

Distribution and Kinetics of Membrane Dielectric Polarization

1. *Long-Term Inactivation of Gating Currents*

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ABSTRACT Gating currents were measured by subtracting the linear component of the capacitative current recorded at very positive or very negative potentials. When the membrane is depolarized for a few minutes, repolarized to the usual holding potential (HP) of -70 mV for 1 ms, and then pulsed to 0 mV, the charge transferred in 2–4 ms is $\sim 50\%$ of that which was transferred during the same pulse holding at -70 mV. This charge decrease, called slow inactivation of the gating current, was found to be consistent with a shift of the charge vs. potential (Q - V) curve to more hyperpolarized potentials. When the HP is 0 mV, the total charge available to move is the same as the total charge available when the HP is -70 mV. The time constants of the fast component of the ON gating current are smaller at depolarized holding potentials than at -70 mV. When the HP is -70 mV and a prepulse of 50 ms duration is given to 0 mV, the Q - V curve is also shifted to more hyperpolarized potentials (charge immobilization), but the effect is not as pronounced as the one obtained by holding at 0 mV. When the HP is 0 mV, a prepulse to -70 mV for 50 ms partially shifts back the Q - V curve, indicating that fast inactivation of the gating charge may be recovered in the presence of slow inactivation. A physical model consisting of a gating particle that interacts with a fast inactivating particle, and a slow inactivating particle, reproduces most of the experimental results.

INTRODUCTION

When the nerve membrane is depolarized, the sodium conductance increases rapidly and then decreases while the depolarization is maintained. Hodgkin and Huxley (1952) described the first process as the activation and the second as the inactivation of the sodium conductance. If the membrane has been depolarized for tens of milliseconds, inactivation will recover within a few milliseconds at voltages near the normal resting potential. If the depolarization

lasts for several seconds, it takes seconds to recover the conductance at the resting potential, and this conductance decrease with prolonged depolarization has been called slow inactivation (Adelman and Palti, 1969; Chandler and Meves, 1970; Rudy, 1978).

The sodium conductance is controlled by membrane potential and this control is achieved by a change in the membrane electric polarization upon a change in membrane potential. When the membrane potential is suddenly modified, the polarization changes, thereby producing a polarization current. The polarization current responsible for the opening and closing of the conductance has been called the gating current (Armstrong and Bezanilla, 1973), and it has been studied in relation to the sodium conductance activation and fast inactivation. It was observed by Bezanilla and Armstrong (1974) that prolonged depolarization abolished most of the gating current attributed to the activation and fast inactivation of the sodium conductance. A similar result was published by Meves and Vogel (1977). Here we have analyzed in more detail the characteristics of gating currents before, during, and after prolonged depolarization and we have found evidence that the movement of the charge responsible for the channel opening and closing is affected but not abolished by prolonged depolarization, which gives an insight into the origin of the slow inactivation of sodium conductance. A short communication of these results has been published (Bezanilla and Taylor, 1979).

METHODS

Squid giant axons from *Loligo pealei* were internally perfused and voltage-clamped according to methods previously described (Bezanilla and Armstrong, 1977). Here we will only detail modifications and improvements of the techniques.

Experimental Chamber

To decrease background noise in the current recording (see, for example, Levis, 1981), the axon chamber was made of three compartments separated by plexiglass partitions with holes through which the axon was threaded. Each compartment had a solution inlet and outlet to exchange the external medium. Membrane current was measured in the center compartment with two large platinized platinum plates and the lateral compartments were connected to ground through two large platinized platinum plates. Reference potential was measured with a pointed glass pipette filled with either artificial sea water-agar or 3 M KCl-agar connected to an Ag-AgCl sintered electrode. The internal electrode was of the "piggy-back" type as described by Chandler and Meves (1965). Temperature was measured by a thermistor in the center compartment and was controlled by a negative feedback circuit powering a peltier cooler in thermal contact with the bottom of the chamber.

Pulse Generation and Recording Electronics

Pulses were generated by storing amplitudes and durations in a bank of random access memories (RAMs) running under program control out of a Nova 3 computer (Data General, Southboro, Mass.). The outputs of the RAMs were connected through optoisolators to a 12-bit deglitched digital-to-analog converter that was connected to the command input of the voltage-clamp circuitry. Unless otherwise noted, the usual pulse procedure was the P-P/4 method described by Bezanilla and Armstrong (1977).

We had the option of changing the potential at which the subtracting pulses were superimposed (the subtracting holding, SHP) to include positive values. The four subtracting pulses could be given in the same direction as the test pulse (P/4) and the resultant current could be subtracted from the current produced by the test pulse. Alternatively, they could be given in the opposite direction to the test pulse (P/-4) and the resultant currents could be added. Current was recorded with a current-to-voltage converter and summed with the output of a transient generator (Bezanilla and Armstrong, 1977). It was then amplified and filtered with a tuneable six-pole Bessel filter with bandwidth from DC to 30 or 50 KHz. The filtered output was fed into a data acquisition module consisting of a sample and hold amplifier, followed by a 12-bit analog-to-digital (A/D) converter running under computer control. The output of the A/D converter was connected by way of optoisolators to the data channel (or direct memory access) of the computer memory and ran under program control. A master program (written in a combination of FORTRAN and assembler languages) controlled the pulse generation, data acquisition, display of the processed input data, and storage in floppy disk units. In each sweep, 1,200 points were sampled at 4-, 5-, or 10- μ s intervals, but we only stored 480 samples obtained by keeping the first 240 points exactly as they were sampled and decimating the rest by keeping 1 of every 4 points after appropriate digital filtering (Otnes and Enochson, 1978).

Solutions

All internal and external solutions used are listed in Table I. In the text we will refer to the solutions as external solution//internal solution. Membrane potentials are not corrected for junction potentials.

Data Analysis

The collected data stored in diskette units were later transferred to hard disk in the Nova 3 computer system. Each sweep could be retrieved and displayed in the oscilloscope. The analysis program allowed us to subtract different traces, subtract base-line, fit base-line, fit exponentials, etc., and the results of the operations were also displayed in the oscilloscope and could be compared with the original data. The analysis of each trace was done by first subtracting the base-line. This was accomplished either by fitting a straight line to the last points of the trace (most of the times forcing it to be a straight line with no slope) or by estimating the base-line from the last points of the trace. The charge was computed as the numerical time integral (2-4 ms) of the current trace.

