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THE DUAL EFFECT OF MEMBRANE POTENTIAL ON SODIUM CONDUCTANCE IN THE GIANT AXON OF *LOLIGO*

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This paper contains a further account of the electrical properties of the giant axon of *Loligo*. It deals with the 'inactivation' process which gradually reduces sodium permeability after it has undergone the initial rise associated with depolarization. Experiments described previously (Hodgkin & Huxley, 1952*a*, *b*) show that the sodium conductance always declines from its initial maximum, but they leave a number of important points unresolved. Thus they give no information about the rate at which repolarization restores the ability of the membrane to respond with its characteristic increase of sodium conductance. Nor do they provide much quantitative evidence about the influence of membrane potential on the process responsible for inactivation. These are the main problems with which this paper is concerned. The experimental method needs no special description, since it was essentially the same as that used previously (Hodgkin, Huxley & Katz, 1952; Hodgkin & Huxley, 1952*b*).

RESULTS

The influence of a small change in membrane potential on the ability of the membrane to undergo its increase in sodium permeability is illustrated by Fig. 1. In this experiment the membrane potential was changed in two steps. The amplitude of the first step was −8 mV. and its duration varied between 0 and 50 msec. This step will be called the conditioning voltage (V_1). It was followed by a second step called the test voltage (V_2) which was kept at a constant amplitude of −44 mV.

Record *A* gives the current observed with the test voltage alone. *B–F* show the effect of preceding this by a conditioning pulse of varying duration. Although the depolarization of 8 mV. was not associated with any appreciable inward current it greatly altered the subsequent response of the nerve. Thus, if the conditioning voltage lasted longer than 20 msec., it reduced the inward

current during the test pulse by about 40%. At intermediate durations the inward current decreased along a smooth exponential curve with a time constant of about 7 msec. The outward current, on the other hand, evidently behaved in a different manner; for it may be seen to approach a final level which was independent of the duration of the conditioning step. This is consistent with the observation that depolarization is associated with a maintained increase in potassium conductance (Hodgkin & Huxley, 1952*a*).

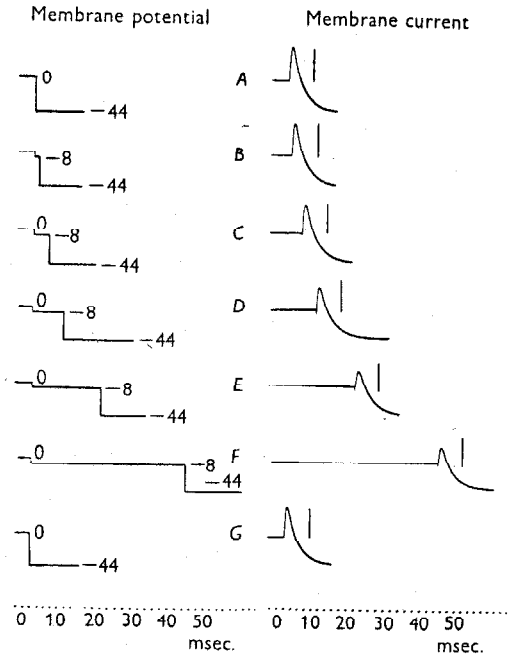

Fig. 1. Development of 'inactivation' during constant depolarization of 8 mV. Left-hand column: time course of membrane potential (the numbers show the displacement of the membrane potential from its resting value in mV.). Right-hand column: time course of membrane current density. Inward current is shown as an upward deflexion. (The vertical lines show the 'sodium current' expected in the absence of a conditioning step; they vary between 1·03 mA./cm.^{2} in *A* and 0·87 mA./cm.^{2} in *G*). Axon 38; compensated feed-back; temperature 5° C.

Fig. 2 illustrates the converse process of raising the membrane potential before applying the test pulse. In this case the conditioning voltage improved the state of the nerve for the inward current increased by about 70% if the first step lasted longer than 15 msec. This finding is not altogether surprising, for the resting potential of isolated squid axons is less than that of other excitable cells (Hodgkin, 1951) and is probably lower than that in the living animal.

