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Phil. Trans. R. Soc. Lond. B 1975 **270**, 449-458
doi: 10.1098/rstb.1975.0022

Phil. Trans. R. Soc. Lond. B. **270**, 449–458 (1975) [449]
Printed in Great Britain

Kinetic properties and inactivation of the gating currents of sodium channels in squid axon

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Associated with the opening and closing of the sodium channels of nerve membrane is a small component of capacitative current, the gating current. After termination of a depolarizing step the gating current and sodium current decay with similar time courses. Both currents decay more rapidly at relatively negative membrane voltages than at positive ones. The gating current that flows during a depolarizing step is diminished by a pre-pulse that inactivates the sodium permeability. A pre-pulse has no effect after inactivation has been destroyed by internal perfusion with the proteolytic enzyme pronase. Gating charge (considered as positive charge) moves outward during a positive voltage step, with voltage dependent kinetics. The time constant of the outward gating current is a maximum at about −10 mV, and has a smaller value at voltages either more positive or negative than this value.

Introduction

In 1952 Hodgkin & Huxley predicted the existence of a small charge movement or 'gating current' associated with the opening and closing of the voltage dependent ionic channels of nerve membrane. This gating charge was postulated to move passively through the membrane when the field changed, and the position or conformation of the structures which carried the charge was postulated to control ionic permeability. For many years the gating current escaped detection, but a small current component with many of the expected properties has recently been measured (Armstrong & Bezanilla 1973; Keynes & Rojas 1973), and there is substantial evidence that this current is associated with the opening and closing of the sodium channels (Bezanilla & Armstrong 1974; Armstrong & Bezanilla 1974; Keynes & Rojas 1974). The observed current is approximately 50 times smaller than the sodium current. The evidence indicates it is capacitative in origin, produced by a relatively slow redistribution of charge within the membrane. It is outward when the channels are opening following a positive step of membrane potential, and inward when the channels are closing, as would be expected of currents generated by the passive movement of charged or dipolar molecules in the membrane field. The following evidence closely links this current to the Na channels. (1) Prolonged depolarization reversibly eliminates gating current and I_{Na} (sodium current), and they recover in parallel when the membrane potential (V_m) is returned to a normal value (e.g. −70 mV). (2) Internal perfusion of an axon with $ZnCl_2$, which is known to abolish I_{Na} (Begenisich & Lynch 1974), also abolishes gating current. (3) When the sodium current is inactivated by a positive pre-pulse (Hodgkin & Huxley 1952), gating current also decreases in amplitude, as described below. (4) When I_{Na} is decreasing (following a depolarization), gating current and I_{Na} have a similar time course.

Study of gating current seems certain to provide more direct evidence of the gating of the ionic permeabilities than has hitherto been available. We describe here some properties of gating current, but the description is far from complete, and there are many obvious questions that remain to be answered.

Methods

Our method for measuring gating current is described in detail elsewhere (Armstrong & Bezanilla 1974), and only a brief summary will be given here. The experiments were performed on voltage-clamped squid giant axons. Ionic current was largely eliminated by replacing external sodium ion with the impermeant ion tris (tris(hydroxymethyl)methyl ammonium ion); by perfusing internally with Cs (the fluoride salt); and by adding tetrodotoxin to the external medium to block ionic movement through the sodium channels. Gating current is small in comparison to the linear portion of capacitative current, making it necessary to sum the current from a positive step with that from an exactly equal negative step. The current that remains is the nonlinear component of capacitative current, and necessarily it is the sum of two component currents. Unfortunately, there is no very direct means of resolving this sum into its components. For large pulses this technique cannot be employed safely, for the membrane potential (V_m) can exceed -200 mV, and at such large potentials the membrane shows evidence of breakdown. For this reason we have employed in some experiments a technique that is described below in the results section. To improve signal to noise ratio, we averaged the current from ten to twenty positive and negative steps. The composition of the solutions mentioned in the text is as follows:

a.s.w.: 440 mM NaCl, 50 mM $MgCl_2$, 10 mM $CaCl_2$
tris s.w.: 440 mM tris chloride, 50 mM $MgCl_2$, 10 mM $CaCl_2$
5 % Na s.w.: 22 mM NaCl, 418 mM tris Cl, 50 mM $MgCl_2$, 10 mM $CaCl_2$
290 CsF: 290 mM CsF, 400 mM sucrose.