Conventions

We use the usual convention of membrane potential defined as internal potential minus the external potential. Positive current represents an outward current.

RESULTS

Recovery from Slow Inactivation

When the membrane potential is held at voltages near zero for periods of many seconds or a few minutes, sodium currents cannot be elicited immediately after repolarization to the normal resting potential (ca. -70 mV) and many seconds at -70 mV are required to recover the sodium conductance (Bezanilla and Armstrong, 1974; Rudy, 1978). In Fig. 1 we show the results

of an experiment designed to test the effect of maintained depolarization on gating currents. The axon was held at -70 mV, a test pulse of 100 mV was given, and the gating charge was measured and assigned a control value of 100%. Then the holding potential was changed to 42 mV for ~ 2 min. After this depolarized period, the holding potential was repolarized to -70 mV, the test pulse of 100 mV was given at the times indicated in Fig. 1, and the current was recorded. The charge is plotted as a function of time after the membrane was repolarized (closed circles) and it can be seen that the charge measured immediately after repolarization is $\sim 65\%$ of the control charge returning to the control value in a nearly exponential time-course. In the same figure we have plotted the results of a similar experiment in which the membrane was depolarized to 42 mV, but the pulse procedure to obtain gating currents was the original $\pm P$ sequence described by Armstrong and Bezanilla (1973). In this procedure, the subtracting pulse is of the same amplitude as the test pulse,

TABLE I

Internal solution:*		TMA-F‡	TMA-glutamate	Trizma-7§				
200	TMAFG	100	100	10				
External solutions:		Trizma-7§	CaCl ₂	MgCl ₂	Tris-Ac	Mg-Ac¶	NaCl	TTX
ASW		10	10	50	—	—	440	—
Tris-Cl-TTX		440	10	50	—	—	—	0.2
Tris-Cl-no-Mg TTX		480	50	—	—	—	—	0.2
Tris-Ac-TTX		—	10	—	440	50	—	0.2

All solutions were adjusted to an osmolality of 960 mosmol/kg and pH = 7.2.

Concentrations are in millimolar.

* Osmolality adjusted with sucrose.

‡ Tetramethylammonium ion.

§ Tris(hydroxymethyl) aminomethane at pH = 7 from Sigma Chemical Co. (Saint Louis, Mo.).

|| Trizma base (Sigma Chemical Co.) neutralized with acetic acid.

¶ Magnesium acetate.

but is of opposite polarity and starts from the same holding potential. The results obtained with the $\pm P$ procedure (squares) seem to show that almost no charge is available to move after the period of maintained depolarization (Bezanilla and Armstrong, 1974), in contrast with the results obtained with the P/4 procedure, in which 50% of the charge still can be displaced after the depolarized period. We will show later that the discrepancy between these results can be explained by a change in the voltage dependence of the charge movement that makes the $\pm P$ procedure totally inadequate.

Charge Distribution Is Affected by the Holding Potential

To explore the mechanisms involved in the charge decrease after maintained depolarization, we studied the gating currents during depolarization. The left panel of Fig. 2 shows typical gating current records obtained at a holding potential of -70 mV and pulsing to the voltage indicated in each trace. The

membrane potential was then held at 0 mV for >2 min and the gating currents were recorded using the pulse protocol indicated at the bottom of the right panel in Fig. 2. The 1-ms prepulse to -70 mV was given to have the same potential difference during the pulse to compare with the records obtained with a holding potential (HP) of -70 mV. (We have compared the charge vs. potential [$Q-V$] curve obtained at HP = 0 with and without the 1-ms prepulse and they are superimposable.)

The currents recorded with a HP of 0 mV and prepulsing for 1 ms to -70 mV are displayed in the right panel of Fig. 2. The most striking difference from the currents obtained at HP = -70 is that the charge transported at 40 mV is much less at HP = 0 and the charge at potentials more negative than -120 mV is larger. It is also apparent that the time-course of the gating

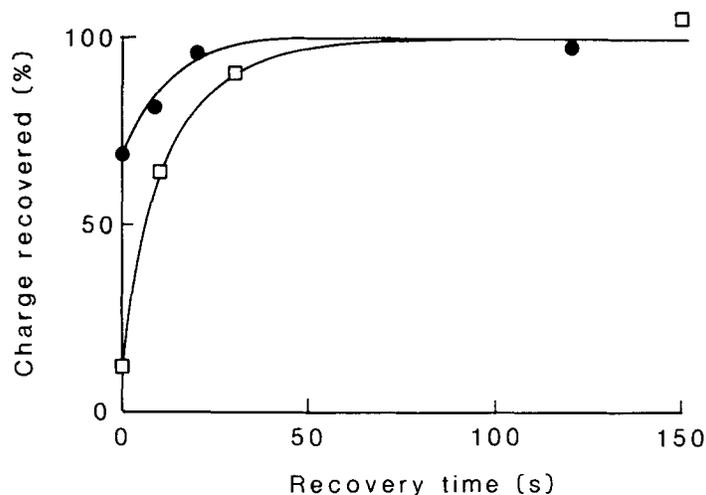


FIGURE 1. Recovery of charge after prolonged depolarization. Open squares represent the charge for a pulse of 100 mV from an HP = -70 mV after the axon was held at 42 mV for 2 min; the procedure used was $\pm P$. Filled circles: same as open squares but the procedure was P/4 with an SHP = 50 mV. 200 Tris-Cl-TTX-no Mg//TMAFG. See Table I. Temperature, 10°C .

currents is faster when the HP = 0, although this only applies to the fast component of the current. This experiment shows that the holding potential is affecting the kinetics and steady-state properties of the charge movement. It is important to notice that the differences are not accounted for by the change in the SHP because it has been observed in conditions using the same SHP. This point will be further discussed with regard to Fig. 4.