A convenient way of expressing these results is to plot the amplitude of the sodium current during the test pulse against the duration of the conditioning

pulse. For this purpose we used the simple method of measurement illustrated by Fig. 3 (inset). This procedure avoids the error introduced by variations of potassium conductance during the first step and should give reasonable results for $V > -15$ mV. With larger depolarizations both the method of measurement and the interpretation of the results become somewhat doubtful, since there may be appreciable sodium current during the conditioning period. Two

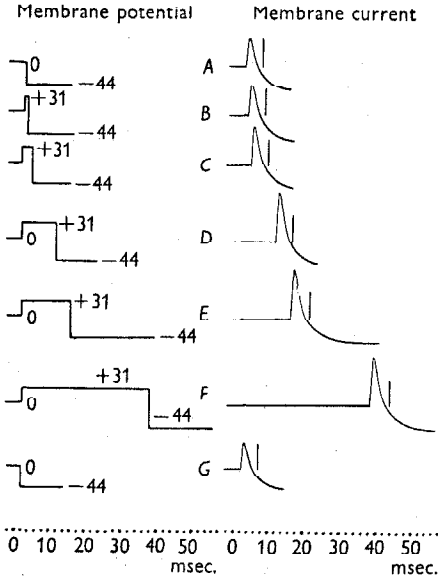

Fig. 2. Removal of 'inactivation' at membrane potential of +31 mV. Experimental details are as in Fig. 1. The vertical lines show the 'sodium current' with no conditioning step; they vary between 0·82 mA./cm.² in *A* and 0·75 mA./cm.² in *G*.

of the curves in Fig. 3 were obtained from the families of records illustrated in Figs. 1 and 2. The other two were determined from similar families obtained on the same axon. All four curves show that inactivation developed or was removed in an approximately exponential manner with a time constant which varied with membrane potential and had a maximum near $V = 0$. They also indicate that inactivation tended to a definite steady level at any particular membrane potential. Values of the exponential time-constant (τ_h) of the inactivation process are given in Table 1.

The influence of membrane potential on the steady level of inactivation is illustrated by the records in Fig. 4. In this experiment the conditioning step lasted long enough to allow inactivation to attain its final level at all voltages. Its amplitude was varied between +46 and −29 mV., while that of the test step was again kept constant at −44 mV. The effect of a small progressive change was allowed for in calculating the vertical lines on each record. These give the inward current expected in the absence of a conditioning step and

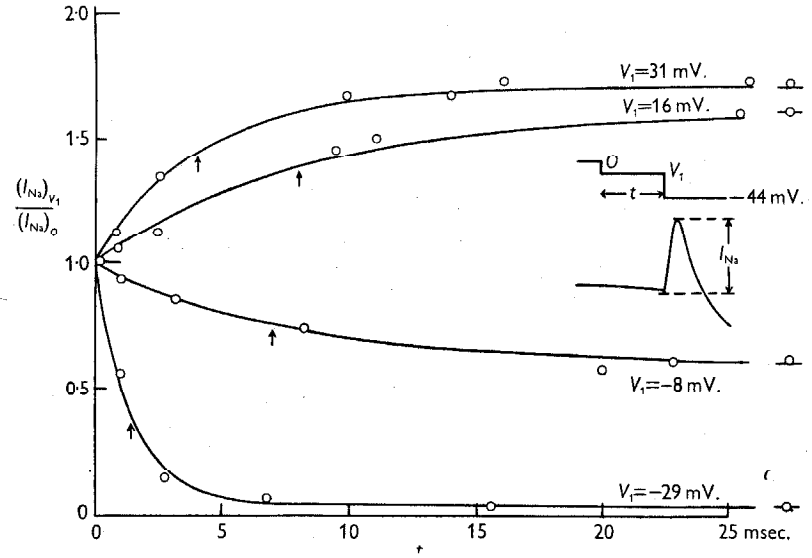

Fig. 3. Time course of inactivation at four different membrane potentials. Abscissa: duration of conditioning step. Ordinate: circles, sodium current (measured as inset) relative to normal sodium current; smooth curve, $y = y_\infty - (y_\infty - 1)\exp(-t/\tau_h)$, where y_∞ is the ordinate at $t = \infty$ and τ_h is the time constant (shown by arrows). Experimental details as in Figs. 1 and 2.

