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Topical Review

Gating of Sodium and Potassium Channels

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Introduction

Voltage-dependent conductances play a fundamental role in the generation and propagation of the action potential. Hodgkin and Huxley (1952*c*) made a complete description of the membrane currents underlying the generation of the impulse. Their description introduced the concepts of separate pathways for sodium and potassium (today called Na and K channels) and the idea of conductances modulated by the membrane potential.

Our present view of the sodium and potassium conductances are based on the idea that the membrane has discrete conducting units called channels. These units are macromolecules highly specialized for ion conduction and embedded in the lipid matrix where they can sense the electrical field across the membrane and respond to its changes.

This article is concerned with the mechanisms by which ion conductances depend on membrane potential, a process called *voltage gating*. Our knowledge of voltage gating is based on measurements of macroscopic currents, analysis of current noise, single channel recordings and gating currents. We will first examine the relationship between macroscopic and microscopic determinations and their relation to gating currents and then results of the different measurements will be analyzed in terms of models.

There are many recent reviews on voltage gating with varying emphasis on the different types of experimental results (Cahalan, 1980; Armstrong, 1981; French & Horn, 1983). This review will emphasize results on gating currents, which are a direct expression of the voltage-dependent process. These currents are small and their detection requires subtraction of large currents. Consequently, the literature on gating current has not been without controversy. Most of the results and interpretations presented here will be obviously biased towards the results obtained in our laboratory and my personal view of the gating process.

Key Words Na-channel · K-channel · voltage-dependence · charge movement · single channel · excitability · axon · squid

The Voltage-Dependent Channel

The sodium channel is a glycoprotein of mol wt 260 kilodaltons (Agnew et al., 1978; Barchi, 1983). The voltage-dependent, delayed-rectifier potassium channel has not been isolated but presumably is also a large protein. The most widely accepted view of these channels is based on the separation of the two main functions, *permeation* and *gating*. In this view, the channel is pictured as having an hydrophilic region, or pore, where ion conduction occurs, and it is in this region where the selection of ions that can traverse the channel occurs. This means that there should be differences in the molecular structures of the sodium and potassium pores to explain their different selectivities. The gating is assumed to be some type of blocking and unblocking of the pore to the passage of ions, although the exact mechanism of this process is unknown at the moment. Regardless of the actual mechanism of the gating process, it is clear that a conformational change of the macromolecule is involved. This conformational change may occur spontaneously with a frequency which depends on the magnitude of the energy barriers encountered in the transition between the different conformations. In the case of the voltage-dependent channel, the frequency of transitions is regulated by the membrane voltage, and this is most likely achieved by a change induced by the membrane field in the energy profile encountered by the gating structure. In what follows, we will concentrate our attention on the gating proper-

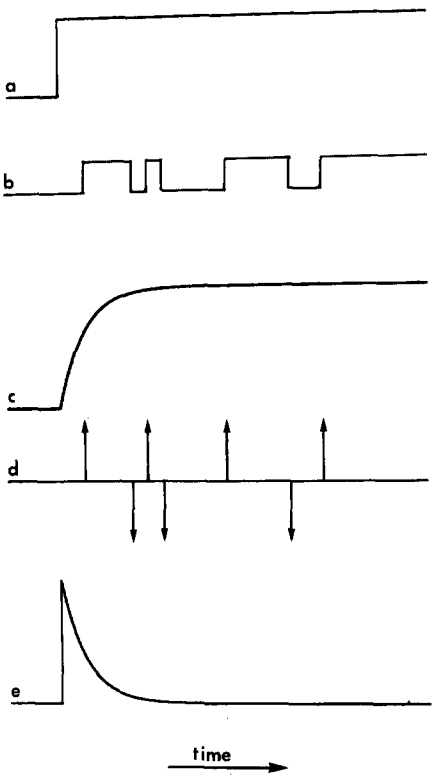

Fig. 1. Time course of currents for the two-state model. (*a*) Voltage step applied to the membrane. (*b*) A single-channel current record. (*c*) The average current produced by a large number of channels. (*d*) The current expected from the gating current sensor as it moves between the two states. This current corresponds to the sensor of the channel pictured, in part *b*. (*e*) The average current (gating current) produced by a large number of gating sensors

ties of the channels and in particular on the gating process controlled by the membrane voltage.

Macroscopic, Microscopic and Gating Currents

We call macroscopic the currents recorded from a large area of membrane such as a segment of a squid giant axon or a node of Ranvier or a whole cell recording with a patch pipette from a GH3 cell. In all these cases the current recorded, under conditions of controlled membrane potential (voltage clamp), corresponds to the sum of a large number of individual conducting units called channels. The understanding of the mechanisms underlying the generation of the macroscopic current will only be possible after the behavior of the individual channels is described along with the function that relates the individual current (microscopic current) with the macroscopic current.

The Two-State Model

A simplified model of a channel can illustrate the connection between macroscopic and microscopic currents. Consider a channel that can only exist in two possible physical states: closed (state C) or open (active state, A). When in state C, no ions can pass through the pore of the channel and in state A ions selected by the selectivity filter can pass readily through the pore producing a *single channel* or *unitary* current. The two states of the channel are represented in the following reaction scheme

$$C \underset{\beta}{\overset{\alpha}{\rightleftarrows}} A \tag{1}$$

in which α and β are rate constants governing the transition between the states. These rate constants depend on the physical structures of the macromolecule responsible for the closed and open conformations. In our case, as we are dealing with voltage-dependent gating, the membrane voltage will also affect the magnitude of the rate constants. The exact mechanism by which the membrane potential affects the rate constants is not known, but it is clear that a *voltage sensor* must be present in the channel molecule to sense the membrane potential and modify the probability that the molecule will undergo a conformational change. The sensor is most likely an electric charge or a dipole that is free to change its orientation in the membrane field. In a simple model in which the charge encounters only one energy barrier the expressions for α and β are given by

$$\alpha = \frac{kT}{h} \exp\left[-\frac{W_C}{kT} + \frac{zedV}{kT}\right]$$
$$\beta = \frac{kT}{h} \exp\left[-\frac{W_A}{kT} - \frac{ze(1-d)V}{kT}\right] \tag{2}$$

where z represents the valence of the charged sensor, V the membrane voltage, e the electronic charge, W_C and W_A the energy differences between the peak of the barrier and the well for the transition between C and A and A and C, respectively, in the absence of electric field, d the fraction of the distance through the field where the peak of the barrier is located, and T, k and h are absolute temperature and the Boltzmann and Planck constants, respectively. These expressions hold when the electric field is considered constant across the membrane; this may not be the case, in general. The effect of the membrane potential is to increase the probability that the channel will be in the open (A) state as V

becomes more positive: although the channel will still make transitions between the two states, it will spend most of its time in the A state.