All solutions were buffered to a pH of about 7.2.

Results

Figure 1 is the sum of the current from five positive and five negative voltage steps applied to an axon immersed in a medium containing only 5 % of the normal sodium ion concentration, the remainder having been replaced by tris. There is a small peak of outward current which cannot be seen in normal seawater, followed by a small inward sodium current. The outward current is what we shall refer to as gating current. Recorded in this way, gating current has a distinct rising phase, which is probably the result of summing a rapidly decaying inward current that occurs during the negative step with a more slowly decaying outward current that flows during the positive step (Armstrong & Bezanilla 1974). The later part of the gating current is obscured by sodium current in this figure.

When all of the external sodium is removed, and tetrodotoxin is added, one records at the end of the pulses, when V_m is returned to its holding value of -70 mV, an inward current that decays with approximately the same time course as I_{Na} (sodium current) recorded in conventional solutions. This is illustrated in figure 2, in which the left traces are I_{Na} tails recorded at various membrane potentials, and the right traces are gating currents, recorded from the same

axon at the same potentials. Table 1 gives the time constant of decay of I_{Na} and I_g from several other axons. The time course of the I_{Na} tails can be seriously affected by the small resistance between the voltage electrodes and the membrane. We were therefore careful to measure this resistance and compensate for it electronically. The results show that on the average I_g decayed only slightly more slowly than I_{Na}. As a further check on the accuracy of our compensation for the series resistance, we measured the decay of I_{Na} in a solution containing 20 % of the normal sodium ion concentration. I_{Na} was so small in this case that the series

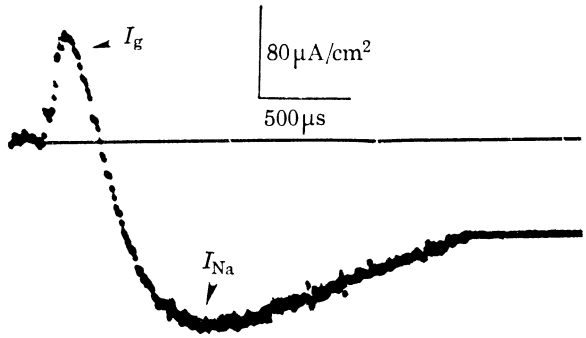

FIGURE 1. Gating current (I_g) and sodium current (I_{Na}) recorded from an axon bathed in 5 % Na s.w. and internally perfused with 290 CsF. The trace is the sum of five positive and five negative pulses of 80 mV amplitude from a holding potential of −70 mV. Temperature: 2 °C.