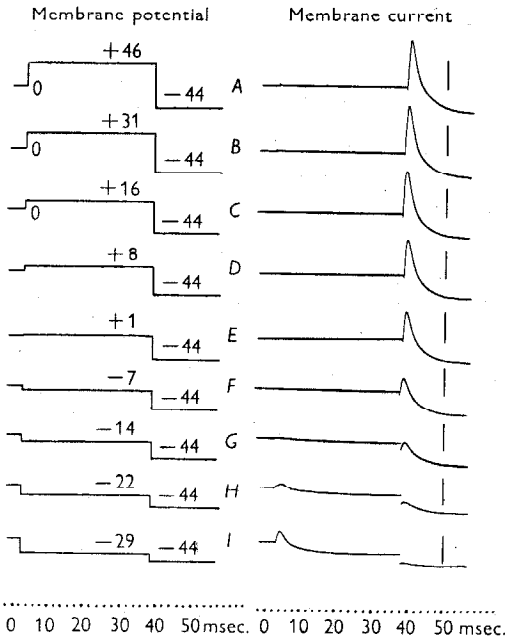

Fig. 4. Influence of membrane potential on 'inactivation' in the steady state. Experimental details are as in Fig. 1. The vertical lines show the sodium current with no conditioning step; they vary between 0·74 mA./cm.^{2} in *A* and 0·70 mA./cm.^{2} in *I*.

were obtained by interpolating between records made with the test step alone. The conditioning voltage clearly had a marked influence on the inward current during the second step, for the amplitude of the sodium current varied between 1·3 mA./cm.^{2} with $V_1 = +46$ mV. and about 0·03 mA./cm.^{2} with $V_1 = -29$ mV.

The quantitative relation between the sodium current during the test pulse and the membrane potential during the conditioning period is given in Fig. 5.

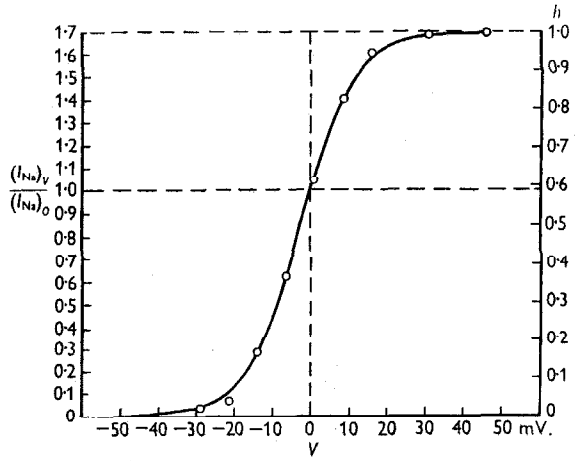

Fig. 5. Influence of membrane potential on 'inactivation' in the steady state. Abscissa: displacement of membrane potential from its resting value during conditioning step. Ordinate: circles, sodium current during test step relative to sodium current in unconditioned test step (left-hand scale) or relative to maximum sodium current (right-hand scale). The smooth curve was drawn according to equation 1 with a value of −2·5 mV. for V_h. This graph is based on the records illustrated in Fig. 4. Sodium currents were determined in the manner shown in Fig. 3.

This shows that the two variables are related by a smooth symmetrical curve which has a definite limiting value at large membrane potentials. In discussing this curve it is convenient to adopt the following nomenclature. We shall denote the ability of the nerve to undergo a change in sodium permeability by a variable, h, which covers a range from 0 to 1 and is proportional to the ordinate in Fig. 5. In these terms $(1-h)$ is a measure of inactivation, while 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. If these definitions are adopted we may say that inactivation is almost complete when $V < -20$ mV. and is almost absent when $V > 30$ mV. At the resting potential h is about 0·6 which implies that inactivation is 40% complete.