This simple model can be used to predict the currents that are experimentally measurable. Assume that the membrane potential has been maintained at a negative level, so that most of the channels are in state C, and then a sudden step of voltage is applied towards a positive value (Fig. 1*a*). Figure 1*b* shows the current expected through a representative single channel, and Fig. 1*c* shows the total macroscopic current representing the addition of a large number of *independent* single-channel currents. The current shown in Fig. 1*d* represents the current produced by the movement of the charged sensor in the particular channel in Fig. 1*b*. In this idealized case they are infinitesimally short and of infinite amplitude (i.e., delta functions in current). This is because the charged sensor has been assumed to exist in only two possible states and the actual transition is instantaneous. The membrane potential modulates the times spent in each of the conformations, but it has no influence on the actual charge transition time across the barrier. When the current produced by the movement of the charged sensors of all channels contributing to Fig. 1*c* are considered, a transient current is obtained called a *gating current* (Fig. 1*e*).

In a real experiment, these three types of current recordings, single-channel current, total ionic current, and gating current could be used to derive the α and β for reaction (1), and a set of measurements made at several voltages could be used to derive the voltage dependence of the rate constants. In this particular case (the two-state model) the values can be derived with any of the three measurements: that is, the measurements are redundant. If single-channel recordings are available, α and β can be obtained from close and open dwell times. From gating current records α and β can be obtained from the time constant of the transient and the maximum value recorded when stepping from a voltage at which all channels are closed. Using macroscopic currents, α and β can be obtained from the transient time constant and the maximum value of the fraction of open channels which requires knowledge of the voltage dependence of the open channel. This last value can be estimated from an instantaneous I-V curve (Hodgkin & Huxley, 1952*a*). The values obtained will only have meaning if the channel investigated has two states, which is rarely the case.

The steady-state properties of the two-state model are summarized in Fig. 2*a* where the gating charge moved (time integral of the gating current) as a function of the membrane potential ($Q(V)$ curve) is shown together with the voltage dependence of the fraction of open channels ($a(V)$ curve). In this case both curves are superimposable and the function, Eq. (4), will be discussed below.

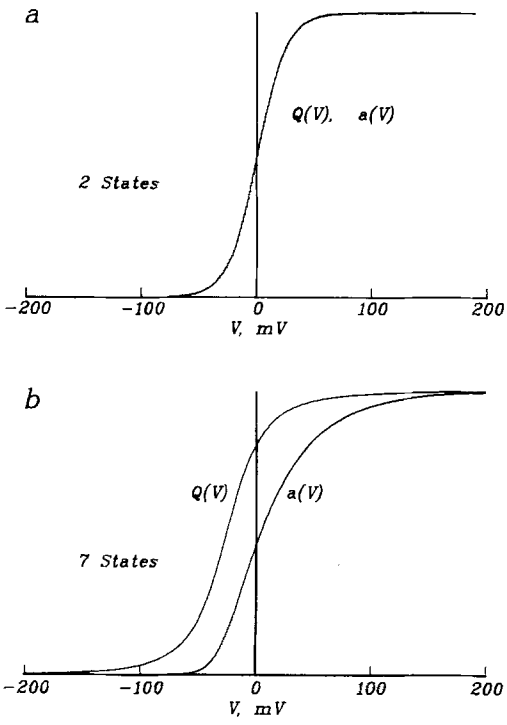

Fig. 2. Voltage dependence of the gating charge ($Q(V)$ curve) and fraction of open channels ($a(V)$ curve) for a two-state model (*a*) and for a seven-state model (6 closed and 1 open state) that simulates sodium activation (*b*). Notice that in the two-state model the $Q(V)$ and $a(V)$ curves superimpose and in the multiple-step model the $Q(V)$ curve is shifted to the left of the $a(V)$ curve

More than Two States

When there are more than two states the three types of measurements are required to derive the values of the many α's and β's involved and, in general, in a multistep model the values derived are not unique. The reason is that in the multistep situation the rate constants are given by very complicated expressions in the eigenvalues of the solution. These eigenvalues are the reciprocals of the time constants of the macroscopic and gating currents and their values are often very similar, making their separation difficult or impossible. Furthermore, the distribution of dwell times of single-channel records are no longer single exponentials, and their separation also becomes difficult or impossible.

