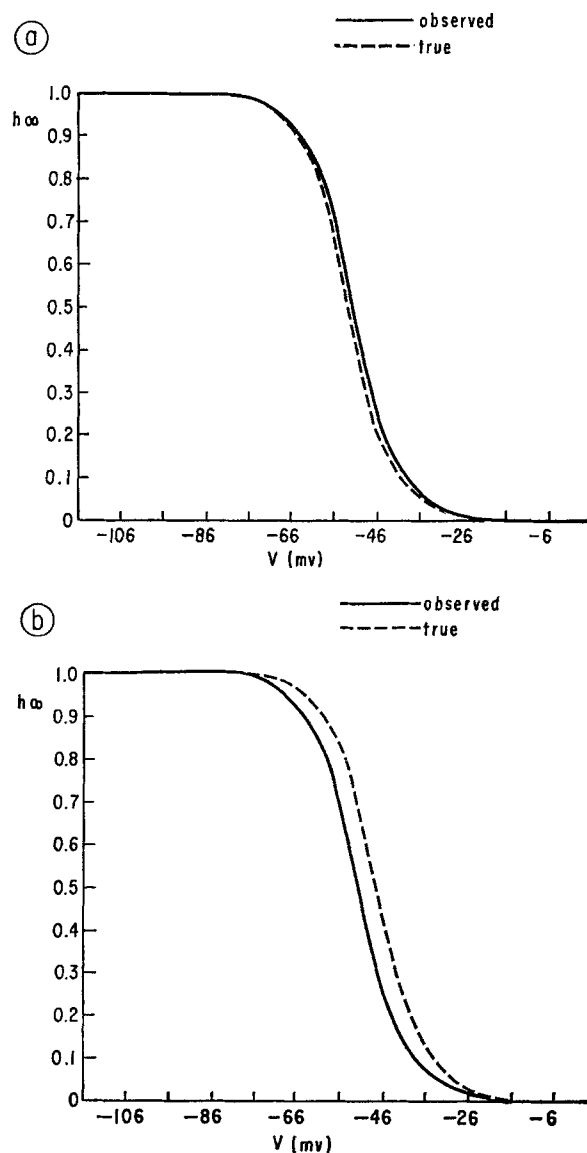
FIGURE 5. A comparison of observed (solid line) and true (dashed line) steady-state inactivation curves computed for a series resistance of 10 ohm cm² and a maximum sodium current of 1 ma/cm². (*b*) is for a test pulse in the region of the negative limb of the sodium current-voltage characteristic. (*a*) is for a test pulse in a positive linear region.

Consider now the general case where a displacement in test pulse potential due to series resistance will change g_{Na}. This is the condition required for the negative limb of the sodium characteristic. For the negative limb $(g'_{Na_{max}}/g'_{Na})$ is always ≥ 1. Therefore experimental h_∞ (V) curves are shifted

to the left. The magnitude of this effect is illustrated in Fig. 5 b. As in Fig. 5 a, the solid curve is the experimental h_∞ (V) relation with an $I_{Na_{max}}$ of 1 ma/cm^2. The dashed curve which is appreciably shifted to the right is the true h_∞ (V) computed from the solid curve assuming again an R_s of 10 ohm cm^2 and taking ($g'_{Na_{max}}/g'_{Na}$) from experimental I_{Na} (V) data. Note that the effect expressed by the coefficient of $h_{\infty(observed)}$ in equation 11 will always be dominated by the conductance ratio for the negative limb. Changes of the coefficient have been neglected in computing the dashed curve of Fig. 5 b. The error introduced will be less than the difference between the solid and dashed curves of Fig. 5 a.

The significance of these effects for the problem of discriminating between Hoyt and Hodgkin-Huxley kinetics is as follows. If the least depolarizing test pulse is on the negative limb (as it must be to demonstrate a Hoyt shift, i.e., one cannot be in a flat region of the conductance-voltage curve), and larger depolarizing test pulses are on the positive limb or in any much less steep region of the conductance-voltage curve, then a residual R_s will produce an apparent Hoyt shift (to the right for larger depolarizing test pulses) even in a system obeying Hodgkin-Huxley kinetics. Moreover as large depolarizing test pulses are continued along the positive limb the shift may saturate. This would also be in apparent agreement with the Hoyt kinetics. The shifts reported by Hoyt and Adelman (1970) seem not to be unambiguously interpretable for just this reason, i.e., there is a shift of unknown magnitude caused by the R_s.