The smooth curve in Fig. 5 was calculated from the equation

$$(h)_{\text{steady state}} = \frac{1}{1+\exp(V_h - V)/7}, \qquad (1)$$

where V is expressed in millivolts and V_h is the value of V at which $h=\frac{1}{2}$ in the steady state. The same equation gave a satisfactory fit in all experiments but there was some variation in the value of V_h. Five experiments with three fresh fibres gave resting values of h between 0·55 and 0·62. In these cases V_h varied between −1·5 and −3·5 mV. On the other hand, two experiments with a fibre which had been used for some time gave a resting h of only about 0·25; V_h was then +7·5 mV. Since the resting potential was found to decline by 10–15 mV. during the course of a long experiment it is reasonable to suppose that the change in V_h arose solely from this cause and that the relation between h and the absolute membrane potential was independent of the condition of the fibre.

In a former paper we examined the relation between the concentration of sodium ions in the external medium and the sodium current through the membrane (Hodgkin & Huxley, 1952*a*). The results were reasonably close to those predicted by the 'independence principle' except that the currents were 20–60% too large in the sodium-deficient solutions. This effect was attributed to the small increase in resting potential associated with the substitution of choline ions for sodium ions. This explanation now seems very reasonable. The resting potential probably increased by about 4 mV. in choline sea water and this would raise h from 0·6 to 0·73 in a fresh fibre and from 0·25 to 0·37 in a fibre which had been used for some time.

The quantitative results obtained in this series of experiments are summarized in Table 1. Most of the experiments were made at 3–7° C. but a temperature of 19° C. was used on one occasion. The results suggest that temperature has little effect on the equilibrium relation between h and V, but greatly alters the rate at which this equilibrium is attained. The Q_{10} of the rate constants cannot be stated with certainty but is clearly of the order of 3.

Two-pulse experiment

This section deals with a single experiment which gave an independent measurement of the time constant of inactivation.

Two pulses of amplitude −44 mV. and duration 1·8 msec. were applied to the membrane. Fig. 6*A* is a record obtained with the second pulse alone. The ionic current was inward and reached a maximum of about 0·25 mA./cm.^{2}. As in all other records, the inward current was not maintained but declined as a result of inactivation. Restoration of the normal membrane potential was associated with a tail of inward current due to the rapid fall of sodium conductance (see Hodgkin & Huxley, 1952*b*). When two pulses were applied in quick succession the effect of the first was similar to that in *A*, but the inward current during the second was reduced to about one half (record *B*). A gradual recovery to the normal level is shown in records *C*–*G*.

The curve in Fig. 7 was obtained by estimating sodium current in the manner

TABLE 1. Experiments with conditioning voltage

Axon	Temperature (° C.)	Variable	Displacement of membrane potential (mV.) −29	−22	−14	−8	−7	0	9	16	31	46
38	5	h* (steady	0·02	0·04	0·17	—	0·37	0·59	0·82	0·94	0·99	1·00
39	19	state)	0·02	0·04	0·09	—	0·28	0·55	0·83	0·94	0·98	0·99
39†	3		0·01	0·03	0·04	—	0·11	0·26	0·50	0·69	0·93	0·99
38	5	h‡ (steady	0·02	—	—	0·43	—	0·58	—	0·92	0·99	—
39	19	state)	0·03	—	—	0·40	—	0·61	—	0·94		—
39†	3		—	—	—	—	—	0·22	—	0·75	0·93	—
37	3		—	0·04	—	0·34	—	0·55	0·81	0·96	—	—
38	5	τ_h‡ (msec.)	1·5	—	—	7	—	[8–10]	—	8	4	—
39	19		0·35	—	—	1·5	—	[1·7–2·1]	—	1·8	—	—
39†	3		—	—	—	—	—	—	—	13	7	—
37	3		—	3	—	6	—	[8–10]	9	7	—	—
38	6	τ_h§ (msec.)	1·3	—	—	6	—	[7–9]	—	7	3·6	—
39	6		1·5	—	—	6	—	[7–9]	—	8	—	—
39†	6		—	—	—	—	—	—	—	9	5	—
37	6		—	2·2	—	4	—	[6–7]	7	5	—	—