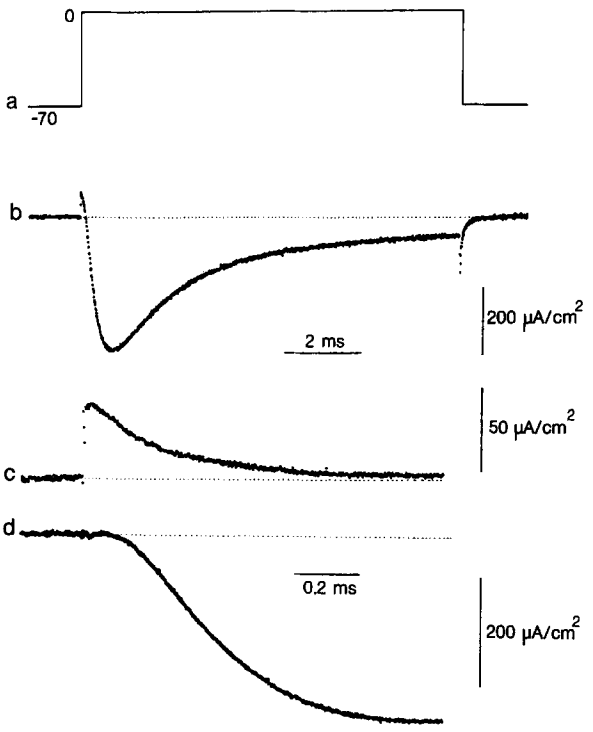

Fig. 3. Sodium and gating current in the squid axon. These records were obtained in internally perfused squid giant axons with solutions containing impermeant cations and bathed in a solution containing $\frac{1}{5}$ of the normal sodium concentration. Linear leakage and linear capacitive currents were subtracted off using subtracting pulses starting at -150 mV using the p/4 procedure. The beginning of the pulse is aligned for all the traces. (*a*) Diagram of the depolarizing pulse from -70 to 0 mV. (*b*) Current recorded for the pulse in *a*. The small transient outward (positive) current corresponds to the gating current, and at the end of the pulse the inward (negative) tail is the sum of the off gating current and the turn-off of the remaining sodium conductance. (*c*) Gating current recorded with higher time resolution. The sample rate is 2 μsec per point, and the external solution contained no sodium and 300 nM tetrodotoxin (TTX). (*d*) Sodium current at higher resolution obtained by subtracting the current in TTX from the current in $\frac{1}{5}$ sodium

An example of the use of two types of measurements is in the case of channels that have several closed states:

$$C_O \underset{\beta_o}{\overset{\alpha_o}{\rightleftarrows}} C_1 \cdots C_i \underset{\beta_i}{\overset{\alpha_i}{\rightleftarrows}} C_{i+1} \cdots\cdots C_n \underset{\beta_n}{\overset{\alpha_n}{\rightleftarrows}} A. \qquad (3)$$

These channels show a characteristic relationship between gating current and macroscopic current: the voltage dependence of the integral of the gating current ($Q(V)$ or Q-V curve) is shifted to the left of the voltage dependence of the fraction of open channels ($a(V)$ or f-V curve (*see* Fig. 2*b*). The magnitude of the shift depends on the number of closed states preceding an open state.

In the case of the sodium and potassium channels, the experimental evidence shows that there are several states involved; the three types of recordings will therefore be required to estimate the multiple α's and β's involved in the gating reaction. The biological preparations available differ in their advantages to record the three types of currents: for example, the best recordings of single sodium channel events have been obtained from cultured mammalian cells and the best macroscopic and gating current recordings have been made on the squid giant axon. Some differences have been found between preparations, which prevents the meaningful integration of the data into a unique model, and detailed modelling will have to be made with caution when using data from different cell types or will have to wait until all the information is available from the same preparation.

The Activation of the Sodium Channel

When a squid axon, with no potassium ions either inside or outside and held at a membrane potential of -70 mV, is suddenly depolarized to 0 mV (Fig. 3*a*), a transient inward current develops. Figure 3*b* shows the current recorded from an axon bathed in $\frac{1}{5}$ of the normal sodium concentration; a small outward current is seen at the beginning of the pulse, corresponding to the gating current. By increasing the sampling rate to 500 kHz it is possible to resolve the currents with more detail. The current shown in Fig. 3*c* corresponds to the gating current recorded with the *P*/4 procedure (Bezanilla & Armstrong, 1977) and in presence of tetrodotoxin. Figure 3*d* shows the current recorded in $\frac{1}{5}$ sodium after the gating current has been subtracted. The macroscopic sodium current differs from the two-state model in two important ways: (i) the current starts with zero slope (Fig. 3*d*), and (ii) as the potential is maintained the current declines (Fig. 3*b*). Both differences are the result of a multistep process, i.e., the lag in the turn-on can be explained by the presence of several closed states as will be discussed below, and the decay is due to the inactivation process which is discussed in detail later.

The Voltage Dependence of the Conductance

The first property to be explained is the voltage dependence of the fraction of open channels. This is a steady-state property and consequently it should not have kinetic complications. Unfortunately, this determination is difficult to make because the inactivation process masks the actual steady-state value

of the activation. The simple measurement of peak sodium current divided by the driving force does not reveal the fraction of open channels, because (i) one must consider the nonlinearity of the open channel characteristics which can be obtained from an instantaneous *I-V* curve (Hodgkin & Huxley, 1952*a*), and (ii) the measurement at the peak current only gives the fraction of channels conducting at time of peak; this fraction is equal to the fraction of open channels that have not been inactivated. To obtain the actual value of the fraction of open channels, inactivation must be subtracted correctly or, better, removed. Stimers, Bezanilla and Taylor (1985) studied the voltage dependence of the fraction of open sodium channels treating the axon with pronase (Armstrong, Bezanilla & Rojas, 1973). The results show that the voltage dependence of activation of the channel measured in absence of inactivation is less steep than estimated previously, because the conductance changes *e*-fold in 7 mV. A similar result was found by Vandenberg and Horn (1984) using whole cell recording in GH_3 cells treated with trypsin to remove inactivation. The steepness of the fraction of open channels has important theoretical consequences because it gives a lower bound to the amount of charge required to gate the channels. In the two-state model, the fraction of open channels $a = A/(A + C)$ is given by (*see* Fig. 2*a*)

$$a(V) = \frac{1}{1 + \exp\left[\frac{W_C - W_A - zeV}{kT}\right]} \tag{4}$$

and the steepness of a with voltage depends on z (the valence of the gating particle times the fraction of the field). In a multistep model, the fraction of open channels a (*see* Fig. 2*b*) may be given by (*cf.* Bezanilla & Taylor, 1982)

$$a = \frac{\exp -\left[\frac{W + zeV}{kT}\right]}{\sum_i \exp -\left[\frac{W_i + z_i eV}{-kT}\right]} \tag{5}$$

with

$$W_i = \sum_{k=0}^{i} w_k$$

and

$$W = \sum_i W_i$$

where $z_i e$ is the accumulated charge moved from C_o to C_i; ze is the total charge transported and w_i are the differences of the energy wells of the individual transitions. In this case, the steepness measured at very negative potentials can be used to estimate ze (Almers, 1978). The experimental results of Stimers et al. (1985) give a minimum of four charges/channel. The total gating charge measured is about 1800 $e/\mu m^2$, and using a total of about 360 channels/μm^2 (Levinson & Meves, 1975; Strichartz, Rogart & Richie, 1979) one finds that there is enough gating charge to explain the steepness of the *f-V* curve.