If the larger test pulse is also on the negative limb of the I_{Na} (V) somewhat different results are obtained depending on the exact form of the I_{Na} (V). For test pulses in a linear region of the I_{Na} (V) there is no shift in the h_∞ (V) curve with changing test pulse. If, rather, in this region the g_{Na}-voltage relation is linear there is a small effect of the test pulse as required by the coefficient of $h_{\infty(observed)}$ in equation 11. The shift produced will be less than that shown in Fig. 5 a and is not experimentally detectable. Of course, if the smaller test pulse is on a less steep region of the g_{Na}-voltage function than the larger, then a shift opposite to that predicted by the Hoyt model will be obtained. In general, as long as the larger test pulse is in a region of the g_{Na}-voltage function at least as steep as that for the smaller test pulse, Hodgkin-Huxley kinetics predict that Hoyt-type shifts will not be observed experimentally.

However, calculations of this sort are only approximate as ΔV is a function of time, and, in addition to the magnitude, the time-course of g'_{Na} depends on the residual R_s. It is necessary therefore to simulate the h_∞ measurements by numerical integration of the Hodgkin-Huxley equations in order to make definitive statements.

Dr. John W. Moore has carried out such studies and has supplied us with his results. The h_∞ curves were computed assuming an R_s of 7 ohm cm^2 and

fitted, also by computer, with an expression of the form of equation 3. Best fit values of V_h and k_h were printed out for each curve. For test pulse displacements from holding of +20 and +30 mv, which span an approximately linear region of the negative conductance characteristic, the computed V_h was found to increase by only 1.4 mv and k_h by about 3% for the larger over the smaller test pulse. The effect is somewhat less if h_∞ values less than 0.01 are neglected. Effects of this magnitude are not experimentally detectable and for all practical purposes these computed curves are indistinguishable. The simulated h_∞ curves were computed with an R_s of 7 ohm cm². In the experiments reported below the residual R_s ranged from 1.1 to a maximum of 5.7 ohm cm², and the sodium currents were somewhat less than for the simulations. It seems very unlikely, then, that the residual R_s in our experiments has introduced any detectable effect

If the larger test pulse for the simulated h_∞ curve is moved into a saturating region of the g_{Na}-voltage curve a clear Hoyt-type shift is seen. The shift increases with test pulse potentials up to +70 mv and saturates at a value of 4 mv. A larger R_s does produce a larger shift. With an R_s of 14 ohm cm² there is a Hoyt-type shift of 6.1 mv between test pulses of +20 and +30 mv (these test pulses no longer span a linear negative conductance region as the +30 mv pulse is now in a saturating region of the g_{Na}-voltage curve) and a shift of 10 mv between test pulses of +20 and +70 mv. In summary, an experimental demonstration of a Hoyt shift for two test pulses in a linear negative slope region of the I_{Na} (V) should indicate coupling between the activation and inactivation processes.

DEMONSTRATION OF SHIFTS IN THE h_∞ (V) RELATION   Fig. 6 *a* shows two experimental h_∞ (V) curves from the same axon determined with test pulses of 11.5 and 31.5 mv. The curves superimpose as required by Hodgkin-Huxley kinetics and are consistent with the results of Chandler and Meves (1965). These results are also consistent with the Hoyt model as both test pulses are well into the positive limb of the I_{Na} (V) (right-hand arrows in Fig. 7), and g_{Na} during the two different test pulses will be the same.

When two more h_∞ (V) curves were determined in this same axon (Fig. 6 *b*) with test pulses now in a linear negative conductance region (Fig. 7, left-hand arrows) a clear effect was seen. The h_∞ (V) curve indicated by the filled circles in Fig. 6 *b* (−18.5 mv test pulse) is shifted to the right by 9 mv relative to the h_∞ (V) curve indicated by the open circles (−33.5 mv test pulse).