To explore further the steady-state distribution of the gating charge, we have plotted the area under the gating current transients to obtain the displaced charge at different potentials for two different holding potentials (-70 and 0 mV) and without using a prepulse to -70 mV. The curves in Fig. 3 have been obtained in the following way. When the HP was -70 mV, pulses positive to -70 produce a charge that is positive and pulses negative to -70

mV produce negative charge. The charge also changes its sign when $HP = 0$, depending on whether the pulse went to positive or negative potentials. To compare the results, we added the absolute value of the most negative charge at $HP = -70$ mV to all the points obtained at this holding potential to convert all the charge to positive values. The same procedure was followed for the points obtained at HP of 0 mV. The results of this procedure are plotted in Fig. 3, where the circles represent the charge recorded at $HP = 0$ mV and the triangles represent the charge at $HP = -70$ mV. It is clear from this figure that the total charge available to move, which is the difference between the

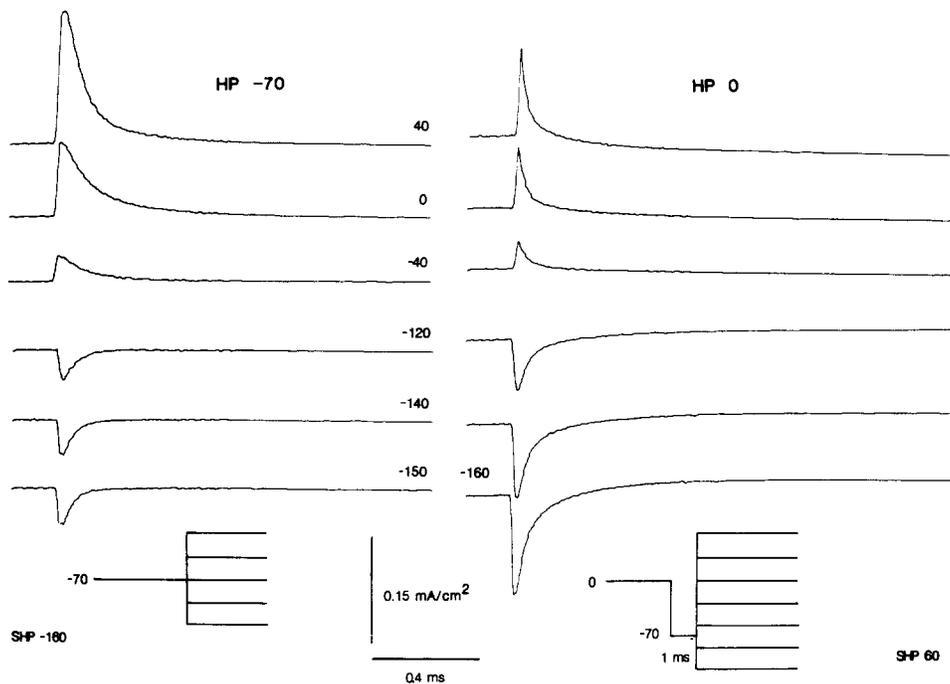


FIGURE 2. Gating currents recorded at two different holding potentials. Pulse patterns are indicated at the bottom of the figure. Left panel: $HP = -70$ mV, $SHP = -160$ mV, P/-4 procedure. Right panel: $HP = 0$ mV, $SHP = 60$ mV, P/4 procedure. Tris-Cl-TTX//200 TMAFG. Temperature, 12°C .

asymptotes at very negative and very positive potentials, is not affected by the change in holding potential. However, the shape of the $Q-V$ curve is changed and its position on the voltage axis is shifted to the left when the HP is changed from -70 to 0 mV. This result can explain the decrease of charge observed after maintained depolarization. If immediately after repolarization from an $HP = 0$ the $Q-V$ curve is still shifted to the left, the charge available to move from -70 to $+30$ mV is $\sim 50\%$ of the charge that is available to move from -70 to 30 mV when the $HP = -70$ mV.

It is important to note that the integrals of the gating currents shown in

Fig. 3 have been obtained by subtracting the linear component of the charge movement using the P/4 procedure. When the holding potential is -70 mV, the charge moving negative to -100 mV is small and the subtracting pulses may be superimposed on a subtracting holding potential (SHP) of about -160 mV and obtain fairly accurate gating current with little contamination of nonlinear charge moved by the subtracting pulses. On the other hand, when the HP = 0, the nonlinear charge moving at potentials more negative than -100 mV is quite large and the subtracting pulse should be superimposed on very negative subtracting holding potentials. An alternative approach is to use positive SHP because the Q - V curve at HP = 0 is flat at positive potentials.

Fig. 4 shows the effects of the subtracting holding potential on the recorded gating currents. In this case, the membrane was held at -70 mV and pulsed to the voltages indicated near the traces. Two SHP were used: -160 mV

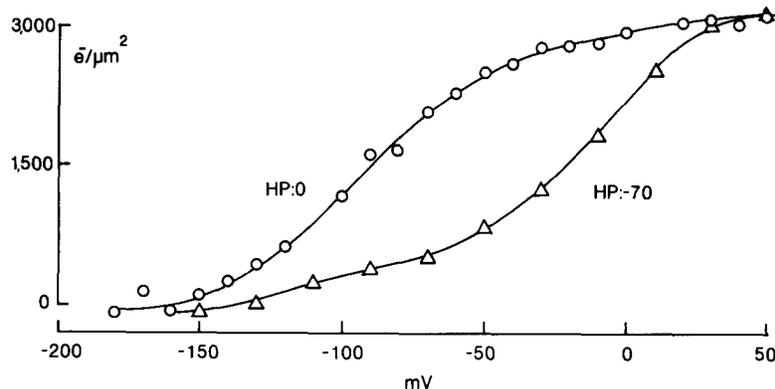


FIGURE 3. Q - V curves for two holding potentials. Open circles: HP = -70 mV, SHP = -160 mV. Open triangles: HP = 0 mV, SHP = 60 mV. The procedure was P/4 or P/-4, depending on the direction of the pulses and the value of the subtracting potential to minimize the invasion of the subtracting pulses into the nonlinear region. Tris-Cl-TTX//200 TMAFG. Temperature, 7.5°C .

(thick traces), and $+60$ mV (thin traces), and the procedure was changed from P/4 to P/-4 (see Methods), according to the direction of the pulse, as indicated in the inset of Fig. 4. It is clear from these results that the value of the SHP can influence the shape of the gating currents and the estimated charge, but the total effect on the charge is small. In this case, the results are consistent with the fact that at HP = -70 , the Q - V curve is flatter at about -160 mV than at $+60$ mV. Consequently, the estimation of the charge should be more accurate when an SHP of -160 mV is used for a HP = -70 and an SHP of 60 mV when the HP = 0 mV.