Several Closed States Produce a Lag in the Sodium Conductance Turn-On

The lag in the activation of the sodium conductance is one of the main pieces of evidence for the existence of several closed states. In the microscopic interpretation of the Hodgkin and Huxley (HH) model (Hodgkin & Huxley, 1952*c*) the activation sequence (the *m* process) contains only one open state and three closed states (*see*, e.g., Fig. 1 of French & Horn, 1983). The activation process in the HH model can be interpreted in terms of three independent identical gating charges that must all be properly located if the channel is to be open. This model is a special case of a four-state sequential model in which the forward rate constants evolving from the most closed to the open state are 3α, 2α, and α and the backward rate constants from open to closed are 3β, 2β, and β. Supporting the presence of multiple closed states is the observed shift to the left of the *Q-V* curve with respect to the *f-V* curve (Armstrong & Gilly, 1979; Stimers et al., 1985). As explained in the previous section, the determination of the fraction of open channels *vs.* voltage (*f-V*) curve can only be determined accurately in absence of inactivation as was done in Stimers et al. (1985) using pronase. These authors found that the *Q-V* curve is shifted by tens of millivolts to the left of the *f-V* curve and this shift includes a prominent 'bump' of extra charge in the *Q-V* curve at potentials more negative than −70 mV (Bezanilla & Armstrong, 1976). The origin of this bump of extra charge was investigated by Taylor and Bezanilla (1983) in a study of the time shift of the sodium and gating currents as a function of initial conditions. The effect of initial conditions on the time course of the potassium conductance was first described by Cole and Moore (1960) who found that a negative prepulse delays the turn-on of the potassium current. The same phenomenon was found for the sodium current by Armstrong and Bezanilla (1974) and Keynes and Rojas (1976) in the squid and by Hahin and Goldman (1978) in *Myxicola*. Taylor and Bezanilla (1983) found that a negative prepulse delays both the sodium and the gating currents by the same amount and that the delay of the gating current is

accompanied by an increase in the charge moved during the transient. Furthermore, the voltage dependence of this charge corresponds to the extra charge observed at $V < -70$ mV in the Q-V curve. These results support the idea that the bump of extra charge is associated with the sodium channel gating and corresponds to the voltage-dependent transitions between closed states, which are favored by negative potentials. As the prepulse is made more negative, most of the channels are shifted towards the most closed states and upon depolarization it will take longer to activate the current because the channels have to evolve through many closed states before they can open. A sequential model with six states (only one being open) accounts for the results of both sodium and gating current.

Several Identical Gating Units Not Possible

The HH model can, in principle, reproduce the time lag of the sodium current activation, but more states must be added to account for the shift of the sodium and gating current by the prepulse. However, this model fails completely to describe the behavior observed at end of the pulse. If after a depolarization the membrane is repolarized to -70 mV a tail of sodium current is recorded (Hodgkin & Huxley, 1952*a*) which corresponds to the deactivation of the conductance or the evolution from the open to the closed states. Gating currents (I_g) recorded for the same pulse pattern shows a decaying transient with a time constant 1.2 times slower than the sodium current (I_{Na}) transient (Bezanilla & Armstrong, 1975; Armstrong & Bezanilla, 1977). The prediction of the HH model is that I_g decays as dm/dt does, i.e., as $\exp(-t/\tau_m)$ and that I_{Na} decays as m^3 which is of the form $\exp(-3t/\tau_m)$. This means that the gating current should be three times slower than the sodium current at pulse turn-off and if there were more states, as is required by the results described in the previous paragraph, the ratio of the time constants should be even larger. The experimental results show that as the returning potential is made more negative after the pulse the ratio goes from 1 (at -60 mV) to a maximum of 1.7 (at -110 mV) (Armstrong & Bezanilla, 1977). This result rules out any model of activation that considers an identical number of independent gating particles. This means that in the general sequential model we cannot assign the 3, 2, 1 relationship between the different α's and β's *a priori* and their individual values will have to be fitted from the experimental data. A unique fit is not likely because there are too many parameters involved; for this reason different types of experiments are required to obtain the values of the rate constants.

The First Step of Activation

The time course of the macroscopic conductance is somewhat insensitive to the relative position of the different alphas and betas in the activation sequence. In contrast, the gating current is extremely sensitive to the order of fast and slow steps in the activation sequence as was clearly illustrated by Armstrong (1981). Therefore, the time of I_g is crucial in assigning values to the alphas and betas between closed states. The presence of a rising phase in the gating current is indicative of a first step slower than the second and the complete absence of a rising phase indicates a first step faster than the second. If I_g starts with zero slope before decaying, it is an indication that the first few steps are equally fast. The original observations of the sodium channel gating current showed the presence of a rising phasing of the order of 60 μsec (Armstrong & Bezanilla, 1974; Armstrong & Gilly, 1979), but more recent experiments show either that there is no rising phase or at least that is faster than 10 μsec (Stimers, Bezanilla & Taylor, 1984). This latter study showed that when a rising phase was observed it was associated with a slow component in the capacitive current indicating that a region of membrane did not follow the command potential accurately. This membrane is most likely located in the region between clefts between adjacent Schwann cells because the use of hypertonic medium outside or hypotonic medium inside eliminates the rising phase together with the slow component of the capacitive transient. The effect of the change in tonicity can be explained by an expansion of the periaxonal space produced by the water flow decreasing the access resistance to the membrane region in between clefts (Stimers et al., 1984). The conclusion from these results is that the first step of the activation sequence is faster than the subsequent ones. Horn and Vandenberg (1984) have found the same result using single-channel recording. Interestingly, we can add that in the subsequent early steps there cannot be a step, carrying a sizeable amount of charge, which is slower than a subsequent one because it would produce a shoulder in the gating current time course which is not observed since I_g decreases monotonically. A slower step than the subsequent one does not produce a noticeable shoulder in I_g if it is closer to the open state: it is therefore not necessary that the steps become progressively slower as the open state is approached, and the possibility of a faster step near the open state is not excluded. In fact, as explained in the

next section, it appears that the transition just before opening may be faster than the previous one.