Hodgkin-Huxley kinetics, even with an uncompensated R_s, predict that h_∞ (V) curves should be essentially independent of the g_{Na} during the test pulse in this region. The shift, then, is generated by something not included in the Hodgkin-Huxley equations, very likely that the inactivation state is a function of the activation state and the processes are coupled.
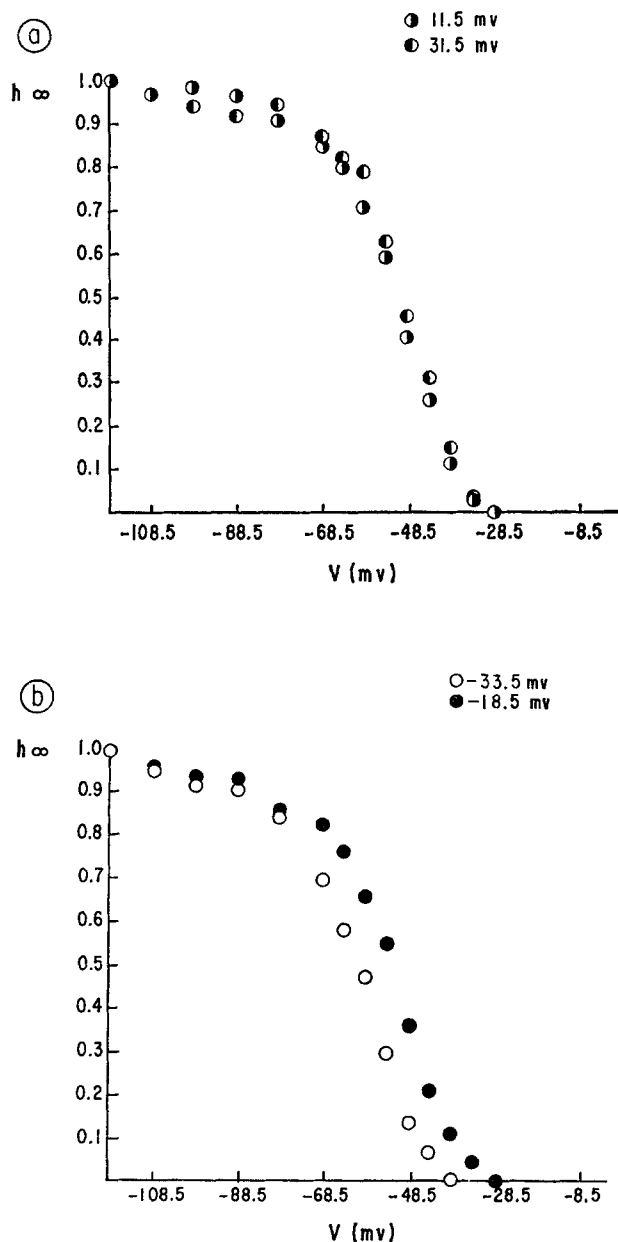
FIGURE 6. Experimental steady-state inactivation curves from a single *Myxicola* axon. (*a*) is for two test pulses in the positive linear region of the sodium current-voltage characteristic. (*b*) is for two test pulses in a negative linear region of the characteristic. Note in (*b*) that the curve described by the open circles can be made to superimpose with that of the filled circles by translating it 9 mv along the voltage axis.

Several features of the data of Fig. 6 *b* are worth noting. First, the shift is fairly substantial (9 mv for a 2.46-fold increase in g_{Na} during the test pulse). Even larger shifts have been seen. Second, the effect of changing the test pulse is simply to translate the whole h_∞ curve along the voltage axis. This was true in each of the four axons examined. It was not necessary therefore to weight very small values of h_∞ in determining the magnitude of the shift. Third, the shift along the voltage axis is reproducible from axon to axon. In Fig. 8 the h_∞ shift is shown as a function of the g_{Na} ratio of the two test pulses. Data are available only over a rather limited range but the function is clearly linear. The solid line in Fig. 8 indicates a 5.8 mv shift for a twofold increase in conductance of the test pulse. In no case was there a discrepancy of more than 10% from this correspondence.
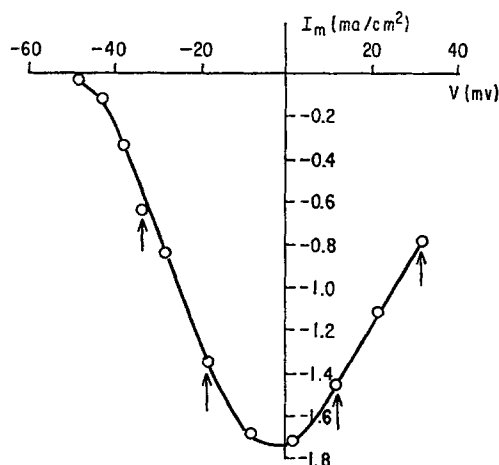

FIGURE 7. Na current-voltage characteristic of the axon of Fig. 6. Arrows indicate the potentials of the test pulses of Fig. 6.