Kinetics of the Charge Movement Is Affected by the Holding Potential

Fig. 2 shows that gating currents are faster when the holding potential is depolarized, as compared with the case of a holding potential of -70 mV.

Fig. 5 presents the voltage dependence of the time constant of the faster exponential of the gating current obtained by fitting two exponentials to the falling phase of the gating current transient. The comparison of the fast time constant at two holding potentials shows that at depolarized holding potential the time constant is less at all potentials and the peak of the curve is shifted to more negative potentials.

Some Charge Can Be Recovered with Short Prepulses

When the membrane potential is held at 0 mV, both fast and slow inactivation are developed, and the shift of the $Q-V$ curve shown in Fig. 3 could be due to both processes. In an attempt to separate the two inactivation processes, the

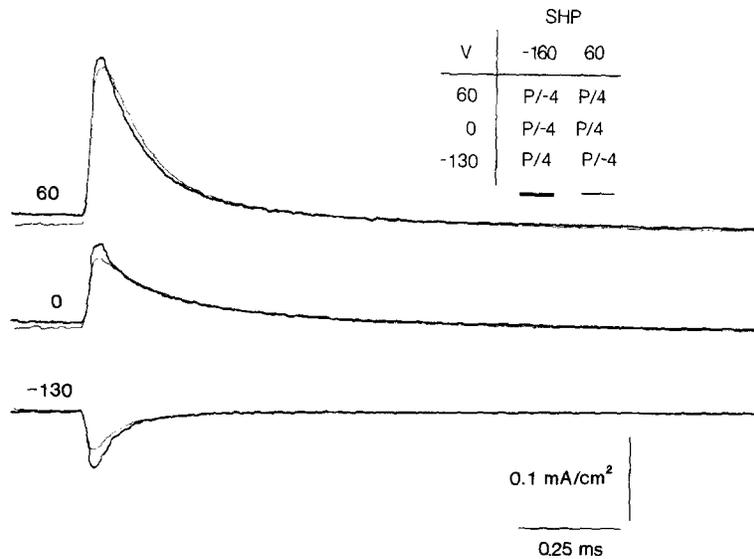


FIGURE 4. Gating currents recorded with different subtraction procedures. The numbers near the traces indicate the membrane potential (V) during the P pulse. HP = -70 mV. P/4 or P/-4 procedure and SHP are indicated in the inset, where the left column shows the parameters for the thick traces and the right column shows the parameters for the thin traces. Tris-CI-TTX//200 TMAFG. Temperature, 7.5°C .

axon was held at 0 mV and the $Q-V$ curve was determined with pulses, preceded by a hyperpolarizing pulse to -70 mV. The pulse protocol and gating current recorded at -160 mV are shown in Fig. 6. Extending the prepulse duration from 1 to 50 ms decreases the charge movement at -160 mV, which indicates that some of the shift was overcome by pulsing the membrane to -70 mV for 50 ms.

The gating current recorded after a 1-ms prepulse to -70 mV shows a slow component similar to the one described and identified by Armstrong and Bezanilla (1977) as the recovery of the charge immobilized by fast inactivation. The slow component is less apparent in the gating current recorded after a 50-

ms prepulse to -70 mV. This type of experiment was repeated for many membrane potentials and the results are plotted in Fig. 7. The charge has been plotted using the same procedure of Fig. 3. The reference $Q-V$ curve (open circles) was obtained with a holding potential of -70 mV. The closed circles are the charge measured at the potential indicated in the abscissa after a prepulse to -70 mV for 1 ms and they trace a curve shifted towards negative potentials as discussed above. When the prepulse to -70 mV is extended to 50 ms, the $Q-V$ curve (open squares) shows a different shape and has a less pronounced shift, although the total charge available to move is still the same.

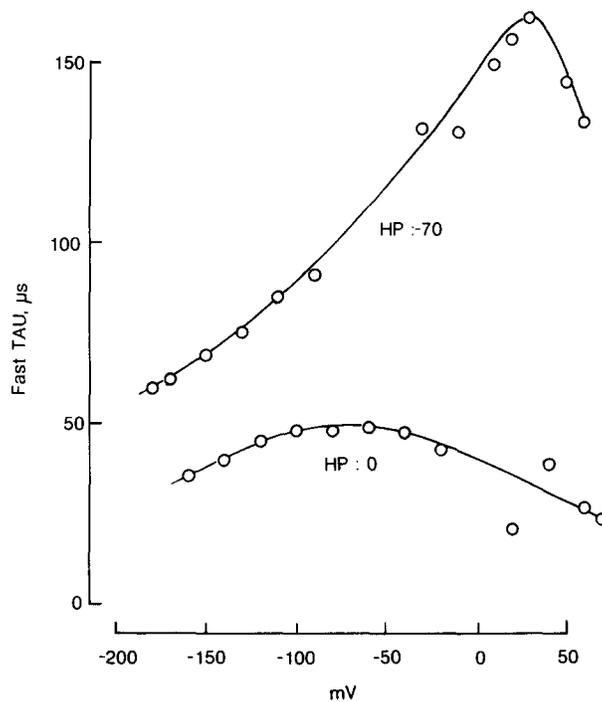


FIGURE 5. Fast time constant of the ON gating current as a function of membrane potential for two different holding potentials. Upper curve: HP = -70 mV. Lower curve: HP = 0 mV. In both cases, SHP = 60 mV. Tris-Cl-TTX//200 TMAFG. Temperature, 7.5°C .

This result indicates that fast inactivation of the gating charge may be at least partially recovered in the presence of slow inactivation.