No Rate-Limiting Step

The next question is whether there is a rate-limiting step in the activation sequence which governs the overall kinetics of activation. It is clear from the results of the previous section that the rate-limiting step, if it exists, must be very close to the open state if it is not the last in the sequence. The protocol of two pulses separated by a variable interval can be used to test for rate-limiting characteristics of the last step (Oxford, 1981). As the interval between pulses is made shorter, fewer channels are allowed to return to the most closed states, and the second pulse tests the activation kinetics starting with most of the channels in the last closed state. If the last step is rate limiting, the main time constant of the sodium current will be unaffected by the pulse interval. Experimentally it was found that the time constant gets smaller as the pulse interval is made shorter, indicating that the last transition is not rate limiting (Stimers, Bezanilla & Taylor, 1983). The actual rate constants of the last steps are not obtainable from these measurements because, as was explained above, the time constants of the gating and sodium currents are the reciprocals of the eigenvalues of the solution to the system of equations and are complicated functions of the original rate constants. The only case in which the measured time constant is close to the reciprocal of the sum of alpha plus beta of one particular step is when that step is rate limiting, meaning that the rate constant has to be many times smaller than any other in the sequence. The longer the sequence is, the larger the required ratio between the rate-limiting step rate constant and all of the others. Single-channel measurements of closed time distributions using the protocol of variable interval between pulses may help in separating the last rate constant. The closing rate constant (beta) of the last step can be obtained from open time distributions in absence of inactivation (Patlak & Horn, 1982; Horn, Vandenberg & Lange, 1984).

Uncharged Steps

Uncharged steps in a sequential activation process corresponds to transitions without charge movement, and they would not contribute directly to the gating current. As a general rule, uncharged steps must not be slower than the fastest activation time constant because they become rate limiting when the membrane is highly depolarized or hyperpolarized as the voltage-dependent rate constants take their maximum values. The transition between the most closed state and the next one cannot be uncharged because it would produce a rising phase in the gating current (*see* Bezanilla & Taylor, 1982). The effect of an uncharged step in the middle of the sequence is to produce a shoulder or even a second peak in the gating current. This effect is less important as the uncharged step gets closer to the open state and is completely unnoticeable if it is faster than any of the charged steps. The last step, however, could be uncharged and not too fast because the sequence may have slower steps as it gets closer to the open state. Conti et al. (1984) have determined the activation volume of the gating reaction by measuring gating currents and ionic currents at high hydrostatic pressures. They found that the activation volume determined from the gating current is less than the volume measured from the sodium current. This result is consistent with the existence of an important conformational change which does not involve charge movement and consequently is electrically silent. This transition would most likely correspond to the last transition before the open state. However, Horn and Vandenberg (1984) have estimated that the last transition is reasonably voltage dependent in mammalian cells.

In summary, the activation of the sodium conductance can be explained by a sequence of six closed states and one open state with voltage-dependent transitions. At depolarizing potentials, during the opening sequence, the transition from the most closed state to the next one is faster than any of the others and the progression towards the open state is made by transitions of the same magnitude or slower.

Inactivation of the Sodium Channel

The decay of the sodium current during a maintained depolarization has been ascribed to a process called inactivation. Hodgkin and Huxley (1952*b*) found that the membrane potential has a dual effect on the sodium conductance: upon depolarization a fast process (activation) increases the conductance and a slower process (inactivation) decreases it. They proposed mainly as a mathematical convenience that both processes are independent. Before gating current measurements were available several authors had proposed a coupling between activation and inactivation (Goldman & Schauf, 1972), and some experimental evidence was consistent with this idea.

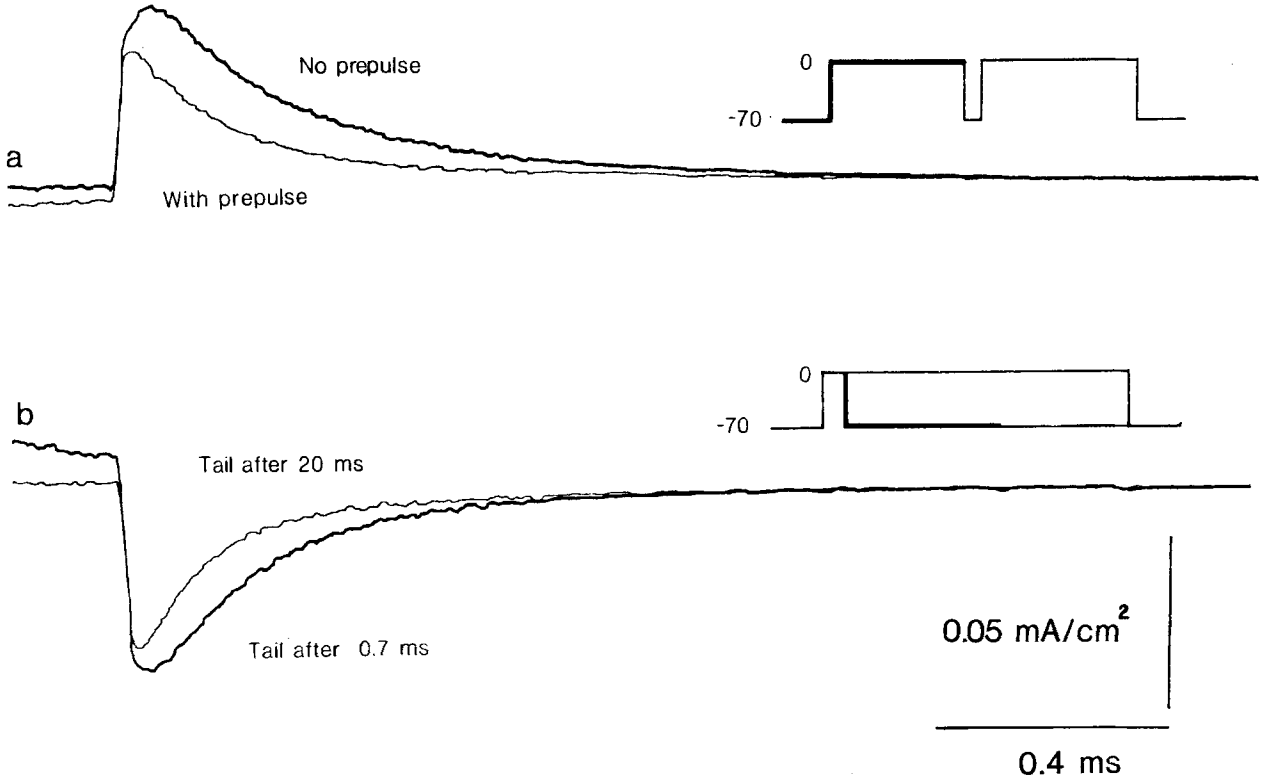

Fig. 4. Inactivation of the gating current. (*a*) Traces correspond to gating currents obtained with (thin trace) and without (thick trace) prepulse. The pulse protocol is indicated in the upper inset. (*b*) Traces correspond to the gating current tails recorded for a 0.7-msec pulse (thick trace) and a 20-msec pulse (thin trace). Lower inset indicates the pulse protocol. Notice that the traces were overlayed at long times because the prepulse was not completely over in *a*, and in *b* there was a sizable gating current at the end of a 0.7-msec pulse