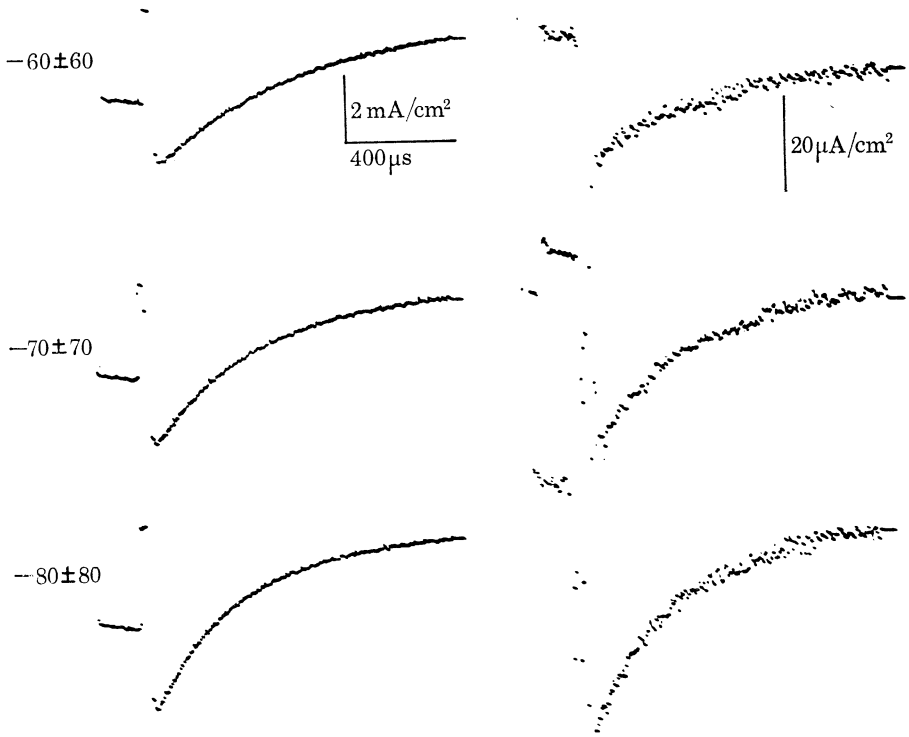

FIGURE 2. Sodium and gating current tails as a function of V_m. Left traces are sodium current at pulse turn-off recorded from an axon bathed in a.s.w. Right traces are gating currents from the same axon recorded in tris s.w. with 300 nM TTX. Holding potential and pulse amplitude are given on the left panel and apply to the right traces also. The sodium currents are the sum of one positive and one negative pulse; while the gating currents traces are the sum of 50 positive and 50 negative pulses. Temperature: 2 °C.

resistance could not have caused much error, and again I_{Na} and I_g are similar in time course, as recorded in the table.

Hodgkin & Huxley demonstrated that I_{Na} tails decay more rapidly at relatively negative membrane potentials. Figure 2 shows that I_g tails are also faster at -80 mV than at -70 or -60 mV, and at all potentials they are similar in time course to I_{Na}.

TABLE 1.

experiment reference	V_m mV	pulse amplitude mV	pulse duration μs	series resistance compensation Ω cm²	[Na] mM	$\tau_{I_{Na}}$ μs	τ_{I_g} μs
JL053C	−70	70	700	5	440	250	—
					0, TTX	—	310
SP053B	−70	90	1000	5	88	151	—
					22	218	—
					0	—	208
SP053D	−70	90	1000	3	88	200	—
					0	—	244
					88	192	—
					0	—	239
					0, TTX	—	260
SP133A	−70	80	300	3	88	120	—
					0, TTX	—	104
	−70	80	1000	3	88	140	—
					0, TTX	—	187

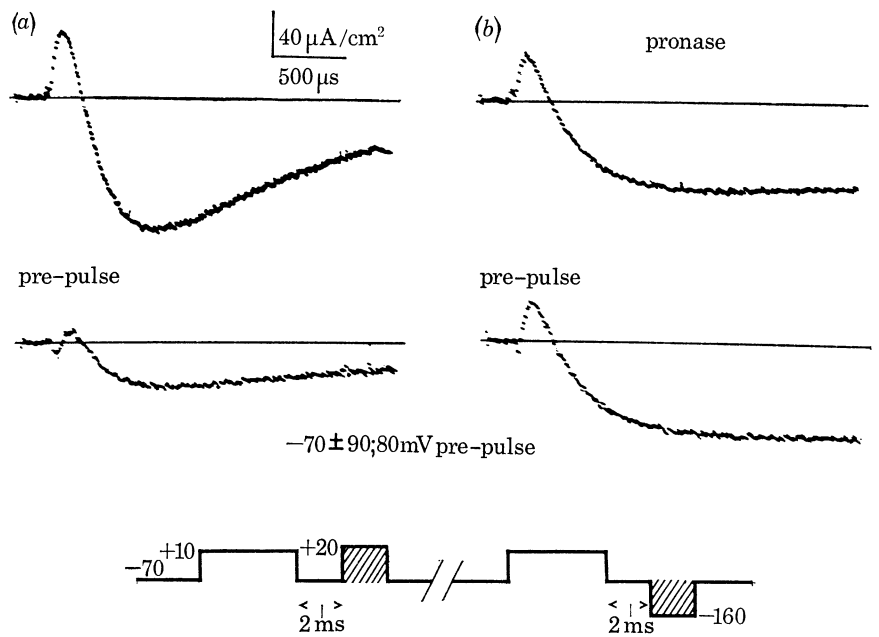

FIGURE 3. The traces show gating current and I_{Na} from an axon in 5 % Na s.w., perfused internally with 290 CsF. The pulse procedure is given below the traces. (*a*) Current traces without (upper) and with (lower) a positive prepulse. The pre-pulse depresses both I_{Na} and I_g. (*b*) Traces from the same axon after pronase treatment. After pronase a pre-pulse has no effect on I_{Na} or I_g. Temperature: 2 °C.