It is interesting to compare the effect of short depolarization alone on the charge movement with the effects of maintained depolarization to assess the contribution of fast and slow inactivation. Fig. 8 shows an experiment where an attempt has been made to separate the effect of short pulses and long depolarization. Charge has been plotted using the same procedure of Fig. 3. The reference $Q-V$ curve (triangles, solid curve), was obtained at a holding potential of -70 mV. The effects of a short depolarization only are shown in

the dotted curve (closed circles), which was obtained at a holding potential of -70 mV and prepulsing for 50 ms to 0 mV before stepping to the potentials indicated in the abscissa. This curve is similar to the curve shown by Armstrong and Bezanilla (1977) and it represents the effects of fast inactivation on the charge movement. The shift can be explained by the splitting of the kinetics of charge movement in two components produced by the depolarizing pulse to 0 mV. The second component is too slow to be included in the integration period at least at potentials positive to -80 mV, but it becomes fast enough at very negative potentials where one sees a total recovery of the

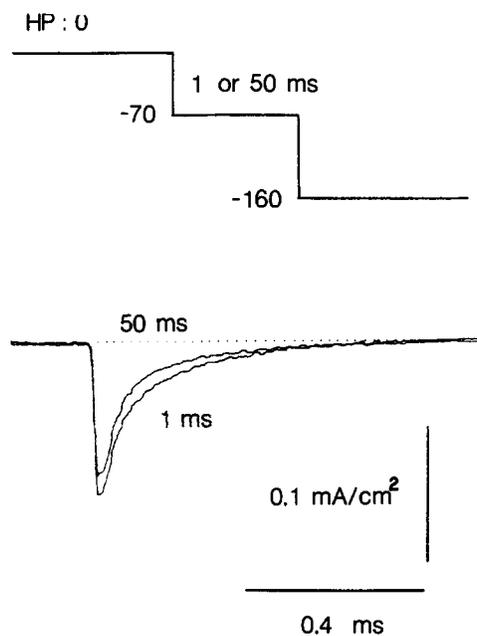


FIGURE 6. Effects of a conditioning prepulse to -70 mV on gating currents at -160 mV for a holding potential of 0 mV. Pulse pattern is indicated at the top of the figure. SHP = 60 mV, P/4 procedure. Tris-Cl-TTX//200 TMAFG. Temperature, 12°C .

charge. The continuous curve through the open circles corresponds to the Q - V curve obtained at a holding potential of 0 mV without prepulse; therefore, it includes the effects of short and prolonged depolarization and shows that extending the depolarization period produces a larger shift of the Q - V curve. The dashed curve through the open squares corresponds to a short recovery interval after a period of maintained depolarization as was shown in Fig. 7. In this case, the curve seems to cross the Q - V curve obtained at continuous holding of 0 mV and shows less recovery of charge negative to -100 mV. This effect was not observed in all axons (e.g., Fig. 7).

Sodium Conductance Does Not Recover with Short Prepulses

It was shown in Fig. 7 that in an axon held at 0 mV, some of the charge can be recovered by prepulsing the membrane potential to -70 mV for 50 ms. A similar experiment to study the sodium conductance is shown in Fig. 9. The top trace (a) is a control record with a holding potential of -70 mV. Records b, c, and d were obtained while the holding potential was 0 mV. When the membrane potential is pulsed for 1 ms to -130 mV (trace b), there is a barely detectable sodium current, although at this potential, fast inactivation of the conductance recovers in about 1 ms (Bezanilla and Armstrong, 1977). By extending the pulse duration to 100 ms, a very small increase is obtained

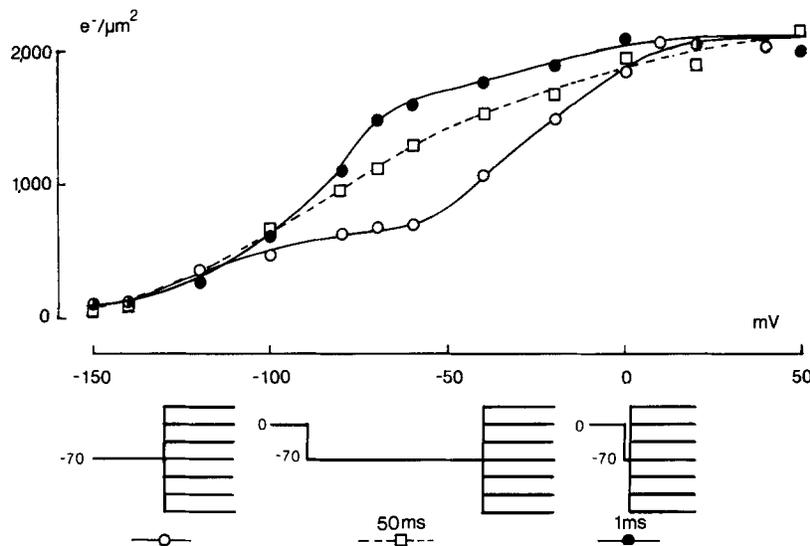


FIGURE 7. Q - V curves at two different holding potentials: recovery of fast in presence of slow inactivation. Pulse patterns are indicated in the bottom of the figure. Open circles: HP = -70 mV, SHP = -160 mV. Closed circles: HP = 0 mV, prepulse to -70 mV for 1 ms, SHP = 60 mV. Open squares: HP = 0 mV, prepulse to -70 mV for 50 ms, SHP = 60 mV; P/4 procedure. Tris-Ac-TTX//200 TMAFG. Temperature, 12°C .

(trace c) and increasing the potential during the prepulse to -170 mV does not produce further recovery of the sodium conductance. This is in contrast to the recovery of the charge observed with a pulse to -170 mV, as seen in Fig. 7 and 8.

DISCUSSION

Previous work has shown that the holding potential has an influence on the voltage-dependent charge movement. For example, Bezanilla and Armstrong (1974) and Armstrong and Bezanilla (1974) reported that after a period of prolonged depolarization, no gating current could be recorded immediately

after repolarization to -70 mV. Keynes et al. (1974) and Meves (1974) studied the effect of holding potential on the current that resulted from the addition of currents produced by pulses of large amplitude but opposite polarity. Again, their results show an effect of the holding potential but they are difficult to interpret because the pulses were driving the membrane potential to different values for each holding potential. Meves (1974) went further in suggesting that the potential at which the charges are evenly distributed is changed by the holding potential.