The Same Gating Charge is Responsible for Activation and Inactivation

Inactivation is also voltage dependent, although with much less dependence than the activation process. A small and slow gating current produced by the movement of the inactivation gating particle is therefore expected to be present together with the activation gating current. Although the gating current shows at least two exponential components during the ON transition (Armstrong & Bezanilla, 1977), both phases are much faster than the inactivation time course and no component with the kinetics of the inactivation was detected. It was quite surprising to find instead that the activation gating current was decreased by a prepulse that produces inactivation of the sodium current (Bezanilla & Armstrong, 1974). This finding is illustrated in Fig. 4*a*. The thick trace is a gating current recorded for a pulse to 0 mV from a holding potential of −70 mV. If the pulse is preceded by a prepulse of 20 msec duration to 0 mV and separated by an interval of 0.7 msec, the gating current recorded is smaller and carries less charge (thin trace of Fig. 4*a*). A prepulse of this length and amplitude also decreases the sodium conductance during the test pulse (Hodgkin & Huxley, 1952*b*), indicating some connection between the charge decrease and the inactivation of the conductance. This connection becomes stronger if the charge returned at the end of a short pulse, when inactivation has not been developed, is compared with the charge returned after a pulse of 20 msec, when inactivation is established. The result is shown in Fig. 4*b* in which the tail after a 0.7-msec pulse (thick trace) spans much more area (i.e., more charge) than the tail for a 20-msec pulse (thin trace).

Armstrong and Bezanilla (1977) found that though the conductance inactivation can be as much as 85–90%, the corresponding charge decrease is to 60–70%. These authors studied the correlation between this charge decrease or charge *immobilization* and the inactivation of the conductance. The results can be summarized as follows: (i) The time course of the charge immobilization with pulse duration is the same as that for the inactivation of the conductance. (ii) The voltage dependence of the charge immobilization is the same as that for the inactivation of the conductance. (iii) The time course of the recovery of the charge immobilization is the same as for the recovery of the inactivation of the conductance. These results

showed a strong correlation between charge immobilization and sodium inactivation, and the authors proposed that the inactivation process is the result of the immobilization of the charge. The charge immobilization is actually due to a change in the charge movement process that splits the charge redistribution kinetics into two components, one of them too slow to be detected in the 2 to 3 msec integration period. For example, the tail of gating current recorded after a 20-msec pulse (thin trace, Fig. 4*b*) has a component of about the same speed of the tail after 0.7 msec but also contains a very slow component that is not easily detected in the record. Armstrong and Bezanilla (1977) showed the presence of this slower component by returning the membrane potential to −150 mV. At this potential the inactivation recovery process has a time constant of about 0.6 msec, and they found that the gating current tail for a 20-msec pulse shows a second component with 0.6 msec time constant. The slow component of the tail can be visualized at −70 mV when the experiment is done at a higher temperature.

Similar results were found by Nonner (1980) in the node of Ranvier, though Meves and Vogel (1977) did not find a strict correlation between gating current immobilization and sodium current inactivation in the squid axon.

The conclusion of these experiments is that the inactivation process is produced by the same gating charge responsible for the activation, when a large fraction of this charge changes to a different set of kinetic states. With these results there is no need for a separate inactivation gating charge because the voltage dependence of the inactivation would be governed by the voltage dependence of the charge which gates the activation process.

Slight Voltage Dependence of the Inactivation Step

Recent work using pronase-treated axons to compare the sodium and gating currents with and without inactivation has shown that the inactivation process must have a voltage dependence of its own to explain the time course of the inactivation at large depolarizations (Stimers et al., 1985). This voltage dependence amounts to less than one electronic charge moving across the total membrane field and is expected to produce a gating current. It is not surprising that this component of gating current has not been detected in the squid axon because it is expected to be very small and slow. Swenson has reported a gating current component with the time course of inactivation in crayfish axons (Swenson, 1983), indicating that the amount of charge involved in the inactivation process in that preparation is probably larger than in the squid axon. It is interesting to note that even in the case of a totally voltage-independent step one expects to find a component of gating current that has the time course of inactivation because the eigenvalues of the solution for the gating current are the same as the eigenvalues for the solution of the conductance. It is presumably not apparent because the coefficient of the exponential bearing the eigenvalue is much smaller than the others, making it difficult to detect.

Inactivation in Single-Channel Recordings

Since the introduction of the patch recording technique (e.g., Hamill et al., 1981) it has been possible to observe single sodium channel events (Sigworth & Neher, 1980) and to test some of the possible models of sodium inactivation which were put forward with the results from macroscopic and gating currents. It must be pointed out that all the measurements available have been made in mammalian cells and are not directly comparable to the results in the squid axon discussed above. The results of Aldrich, Corey, and Stevens (1983) show that the mean open time of the sodium channel in neuroblastoma cells is quite short (about 0.5 msec) and independent of voltage. This result along with the fact that they rarely see reopenings (i.e., the inactivation state is absorbing) indicate that the inactivation time constant is essentially voltage independent and much faster than the decay of the current (the 'macroscopic' inactivation). Their results are consistent with the idea that the activation process has a slow component that is responsible for the time course of the average current and as the current is decaying, some channels are still opening for the first time. The results of Patlak and Horn (1982) in N-bromoacetamide (NBA)-treated patches of rat myotubes are consistent with the existence of slow components of activation. NBA-treated patches do not show inactivation, and they show a probability of opening which keeps rising after the peak of the opening probability of untreated patches. This is quite different from the results in pronase-treated squid axons, which show that the activation phase is essentially over by the time of the peak of the sodium current recorded with intact inactivation (Armstrong et al., 1973; Stimers, et al., 1985). Vandenberg and Horn (1984) recorded both total current and single channels from GH_3 cells, a pituitary cell line. They treated the cells with trypsin to remove inactivation and found a similar result as in the squid axon. In contrast to the results of Aldrich et al. (1983), their single-channel recordings show reopenings of the channel during the depolarization, and the mean open time is a function of voltage. The discrepancies between these two groups have not