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Inactivation of gating current

Hodgkin & Huxley demonstrated that I_{Na} inactivates after a few milliseconds if V_m is held at a value positive to the normal resting potential. Inactivation can be demonstrated by the pulse pattern shown in the lower part of figure 3: a pre-pulse is applied, and then after an interval too short to allow significant recovery from inactivation, the current is summed from test pulses (shaded) of opposite direction. Part *a* of the figure shows the response of an axon in 5% Na seawater in the absence (upper trace) and presence of a pre-pulse. The pre-pulse inactivates I_{Na} almost completely, and markedly reduces the amplitude of the gating current. After internal perfusion with pronase, which is known to destroy inactivation (Armstrong, Bezanilla & Rojas 1973), the pre-pulse affects neither I_{Na} nor I_g. This experiment has been criticized on the grounds that the pre-pulse should also alternate in polarity as does the test pulse. Experiments of this type have been performed recently, and they confirm that inactivation affects I_g, though with the symmetrical pattern the effect is somewhat less marked than in figure 3.

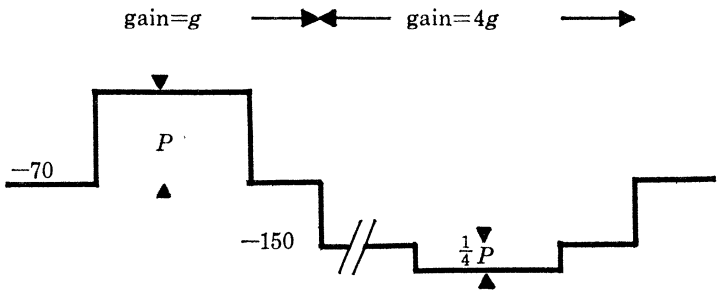


FIGURE 4. Divided pulse procedure. In the first part of the cycle the membrane is held at −70 or −80 mV and a positive pulse of amplitude P is applied. In the second part of the cycle the membrane potential is held at −150 mV and a negative pulse of amplitude $\frac{1}{4}P$ is applied. The current produced by the negative pulse is multiplied by four before being added to the positive pulse current.

The distribution of gating charge as a function of voltage, and the kinetics of its movement

The sodium conductance (g_{Na}) increases sharply as V_m is made positive relative to the normal resting potential, and the kinetics of the increase depend on V_m. Gating charge distribution and kinetics also depend on V_m, as shown in figures 4 and 5 (cf. Keynes & Rojas 1974). The simplest method of measuring gating charge movement is to hold V_m at −70 or −100 mV, sum the current from positive and negative pulses as has been described, and integrate the resulting current–time curve to determine total charge movement. This method has two defects. First, in order to cover a sufficiently wide potential range it is necessary to take V_m to dangerously negative potentials. The second problem is that inward gating charge movement during the negative step from the holding potential makes an unknown contribution to the current sum, resulting in a distorted curve of charge distribution as a function of voltage. To alleviate these problems we devised the procedure shown in figure 4. The main purpose is to make the negative voltage step cover a very negative voltage range where, we hope, there is little gating charge movement. The negative step must be small to prevent membrane breakdown, so we made it one quarter the size of the positive pulse, and compensated for this by multiplying the negative step current by four before adding it to the positive step current. Evidence to show that there

is relatively little gating charge movement in this negative voltage range has been presented by Armstrong & Bezanilla (1974).

When recorded in this way, gating current has little or none of the rising phase that is so evident in figure 1, and the amplitude of the outward current is greater than with the simple procedure. Since the positive step covers the same voltage range in both procedures and thus produces the same gating current, it seems clear that the divided pulse procedure significantly reduces gating charge movement during the negative step (cf. Armstrong & Bezanilla 1974). The falling phase of gating current recorded with the divided pulse procedure has at least two components, a rapid, approximately exponential component and a slower component of small amplitude. The origin of this rather ill-defined slow component is unknown at present, but we speculate that it may be associated with the potassium conductance.