We have attempted to describe the voltage dependence of the charge

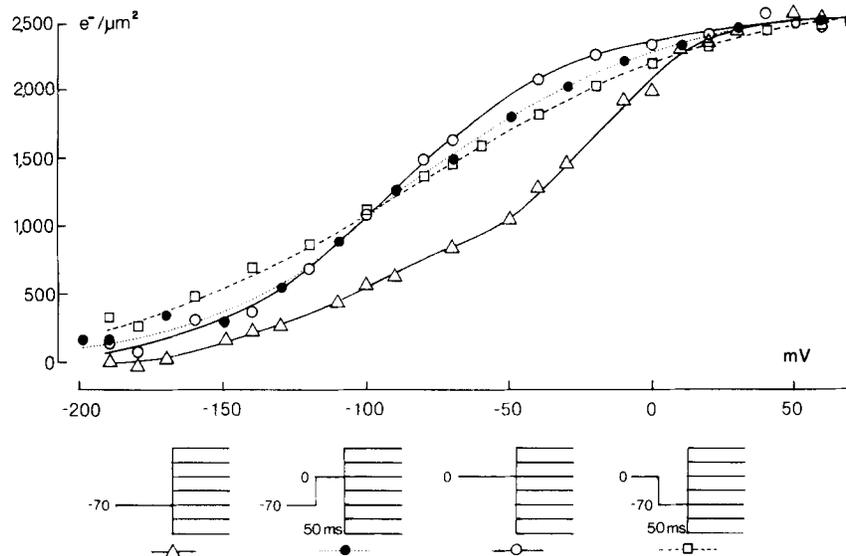


FIGURE 8. Q - V curves at two different holding potentials: development of fast without slow inactivation and recovery of fast in presence of slow inactivation. Pulse patterns are indicated in the bottom of the figure. Open triangles: HP = -70 mV, SHP = 60 mV. Closed circles: HP = -70 mV, prepulse to 0 mV for 50 ms, SHP = 60 mV. Open circles: HP = 0 mV, SHP = 70 mV. Open squares: HP = 0 mV, prepulse to -70 mV for 50 ms, SHP = 70 mV. P/4 or P/-4 used to minimize invasion of subtracting pulses in the nonlinear region. Tris-Cl-TTX//200 TMAFG. Temperature, 7.5°C .

distribution as a function of the holding potential and correlate it with the "slow inactivation" of the charge movement. At the same time, we have searched for a relationship between fast and slow inactivation of the charge movement.

The shift of the Q - V curve toward negative potentials under conditions of maintained depolarization seems to be produced by at least two processes: one that develops within 50 ms and corresponds to the fast inactivation or immobilization of the gating charge, and a second process that develops within several seconds, which we will call slow inactivation of the gating charge.

The charge immobilization observed after a period of maintained depolarization can be understood in terms of the shift of the $Q-V$ curve on the voltage axis. The total disappearance of the charge observed with the $\pm P$ procedure can also be explained by the shift of the $Q-V$ curve because the $Q-V$ for maintained depolarization shows half of the charge at about -70 mV and the identical pulses will subtract not only the linear component but also the nonlinear charge movement. In this interpretation, it is implicit that the $Q-V$ curve measured during maintained depolarization does not change instantaneously after the membrane is repolarized to -70 mV but rather in the first 10–50 ms it will be shifted partially back to more positive potentials because of the recovery of the fast inactivation, and in seconds it will become the $Q-V$

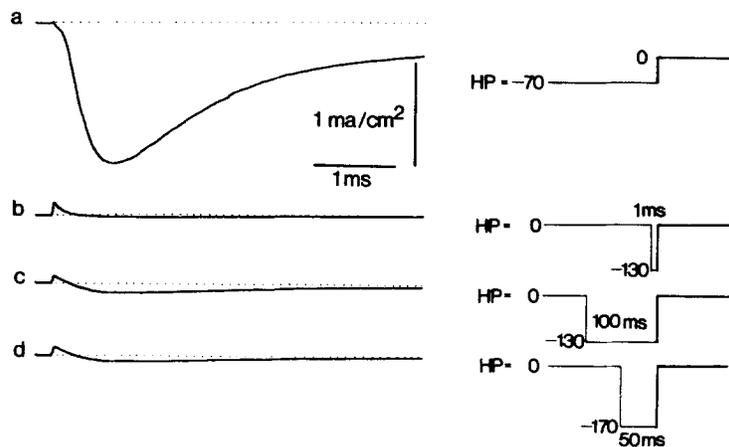


FIGURE 9. Lack of recovery of fast inactivation of the sodium conductance with prepulses to negative potentials. (a) Sodium current recorded in ASW//200 TMAFG after recovery from TTX. HP = -70 mV, SHP = 80 mV. (b), (c), and (d) The HP was 0 mV and the prepulses are indicated at the right of each record. The thicker part of the voltage trace corresponds to the time sampled and shown as the current trace. ASW//200 TMAFG after recovery from TTX, same axon as in (a). SHP = 80 mV; P/4 procedure. Temperature, 12°C .

distribution observed at a holding potential of -70 mV. The actual time-course of the displacement of the $Q-V$ curve has not been measured (except for one point, e.g., Fig. 1) because the measurement would take longer than the time it takes to recover inactivation. The shift to the left of the $Q-V$ curve with prolonged depolarization can also explain the results of Meves and Vogel (1977), in which they reported an almost complete blockage of gating charge when the membrane potential was pulsed from -30 to 75 mV.

The change of the voltage dependence of the $Q-V$ curve with the holding potential is measured after the linear component of the capacitive current has been subtracted. We have discussed in Results that there are sometimes problems with this subtraction. Depending on the holding potential, the subtracting holding potential should be selected in the positive or negative

region. It is highly desirable to obtain the voltage dependence of the nonlinear charge movement without the use of large pulses and subtracting techniques. We will show in the next paper (Fernández et al., 1982) that the voltage dependence of the membrane capacitance can be demonstrated without subtraction by using frequency domain analysis, and the results are consistent with the Q - V distributions presented here.

The measurements of charge vs. potential have been obtained by integrating the gating current transients for 2–4 ms. If the integration period were extended indefinitely, the Q - V curve would correspond to the steady-state distribution of charge vs. holding potential and it would not depend on the initial conditions. Although this experiment is not practical, a similar experiment can be done in the frequency domain to obtain the dependence of the capacitance C on the holding potential (Fernández et al., 1982) and the distribution of the capacitance lies in between the C - V obtained at HP = -70 mV and the C - V obtained at an HP = 0. All these results indicate that the distribution of charge vs. potential is a function of the time the membrane has been held at a given voltage and that the system is nonstationary. Consequently, interpreting the measured charge movements as if they were in steady-state is only meaningful if the integration period is shorter than the time it takes for the system to evolve to a new steady state. This is always true for the slow inactivation experiments described in this paper because the inactivation process develops in several seconds and the integration periods are always <4 ms.