been explained, but the differences between the results with trypsin and NBA could be the result of a change in the activation kinetics produced by NBA. Neither pronase in squid axon (Armstrong et al., 1973; Stimers et al., 1985) nor trypsin treatment in GH_3 cells (Vandenberg & Horn, 1984) appear to affect activation properties.

A Model for the Sodium Channel

The results of gating charge immobilization have suggested a kinetic model for sodium inactivation consisting of an inactivating particle that could fit in the inside mouth of the channel and block the current when located in position (Armstrong & Bezanilla, 1977). There are several substances that simulate inactivation in axons with normal inactivation removed (*see* Brodwick & Eaton, 1982; Armstrong, 1981; Yeh, 1982), and their mode of action can be explained also as a blocking effect by fitting in the mouth of the channel, lending some support to this view.

A physical model of inactivation can be proposed if one allows interaction between the activating and inactivating particle (Bezanilla et al., 1982*a*). In this model, the inactivating particle is assumed to have two stable positions: in the mouth (blocking) and out of the mouth (unblocking) and the transition between the two positions is separated by a large energy barrier. The interaction with the activating (gating) particle makes the well of energy deeper for both the activating and inactivating particles when they are close together, stabilizing this conformation (open and inactivated). The model will multiply by two the number of states proposed for the activation alone. In the original formulation, only the last transition of the activation sequence was considered, making the treatment of only four states quite possible. However, any realistic model of the sodium channel (Armstrong & Bezanilla, 1977; Armstrong & Gilly, 1979; Greeff, Keynes & van Helden 1982; Keynes, 1983; Horn & Vandenberg, 1984) will have to consider more states to account for all the features of activation (*see* above). This view of the inactivation process multiplies by two the number of states considered for activation alone, and there is no *a priori* reason to discard any of the resultant states. An attempt to use several activating states combined with the interaction of the inactivating particle was made by Bezanilla and Taylor (1982), but the curve fitting becomes unwieldy.

The recent evidence for a gating charge associated with the inactivating step (Vandenberg & Horn, 1984; Stimers et al., 1985) makes it necessary to assign some charge to the inactivating particle, and this charge must move some distance in the membrane field. This charge may be the way the inactivating particle interacts with the charge of the activation gating particle. For example, in the model proposed by Armstrong (1981) when the channel opens the exposed charge on the inside of the membrane produced by the shift of the macromolecular chains might interact electrically with the charge of the inactivating particle, producing a stable open-inactivated state. The sequence of activation steps and the charged inactivated step with interaction was used by Stimers et al. (1985) in the model proposed to explain the results obtained with and without pronase.

The identification of the actual chemical groups responsible for the charge movement recorded as gating currents is not too distant in the future since the primary structure of the macromolecule has recently been described (Noda et al., 1984) and the reconstitution in bilayers has been achieved (Rosenberg, Tomiko & Agnew, 1984). The description of the three-dimensional structure will require extensive modelling and information derived from crystal structure (yet to be produced) and the time and frequency domain recording of the charge movement responsible for the channel gating. Pharmacological modifications and targeted modifications of the aminoacid sequence of the protein will help in the identification of the gating groups.

An example of the type of information provided by pharmacological agents has been provided by the effect of chloroform. The movement of tetraphenylboron and dipicrylamine in a lipid bilayer has usually been considered as a model of gating current (Andersen & Fuchs, 1975). Fernandez, Bezanilla and Taylor (1982) found that the effect of chloroform on the movement of dipicrylamine in the squid axon is to change its rate of translocation. This is in contrast with the effect of chloroform on the gating current of the sodium channel, which is to completely block the fast component without changing the kinetics. This implies that the charges responsible for channel gating do not move in the bilayer the way dipicrylamine does, being buried in the macromolecule and surrounded by a different environment. These charges most likely correspond to several charged residues found in the protein sequence that move a small distance, effectively translocating charge from one side to the other of the membrane by neutralizing charges in one side and exposing it at the opposite side. This exposed gating activating charge may in turn interact with the movable region of the protein that acts as the inactivating particle, which blocks conduction resulting in inactivation.

Activation of the Delayed Rectifier (K Channel)

There are many kinds of potassium channels and different types are found in many preparations (*see*, e.g., Hille, 1984). In this section we will refer only to the voltage-dependent potassium conductance, or delayed rectifier, described by Hodgkin and Huxley (1952*c*).

In the squid axon, potassium currents develop with a pronounced delay, and they do not decay significantly during a depolarization maintained for a few tens of milliseconds. These characteristics should make the study of the conductance simpler, but there are several complicating factors that have made the understanding of the activation more difficult than the sodium channel. The actual time course of the fraction of open channels cannot be derived directly from the time course of the current during a depolarization. This is because the maintained current accumulates potassium in the periaxonal space and the driving force changes as the current develops (Frankenhaeuser & Hodgkin, 1956). It is then necessary to obtain instantaneous *I-V* curves for each depolarization and as a function of time. This experimental procedure is rarely done and instead the available estimates of the fraction of open channel *vs.* time are obtained from the currents corrected for the K accumulation in the periaxonal space. The gating currents of the potassium channel have only been recently recorded; they do not yet have the signal-to-noise ratio of the sodium gating currents and are contaminated by the latter, making the quantitative study less accurate. Single-channel recordings from the K channel in the squid axon have been obtained (Conti & Neher, 1980; Llano & Bezanilla, 1983, 1985), making it now possible to record the three basic electric expressions in the same preparation.