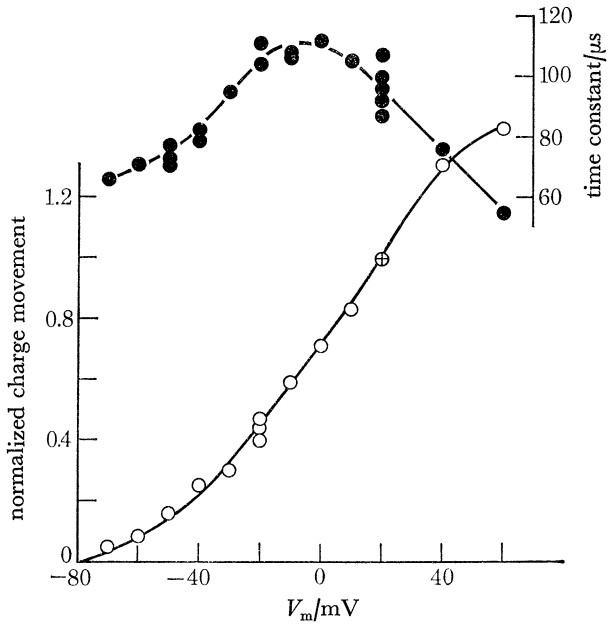


FIGURE 5. Charge movement and time constants of gating current as a function of V_m during the positive step. Upper part. Each point represents the time constant of the fast exponential component of gating current. Lower part. Each point is the total charge movement in the fast exponential component. Each determination has been normalized with respect to the charge movement for a pulse of 100 mV, which was repeated several times during the experiment. The points were obtained in random order using the pulse scheme given in figure 4 with a holding potential of −80 mV. The axon was bathed in tris s.w. with TTX and internally perfused with 290 CsF. Temperature: 8 °C.

The curves in figures 5 and 6 give the total charge movement in the fast component as a function of V_m during the positive step, which started from a holding potential of −80 (figure 5) or −70 mV (figure 6). The points in both curves were taken at random and scaled relative to a frequently repeated control determination with a positive pulse of 100 mV amplitude. This procedure was necessary to correct for the gradual deterioration of the axon during the experiments.

The experiment of figure 5 concentrated primarily on relatively small depolarizations, and it is clear that there is relatively little charge movement near −70 mV. The curve then climbs more steeply, and shows some evidence of saturation at 40 to 60 mV positive. In figure 6 there are many points in the 30–90 mV range, and clear evidence of saturation; that is, all of the

charge that moves in the fast component seems to be in 'open' position for V_m of 30 mV or above. The exact shape of these curves, of course, depends on the method of analysis, and particularly on our decision to integrate only the fast component. We made this choice in part because the area of the slow component is critically dependent on the choice of a base-line, and the area of the slow component therefore is rather variable in successive determinations at the same voltage. None the less, we have seen evidence of saturation of total charge movement in some experiments, and we think the saturation shown in figure 6 is genuine. The midpoint of the distribution in figure 5 is at about 0 mV, while in figure 6 it is about -15 mV. We have no explanation for this difference, but it may be related to the fact that saturation is less evident in figure 5, where the midpoint is more positive. Both curves show that the distribution of gating charge as a function of voltage is much less steep than the g_{Na}–V curve determined by Hodgkin & Huxley (1952).

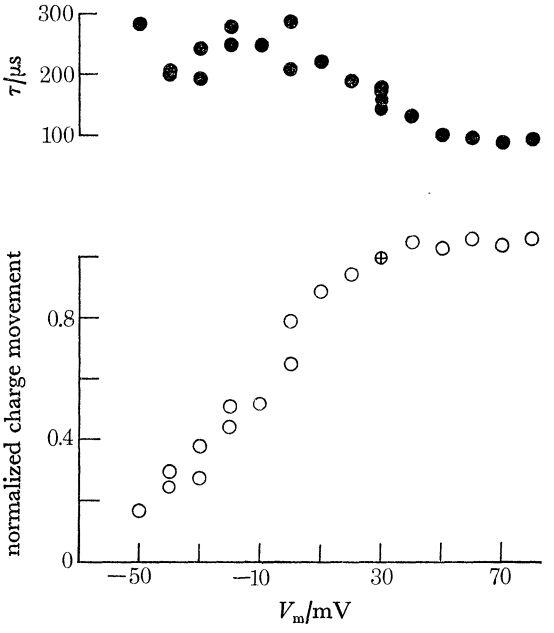


FIGURE 6. Charge movement and time constants of gating as a function of V_m during the positive step. Same procedure as figure 5. Temperature: 2 °C. Pulse scheme of figure 4 with a holding potential of -70 mV.

The upper points in figures 5 and 6 give the time constants of the exponential fitted to the fast component of gating current. It can be seen that in both cases the time constant is a maximum at about the midpoint of the charge–voltage curve, and that it is smaller for V_m either negative or positive to this point.