There is an important difference between fast and slow inactivation of the gating charge that deserves discussion. After the fast inactivation of charge has been developed by a 50-ms pulse to 0 mV, it is possible to recover all the charge by stepping back to -160 mV, but only a fraction of the charge is detected when stepping back to -70 mV. The explanation for this (Armstrong and Bezanilla, 1977) is that after the charge has been fast-inactivated by the 50-ms pulse, it moves with at least two time constants; the slow one is too long to be detected within the integration period of ~ 2 – 3 ms at -70 mV but becomes of the order of 0.6 ms at -160 mV, where it can be detected easily. When a similar experiment is done after developing the slow inactivation process by holding the membrane potential at 0 mV, it is again possible to recover all the charge at about -160 or -180 mV. In this case it is possible to recover the fast inactivation first by stepping back to -70 mV for 50 ms and all the charge moves back with no noticeable slow component (see Figs. 7 and 8). If after developing fast inactivation the charge is recovered by pulsing to -160 mV and then the membrane is depolarized again, it is possible to elicit a normal sodium current, which indicates that the fast inactivation of conductance has recovered. The equivalent experiment with prolonged depolarization shows that, although all the charge is recovered, almost no sodium current can be elicited (see Fig. 9). This means that the recovery of all the charge after pulsing to -160 mV does not recover the slow inactivation of the sodium conductance.

An appealing explanation for the charge recorded from an axon that is

pulsed during maintained depolarization is that it corresponds to the gating charge normally responsible for the activation of the sodium conductance that has gone into a different set of physical states that still allow transitions across the membrane but cannot open the channel. The new states show different steady-state and kinetic properties for the charge movement and these changes are related to the inability of the charge movement to make the channel conducting or nonconducting.

Our results show that the voltage dependence of the charge moved during a pulse is changed by both the holding potential and a 50-ms conditioning prepulse. The effects of either short or long conditioning depolarization are to shift the Q - V curve to the left and the effects are additive. Furthermore, it is possible to partially reverse the shift by stepping back to -70 mV for 50 ms, which suggests that the short-term effects can be reversed in the presence of the slow effects. Although additional experiments with fast inactivation removed will be necessary to make sure that some of this reversal is not part of the initial recovery from slow inactivation, we feel certain that our results do not support a sequential model in which slow inactivation can only be reached via fast inactivation. The minimum models possible must consider an alternative pathway between the active and the slow-inactivated states as proposed by Rudy (1978) or by Khodorov (1979). However, their models cannot explain all the observations of the effects of short and prolonged depolarization on conductance and at the same time the kinetics and steady state of the charge responsible for the gating process.

A Model for the Inactivation of Charge Movement

We consider here a simplified model that accounts for a number of observations reported in this paper and predicts many of the results obtained in experiments designed to measure the voltage dependence of the membrane capacitance as reported in the following paper (Fernández et al., 1982).

We will first address the effects of prolonged depolarization. The model considers a charge group (or dipole) that can move (or rotate) within the membrane field. For simplicity we will assume that the charge has only two stable positions, one that closes the channel and another that opens it. This charge must overcome an energy barrier to go from one position to the other and the barrier height and the wells are affected by the membrane potential. A second particle or dipole is able to move between two stable positions, one that does not affect the pore and another that blocks or slow-inactivates it. As a first approximation, we have assumed that the barrier height encountered by the second particle is not affected by the membrane field. When the two particles are close together they can interact, increasing the depth of the well for each of them. The shaded states in Fig. 10 are a physical representation of the four possible states of the channel containing these two particles (the nonshaded states will be explained later). Also pictured in Fig. 10 are the rate constants for the transitions between the states. W_s is the interaction energy in units of kT that deepens the wells for both particles and this energy appears as $\exp(-W_s)$ in the rate constants leaving the state where interaction occurs.

The expressions for α and β (e.g., Woodbury [1971]) are:

$$\alpha = \frac{kT}{h} \exp - \left(W_1 - \frac{ezV}{2kT} \right), \quad \beta = \frac{kT}{h} \exp - \left(W_2 + \frac{ezV}{2kT} \right),$$

where W_1 and W_2 are the differences in kT units from the well to the barrier peak, e is the electronic charge, z is the valence, k is the Boltzmann constant, T is the absolute temperature, and h is the Planck constant. In the hyperpo-

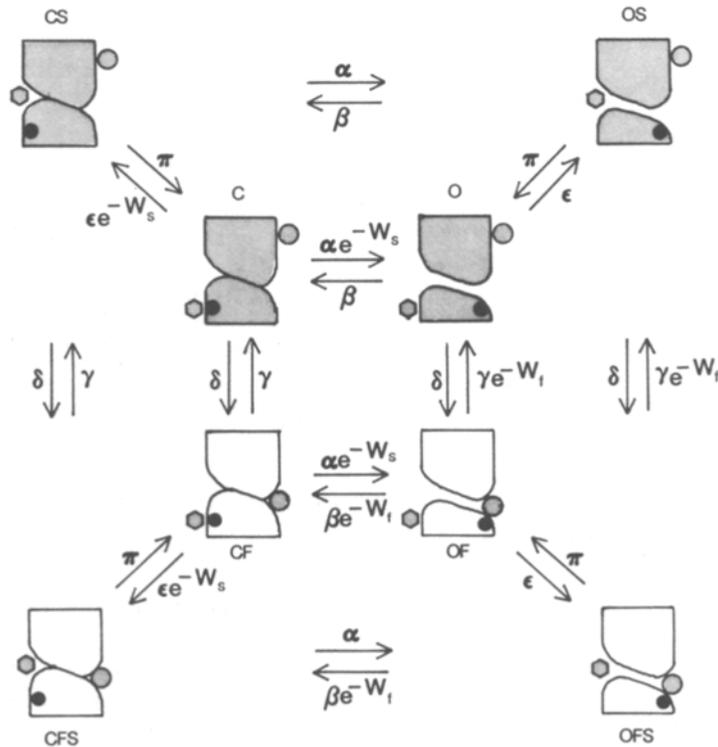


FIGURE 10. A four-state and an eight-state model for fast and slow inactivation. The four shaded states represent the four-state model. The set of all the states represents the eight-state model. The labels are combinations of the following symbols: C = closed, F = fast inactivated, S = slow inactivated, O = open. Black circle: gating particle; gray circle: fast inactivating particle; gray hexagon: slow inactivating particle. For details, see text.