This quantitative agreement between preparations is further confirmation that the shifts are not generated artefactually by an uncompensated R_s. One of the axons for which data are shown in Fig. 8 had a residual R_s of only 1.1 ohm cm². With an $I_{Na_{max}}$ of < 1 ma/cm² this should produce very little error. Another had a residual R_s of 5.7 ohm cm² indicating that the shift is not correlated with R_s. The data of Fig. 8 are also interesting in that they define in a simple way something about the nature of the coupling.

It is somewhat difficult experimentally to extend the range of these observations. Small test pulses must be well onto the linear negative region of the I_{Na} (V) to give stable currents, and large test pulses must be below the region of a saturating g_{Na}-voltage relation. This puts a limit to how large a conductance ratio can be tested and small ratios require the detection of shifts of a very few millivolts.

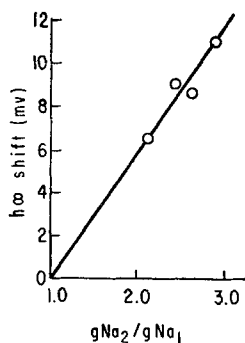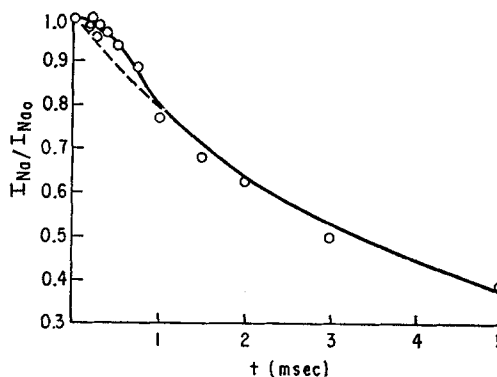FIGURE 8   FIGURE 9

FIGURE 8.   Shift of the steady-state inactivation curve as a function of the Na conductance ratio of the test pulses. The solid line has been drawn to indicate no shift for a conductance ratio of 1.

FIGURE 9.   Sodium current relative to that with no prepulse (I_{Na}/I_{Na_o}) as a function of prepulse duration. The dashed line (solid where not different) is drawn according to

$$I_{Na}/I_{Na_o} = 0.167 + 0.833\ e^{-t/3.64}.$$

The solid line where different from the dashed is drawn by eye for visual reference. Test and prepulse potential were −20.5 mv, the interval between them was 5 msec, and temperature was 5°C.

The Effect of the Rise in g_{Na} of the Prepulse on the Rate of Inactivation

Chandler et al. (1965) have argued that if the activation and inactivation processes are coupled one might expect to see a delay in the development of inactivation, i.e. some sort of shoulder in the I_{Na}/I_{Na_o} (t) curve (Fig. 1), owing to the time taken for the rise of g_{Na} during the prepulse. For independent kinetics no appreciable delay is expected. Although the failure to detect a delay may not be a critical test of coupling (Hoyt, 1968) it seemed of interest to look for such delays in *Myxicola*.

The experimental design was similar to that of the two-pulse experiment of Chandler et al. (1965). Test and prepulse potentials were fixed and prepulse duration varied. A fixed delay, i.e. a step back to the holding potential of fixed duration, was included between the pre- and test pulses. The delay was selected to be long relative to τ_m. In this way I_{Na}/I_{Na_o} could be measured for very short prepulse durations.

In Fig. 9 the data of one such experiment are illustrated. The potential of both the pre- and test pulses was −20.5 mv. The dashed line (solid where they are not different) is drawn according to

$$I_{Na}/I_{Na_o} = 0.167 + 0.833\ e^{-t/3.64}.$$

The solid line, where different from the dashed, has been drawn by eye for

visual reference. There is a clear delay in the I_{Na}/I_{Na_o} (t) curve consistent with the results of Armstrong (1970) on *Dosidicus* axons. Values for brief pre-pulses differ from I_{Na_o} less than expected for the simple exponential decay which characterizes the function for long prepulses (see also Fig. 1). Each of the four axons tested showed such shoulders, providing an independent line of evidence for activation-inactivation coupling. However, there is no obvious delay in the data of Chandler et al. (1965). The reason for this difference be-tween the various observations is not clear (however, see Hoyt, 1968).

The time to peak of the I_{Na} for this experiment was 1.3 msec, which is very similar to that of the experiment illustrated by Chandler et al. Note that the duration of the delay in Fig. 9 is substantially less than that expected for the specific coupling model considered by these authors.