DISCUSSION

The Hodgkin–Huxley equations are a convenient point for beginning a discussion of gating currents. In terms of their formulation, it is most reasonable to identify gating current with the movement of their charged m particles, which control activation of g_{Na}, and this makes the predicted gating current proportional to dm/dt. For a positive step from the resting potential (which we assume to be -60 mV), dm/dt is positive in sign, which means that the current would be outward, and it decays exponentially. For a negative step, the gating current generated

by m particle movement would be inward, and again it decays exponentially but with a faster time constant than the positive current. When outward and inward 'm current' from equal but opposite pulses are added together, the result is the curve shown in figure 7, which has a rather startling qualitative resemblance to the experimental traces. Initially there is a fast inward tail of current, which in fact is often observed for pulses from a holding potential of -60 mV. This is followed by a rising outward current which peaks and then decays in a way similar to the experimental records. At the termination of the pulses, when V_m is returned to -60 mV, m current is inward and it decays exponentially, as the experimental records appear to do.

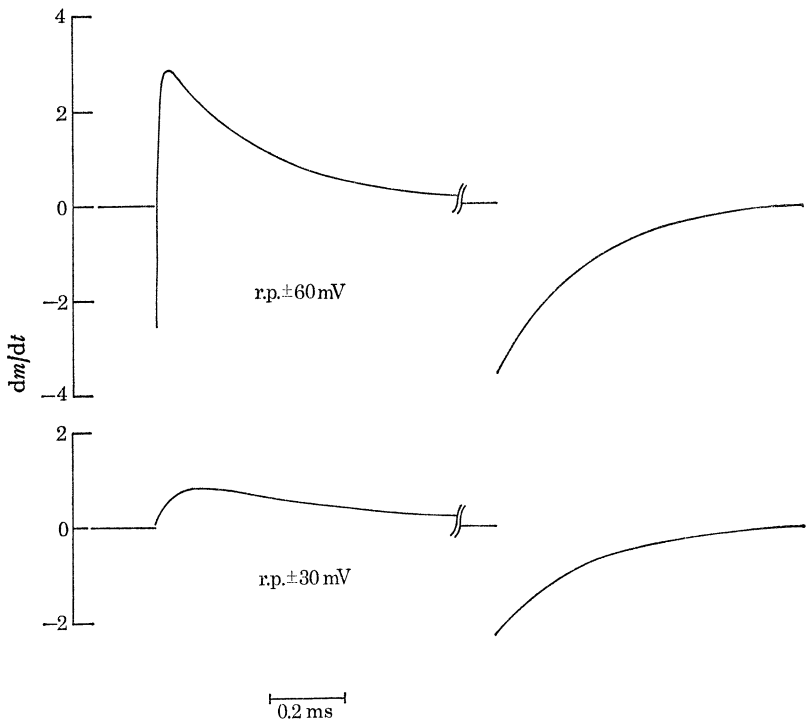


FIGURE 7. Gating current or 'm current' predicted by the Hodgkin & Huxley equations. Each trace is the sum of dm/dt for the positive and negative pulse of the amplitude indicated, from the resting potential.

In spite of this qualitative resemblance, there are two points at which our results diverge from the Hodgkin & Huxley predictions. The first is that gating current is affected by inactivation of g_{Na}, as discussed below, while dm/dt of their formulation is not. The second difference is in the relative rate of decay of I_{Na} and gating current. In their equations, if m is assumed to be zero (a good approximation at -70 mV), I_{Na} decays according to the equation

$$I_{Na} = \bar{g}_{Na}\, m_0^3\, e^{-3t/\tau_m},$$

(Please refer to Hodgkin & Huxley 1952 for an explanation of unfamiliar terms.) Under the same conditions

$$dm/dt = m_0\, e^{-t/\tau_m}.$$

m current thus decays three times more slowly than I_{Na}. The data in table 1, however, show that gating current and I_{Na} decay at about the same rate.