larized case, β is much larger than α and most of the channels will be in states *closed* and *closed/slow-inactivated*. π and ϵ are considered small with respect to α and β to predict the slow time-course of slow inactivation and they have been adjusted to have the majority of the channels in state *closed*. For a strong depolarization, α increases and the *open* state is populated and eventually most of the channels end up in the *open/slow-inactivated* state. Note that a gating current with decay time constant close to $1/(\alpha e^{-W_s} + \beta)$ will be observed. If

after being at a depolarized potential for a long time the membrane voltage is repolarized, β will increase and most of the channels will go to *closed/slow-inactivated* state and a gating current with time constant close to $1/(\alpha + \beta)$ will be observed. The $Q-V$ curve measured when starting at a depolarized potential will be shifted to the left due to the factor e^{-W_s} that affects the rate constant α . It is clear then that this scheme predicts the shift of the $Q-V$ curves and the change of the time constants when the holding potential is modified. Furthermore, this model explains the fact that holding at positive potentials one is able to obtain gating currents produced by charge moving between states *closed/slow-inactivated* and *open/slow-inactivated*, but no conductance is observed unless a long time is allowed at negative potentials to populate the *closed* state.

To predict changes produced by short depolarizations, fast inactivation has to be added to this model. We can envision the fast inactivation process in a similar way as described for slow inactivation. The experiments done with inactivation removed by pronase (Armstrong et al., 1973; Armstrong and Bezanilla, 1977) and experiments done with other agents that affect inactivation (Nonner et al., 1980; Eaton et al., 1980) are consistent with the idea of a blocking particle being responsible for inactivation and this is the main reason we have located the inactivating particle in the interior of the membrane (Fig. 10). Figs. 7 and 8 are consistent with the idea that fast and slow inactivation of the gating charge can proceed independently and we have represented the fast-inactivating particle as being independent and having two stable positions increasing to eight the number of possible states of the physical model as it is pictured in Fig. 10. Although the diagram appears to be much more complex than the four-state model (represented as the group of shaded states in Fig. 10), it only has two new voltage-independent rate constants and a new interaction term, W_f , and most of the rate constants are not independent of each other.

Since there are four transitions in the eight-state model (Fig. 10) that involve charge movements within the field of the membrane, any predicted $Q-V$ curve would be the sum of four curves. For integrations of 2–4 ms, the individual $Q-V$ curves are not necessarily symmetrical and in some cases are not even monotonic. We suggest that this may be a reason the normal $Q-V$ curve for SH = -70 mV (cf. curve with open triangles in Fig. 8) is not symmetrical and frequently contains a “bump” around -70 to -100 mV.

Simulations have been done with both models and most of the results of the four-state model (the shaded portion of Fig. 10) will be presented in the next paper, where predictions have been tested against results obtained in the frequency domain. The most important features of the $Q-V$ curves are reproduced by the model as shown in Fig. 11. The right-most solid curve corresponds to the $Q-V$ curve integrating the current transients for 3.5 ms when the holding potential was -70 mV. The left-most curve was obtained with a holding potential of 0 mV. The dotted curve corresponds to a holding potential of 0 mV but stepping to -70 mV for 50 ms (to recover fast inactivation) before stepping to the voltages indicated in the abscissa. The dashed curve was obtained with a holding potential of -70 mV but before obtaining the $Q-V$

curve the membrane was depolarized to 0 mV for 50 ms to establish fast inactivation.

We have not attempted to fit the experimental data to this model because it is already a simplified scheme of all the possible states involved in gating. For example, the fact that we have pictured the gating particle as having only two states will not account for the delay in the Na conductance turn-on, nor will it predict the presence of a rising phase in the gating current (Armstrong and Gilly, 1979). When the gating particle is modeled with several stable positions it will add to the present model eight more states per additional state of the gating particle (Bezanilla and Taylor, 1981). It is clear that without additional, independent information, fitting of such a model does not promise to be very profitable.

However, it is instructive to see that many of the features of slow and fast

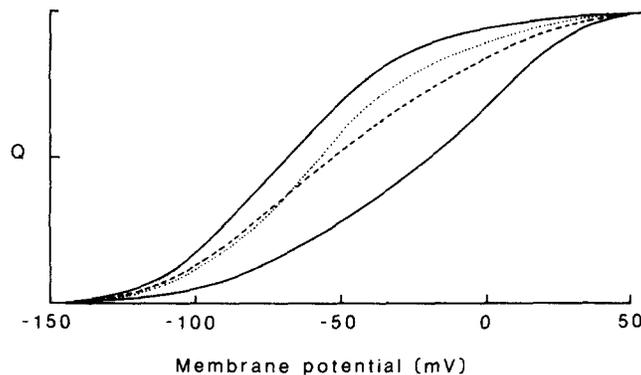


FIGURE 11. Q - V curves predicted by the eight-state model. Right-most continuous curve: HP = -70 mV. Left-most continuous curve: HP = 0 mV. Dotted curve: HP = 0 mV, prepulse to -70 mV for 50 ms. Dashed curve: HP = -70 mV, prepulse to 0 mV for 50 ms. Parameters used: $W_f = 4$, $W_s = 3$, $\gamma = 7,500 \text{ s}^{-1}$, $\delta = 1,500 \text{ s}^{-1}$, $\pi = 0.01 \text{ s}^{-1}$, $\epsilon = 0.05472 \text{ s}^{-1}$, $z = 1.6$, $W_1 = 20.55$, $W_2 = 22.09$.

inactivation can be accounted for by the movement of a single charged particle in the membrane field that can interact with two other independent particles that need not move in the membrane field. The effect of pronase can be pictured as removing the interaction of one of the particles. The model predicts that a modification of the interaction of the slow-inactivating particle should produce drastic changes in the shape of the Q - V curves and in the time constants of charge movement. This effect could be investigated with modifiers of slow inactivation (see, for example, Starkus and Shrager, 1978).

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