Two-pulse experiment

Axon 31 at 4·5° C. $\tau_h = 1{\cdot}8$ msec. at $V = -44$ mV. $\tau_h = 12$ msec. at $V = 0$
Axon 31 at 6° C. τ_h§ $= 1{\cdot}5$ msec. at $V = -44$ mV. τ_h§ $= 10$ msec. at $V = 0$

* Measurements made by methods illustrated in Figs. 4 and 5.
† The axon had been used for some time and was in poor condition when these measurements were made.
‡ Methods illustrated in Figs. 1–3.
§ Calculated from above assuming Q_{10} of 3.
[ ] Interpolated.
h is the fraction of the sodium system which is rapidly available, $(1-h)$ is the fraction inactivated.
τ_h *determines the rate at which* h *approaches its steady state.*

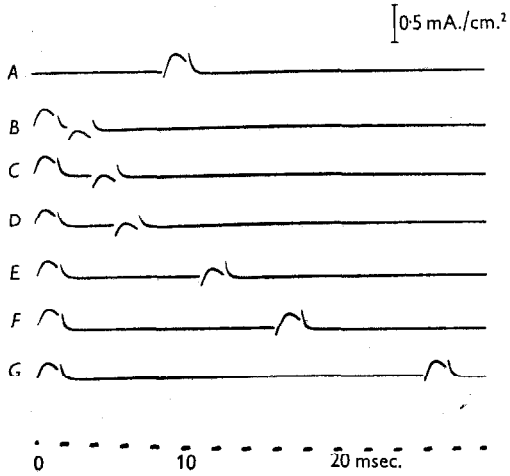

Fig. 6. Membrane currents associated with two square waves applied in succession. The amplitude of each square wave was −44 mV. and the duration 1·8 msec. Record *A* shows the second square wave alone, *B–G* both square waves at various intervals. Axon 31; uncompensated feed-back; temperature 4·5° C.

shown in Fig. 3 and plotting this against the interval between the two pulses. It will be seen that recovery from inactivation took place in an approximately exponential manner with a time constant of about 12 msec. A similar curve and a similar time constant were obtained by plotting

$$\left(\frac{dI_{Na}}{dt}\right)_{max.} \quad \text{instead of} \quad (I_{Na})_{max.}.$$

This time constant is clearly of the same order as that given by the method using weak conditioning voltages (see Table 1). An estimate of the inactivation time constant at -44 mV. may be obtained by extrapolating the curve in Fig. 7 to zero time. This indicates that the available fraction of the sodium-carrying system was reduced to 0·37 at the end of a pulse of amplitude -44 mV. and duration 1·8 msec. Hence the inactivation time constant at -44 mV. is about 1·8 msec., which is of the same order as the values obtained with large depolarizations by the first method (Table 1). It is also in satisfactory agreement with the time constant obtained by fitting a curve to the variation of sodium conductance during a maintained depolarization of 40–50 mV. (Hodgkin & Huxley, 1952*c*).