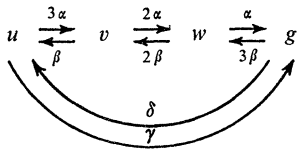
A kinetic scheme of the Hodgkin & Huxley m^3 process is helpful in understanding this problem, and in suggesting a remedy for it. The scheme

$$u \underset{\beta}{\overset{3\alpha}{\rightleftharpoons}} v \underset{2\beta}{\overset{2\alpha}{\rightleftharpoons}} w \underset{3\beta}{\overset{\alpha}{\rightleftharpoons}} g$$

is equivalent to the m^3 formulation if g is taken as the fraction of the channels in the conducting (m^3) state, and u, v and w represent respectively the fraction with three, two or one of the m particles in 'channel closed' position. Closing a channel at the end of a positive step requires simply that it go from state g to state w. m current, however, is produced at each of the steps, and continues after the channel has closed, as it progresses from w to v to u. More precisely if α is taken as zero, which is a reasonable approximation at -70 mV, m current is equal to

$$\tfrac{1}{3}(3\beta g + 2\beta w + \beta v).$$

There are at least two ways that this kinetic version of Hodgkin & Huxley formulation can be modified to bring it into better accord with the data on the gating current tails. One modification adds a direct path from g to u, with rate constant γ in the forward direction and δ in the reverse direction.



To fit the data, γ must be small at all membrane potentials; and δ must be small compared to α at positive potentials, but large compared to β at negative potentials when the channels are closed or closing. On repolarization, assuming α is zero, gating current then would be proportional to

$$-(\delta g + 3\beta g + 2\beta w + \beta v)$$

and the dominant term would be $-\delta g$. Closing of the sodium channels would occur at the rate

$$-(\delta g + 3\beta g).$$

Since the dominant term again would be $-\delta g$, gating current and sodium current would have approximately the same time course.

A second way to obtain a better fit to the turn-off gating current is to assume that the three hypothetical charged particles controlling g_{Na} are not identical, but instead, for example, that one of the particles has kinetics that are much faster than those of the other two particles. This scheme predicts reasonable turn-on kinetics for g_{Na}; and predicts gating currents with two exponential components, one of them small and slow in comparison to the other. (In the results section it was noted that we often see a small slow component of gating current, and we suggested that it may be associated with g_K. Obviously another possibility is that it pertains instead to g_{Na}.) At the end of a positive pulse, g_{Na} turns off at a rate given by the sum of the rate constants for the movement of the three particles, and since one of the particles moves faster than the other two, the sum is approximately equal to the rate constant of the fastest particle. Gating current is proportional to the sum of two exponentials (for the three particles), and has a time course similar to that of g_{Na}, except that there is a small slow component, generated by the movement of the slower particles, that continues after g_{Na} has fallen to its steady state value.

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The other major point of difference between our results and dm/dt is that gating current is affected by inactivation. In the absence of experimental evidence demanding a more complex formulation, Hodgkin & Huxley chose, for mathematical simplicity, to make m^3, the activation factor, and h, the inactivation factor, completely independent of each other. The pre-pulse experiment of figure 3 shows instead that gating current, unlike dm/dt, is reduced by an inactivating pre-pulse. The relation between activation and inactivation remains to be worked out in detail, but the evidence indicates that they are not independent (cf. Goldman & Schauf 1972).

It is relevant at this point to make a distinction between the inactivation process described by Hodgkin & Huxley, which occurs in milliseconds and requires only milliseconds for recovery; and a slow inactivation that is established after a depolarization of many seconds, and recovery also requires many seconds. We find that if V_m is held at 56 mV for 2 min, no gating current can be recorded initially when V_m is returned to a holding value of -70 mV, but that gating current does recover completely in amplitude, with a half-time of about 30 s at 2 °C (Bezanilla & Armstrong 1974; Armstrong & Bezanilla 1974). We suggest, therefore, that the transients recorded from a depolarized membrane are of questionable origin, and may not be related to normal activation of the Na channels.

A final problem that we wish to raise, but not solve, is that the curve relating gating charge and voltage is much less steep than the g_{Na}–V_m relation of Hodgkin & Huxley. At first glance it seems this problem can be solved by saying that g_{Na} is a power function of the gating charge in 'open' position, just as in the Hodgkin & Huxley formulation g_{Na} is proportional to m^3, while the gating charge in open position is m. It works out, however, that the predicted g_{Na}–V_m curve is steepened relative to the gating charge–voltage curve primarily in the very negative voltage range; but in the range where g_{Na} is easily measurable the relative steepening is by less than a factor of two. The predicted curve, then, is much less steep than the experimental one. The relation between the gating charge–V_m curve and the g_{Na}–V_m curve is apparently not a power relation, but a more complicated one. In consequence, we suggest that until this relation is worked out completely, estimates of channel density based on total gating charge movement must be viewed with caution.

The work was supported by U.S.P.H.S. grant no. 2R01 NS 08951.

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