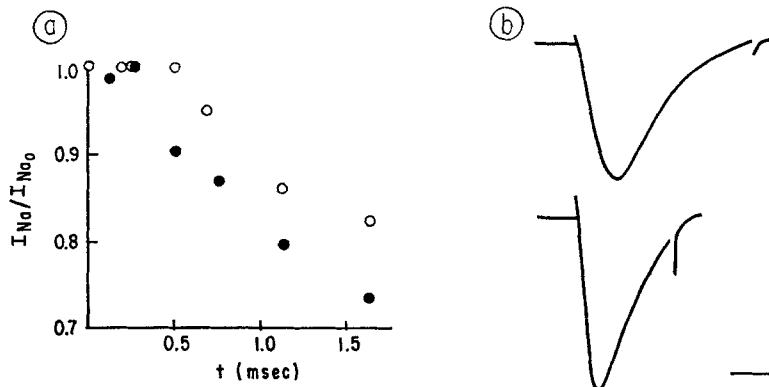It is somewhat difficult to pick quantitative features of the delay to corre-

Figure 10.   (a) I_{Na}/I_{Na_o} as a function of prepulse duration for prepulse potentials of −14.5 mv (open circles) and 5.5 mv (filled circles). (b) time-course of the I_{Na} during the two prepulses. The upper tracing corresponds to the open circles in (a) and the lower tracing to the filled circles. Test pulse potential was −14.5 mv, the interval be-tween pulses was 7.5 msec, and temperature was 2°C for both sets of observations. Scale: 0.15 ma/cm², 2.5 msec.

late with the rise of g_{Na} of the prepulse. However, some information may be gained from the experiment of Fig. 10. In Fig. 10 a we see two I_{Na}/I_{Na_o} (t) curves again from a two-pulse experiment and in Fig. 10 b the corresponding I_{Na} currents during the prepulses are shown. For the first run (open circles in Fig. 10 a and upper trace in Fig. 10 b) the test and prepulse potentials were both −14.5 mv. The second run (filled circles and lower trace) is from the same axon, with the same test pulse potential and the same delay between pre- and test pulses but with a prepulse potential of 5.5 mv. Temperature was 2°C.

The rate of inactivation increases with the increased prepulse potential in the usual way. However, the duration of the initial plateau also decreases with the larger prepulse (but was found to be independent of the test pulse

potential). The ratio of the duration of the initial plateaus (0.25 and 0.5 msec) is roughly similar to the ratio of the times to peak of the I_{Na} during the prepulses (1.25 and 2.75 msec). This suggests that these brief plateaus are in fact generated in some way by the time taken for the development of the g_{Na} of the prepulse, and that the delays in the I_{Na}/I_{Na_0} (t) curve are possibly some function of the early rate of rise of the g_{Na} during the prepulse.

DISCUSSION

The inactivation of the I_{Na} in *Myxicola* giant axons seems to be coupled to the activation process. The measured values of h_∞ depend on the g_{Na} during the test pulse, and the early rate of development of inactivation is some function of the rise of g_{Na} during the prepulse. If the g_{Na} is taken as a measure of the activation of the transient channels, then these results indicate that the inactivation state is a function of the activation state. Activation and inactivation are therefore not independent processes, but rather represent different states of a single system. In the light of these observations it seems likely that the $h_\infty(V)$ shifts in squid axons reported by Hoyt and Adelman (1970) are due at least in part to activation-inactivation coupling also.

The shift of the $h_\infty(V)$ relation along the voltage axis is a linear function of the g_{Na} ratio during the test pulses, showing about a 6 mv shift for a twofold increase in g_{Na}. This provides, in a simple way, information about the nature of the coupling. One would like to see models for excitation account for this relationship.

Chandler et al. (1965) have discussed a specific coupling model: the rate of inactivation is proportional to the g_{Na} during the prepulse. This requires that the I_{Na}/I_{Na_0} (t) curve show simple exponential decay only for times longer than that needed to reach peak I_{Na} of the prepulse and that its slope be maximum at the time of peak prepulse I_{Na}. The data of Figs. 9 and 10 are inconsistent with this model. Moreover, the Chandler et al. model does not predict a translation of the $h_\infty(V)$ along the voltage axis with changing test pulse.

Lastly, it might be pointed out that there is an alternative interpretation to these data, that the sodium conductance is governed by two or more independent variables the behaviors of which are not now known, but which are quite different from the h and m functions proposed by Hodgkin and Huxley. In this view, experimental demonstrations of a dependency of inactivation on activation would arise somehow artefactually from the experimental procedures. With either view it is equally required that m and h cannot be reflective of the underlying physical variable or variables.

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