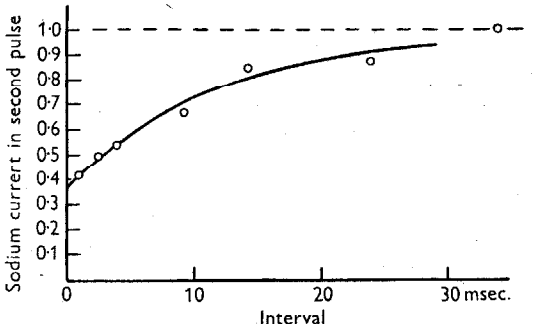

Fig. 7. Recovery from inactivation. Abscissa: interval between end of first pulse and beginning of second pulse. Ordinate: sodium current in second pulse measured as shown in Fig. 3 and expressed as a fraction of the sodium current in an unconditioned pulse. The circles are experimental points derived from the records in Fig. 6. The smooth curve is drawn according to the expression $1-0{\cdot}63 \exp(-t/\tau_h)$, where $\tau_h = 12$ msec.

The two-pulse experiment is interesting because it emphasizes the difference between the rapid fall of sodium conductance associated with repolarization and the slower decline during a maintained depolarization. Both events lead to a decrease in sodium current, but the underlying mechanisms are clearly different. In the first case it must be supposed that repolarization converts active membrane into resting membrane; in the second that prolonged depolarization turns it into a refractory or inactivated condition from which it recovers at a relatively slow rate when the fibre is repolarized. It cannot be argued that repolarization reduces sodium conductance by making the active

membrane refractory. If this were so, one would expect that the inward current during the second pulse would be reduced to zero at short intervals, instead of to 37 % as in Fig. 7. The reduction to 37 % is clearly associated with the incomplete decline of sodium conductance during the first pulse and not with the rapid and complete decline due to repolarization at the end of the first pulse.

DISCUSSION

The experimental evidence in this paper and in those which precede it (Hodgkin & Huxley, 1952*a*, *b*) suggests that the membrane potential has two distinct influences on the system which allows sodium ions to flow through the membrane. The early effects of changes in membrane potential are a rapid increase in sodium conductance when the fibre is depolarized and a rapid decrease when it is repolarized. The late effects are a slow onset of a refractory or inactive condition during a maintained depolarization and a slow recovery following repolarization. A membrane in the refractory or inactive condition resembles one in the resting state in having a low sodium conductance. It differs in that it cannot undergo an increase in sodium conductance if the fibre is depolarized. The difference allows inactivation to be measured by methods such as those described in this paper. The results show that both the final level of inactivation and the rate at which this level is approached are greatly influenced by membrane potential. At high membrane potentials inactivation appears to be absent, at low membrane potentials it approaches completion with a time constant of about 1·5 msec. at 6° C. This conclusion is clearly consistent with former evidence which suggests that the sodium conductance declines to a low level with a time constant of 1–2 msec. during a large and maintained depolarization (Hodgkin & Huxley, 1952*a*). Both sets of experiments may be summarized by stating that changes in sodium conductance are transient over a wide range of membrane potentials.

The persistence of inactivation after a depolarization is clearly connected with the existence of a refractory state and with accommodation. It is not the only factor concerned, since the persistence of the raised potassium conductance will also help to hold the membrane potential at a positive value and will therefore tend to make the fibre inexcitable. The relative importance of the two processes can only be judged by numerical analysis of the type described in the final paper of this series (Hodgkin & Huxley, 1952*c*).

SUMMARY

1. Small changes in the membrane potential of the giant axon of *Loligo* are associated with large alterations in the ability of the surface membrane to undergo its characteristic increase in sodium conductance.

2. A steady depolarization of 10 mV. reduces the sodium current associated with a sudden depolarization of 45 mV. by about 60 %. A steady rise of 10 mV.

increases the sodium current associated with subsequent depolarization by about 50%.

3. These effects are described by stating that depolarization gradually inactivates the system which enables sodium ions to cross the membrane.

4. In the steady state, inactivation appears to be almost complete if the membrane potential is reduced by 30 mV. and is almost absent if it is increased by 30 mV. Between these limits the amount of inactivation is determined by a smooth symmetrical curve and is about 40% complete in a resting fibre at the beginning of an experiment.

5. At 6° C. the time constant of the inactivation process is about 10 msec. with $V=0$, about 1·5 msec. with $V=-30$ mV. and about 5 msec. at $V=+30$ mV.

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