Gating Current of the Potassium Channel

The difficulties in recording the potassium gating current are (i) the charge expected from the voltage dependence and number of channels is small; (ii) the expected kinetics is significantly slower than the sodium gating, making them hard to resolve; (iii) as the sodium gating currents are much larger they will make the separation of both currents difficult; (iv) at present there is no blocking toxin for the potassium channel with mode of action similar to tetrodotoxin in sodium channels, and elimination of potassium for a prolonged period abolishes the potassium conductance irreversibly.

Gilly and Armstrong (1980) described a component of the total gating current in the squid that disappeared after removal of the potassium bathing the membrane. As mentioned above, the removal of potassium in both sides of the membrane eliminates irreversibly the potassium conductance in perfused squid axon (Chandler & Meves, 1970; Almers & Armstrong, 1980); it is therefore attractive to think that the gating current component eliminated might be responsible for the potassium activation. This component, however, is faster than the main component of the ionic current activation, and as it is difficult to study it has only been done at a single potential.

The first two difficulties in recording the K gating current were overcome by increasing the temperature to speed up the kinetics and resolve the charge movement above the background noise (Bezanilla, White & Taylor, 1982*b*). The contamination by sodium gating current was minimized, producing slow inactivation of the sodium channel by holding at −60 mV and blocking part of the sodium gating current with dibucaine (Gilly & Armstrong, 1980). More recently, a further improvement was obtained by replacing chloride by nitrate which partially blocks sodium gating (White & Bezanilla, 1985). Figure 5 shows recordings of gating currents made at high temperature. It is clear that a new slower component of the gating current appears (Fig. 5*a*) and that it becomes more obvious when a large fraction of the sodium gating is blocked by dibucaine (Fig. 5*b*). In Fig. 5*c* a potassium current recorded in the same axon before potassium was removed is shown for comparison. The new slow component of the gating current has the same time course of the potassium current after its initial lag.

Identical Subunits are Inconsistent with Deactivation

The turn-off of the K conductance and gating current have similar time courses (Bezanilla et al., 1982b; White & Bezanilla, 1985). This result rules out models with a number of identical independent activating particles for the same reasons discussed in connection with the deactivation of the Na conductance.

Large Number of Closed States

The results of Cole and Moore (1960), showing an increase in the turn-on delay when the initial potential is made more negative, provided a clear indication that the K channel has a large number of closed states. They proposed that the parameter n of Hodgkin and Huxley (1952*c*) had to be raised to the 25th power to adjust the data properly, but the

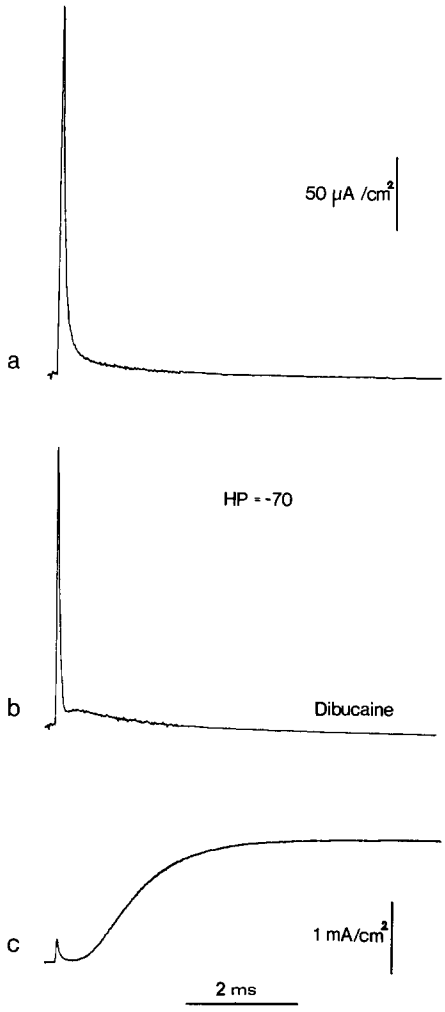

Fig. 5. Gating current of the potassium channel. (*a*) The total gating current recorded at 20°C at a holding potential of −70 mV. Addition of dibucaine (*b*) decreases the first fast component of the current (sodium gating current) and shows with more detail the time course of the slow component of the current (potassium gating current), which can be compared with the time course of the potassium current (*c*) recorded under the same conditions in the same axon before the removal of potassium

results mentioned in the previous paragraph discards this type of model. The gating current results confirm the existence of many closed states. Figure 6 shows the results of an experiment to reproduce the Cole-Moore shift (1960) in the potassium current. In the same axon the potassium gating currents were also recorded under the same pulse protocol and a shift in the gating current is observed. In addition, the charge moved in the presence of the negative prepulse is larger than without a prepulse, indicating that at negative potentials there is charge movement associated with the potassium channel activation. White and Bezanilla (1985) showed a large separation between the *Q-V* and the *f-V* curves: the center points of both distributions were separated by about 40 mV. There are some uncertainties with regard to the actual shape of the *Q-V* curve due to the remaining contamination of the *K* gating currents by Na gating current, but it is clear that the *Q-V* curve is displaced a few tens of mV to the left of the *f-V* curve, showing that a large amount of charge must move before the K channel opens. It is possible to predict, for specific sequential models, the number of states required to produce a given separation between the *Q-V* and *f-V* curves (Bezanilla, 1982), and in this case it gives about 16 states. White and Bezanilla (1985) fitted the conductance and gating currents with a 16-state sequential model that reasonably reproduced the experimental observations. The sharpness in the ionic current rise after the delay was not appropriately reproduced, and this point will be discussed in detail below.

First and Last Transitions

If the test pulse is preceded by a negative potential, the K gating current shows a definitive rising phase (White & Bezanilla, 1985). This result demonstrates that the first step of the sequential activation of the K channel is slower and/or less voltage dependent than the subsequent ones.

The result of the experiment with two depolarizing pulses separated by a variable interval reveals, as it is in the case of the sodium current, that the last step is not rate limiting in the activation sequence (White & Bezanilla, 1985). The results obtained with single-channel recording (Conti & Neher, 1980) show significant flicker during the open period of the K channel and this result is also consistent with a fast last step.

Modelling of the Potassium Channel

The present data available from macroscopic currents, gating currents, and single-channel recordings give us no information about the steps in the middle of the sequence, and any modelling can be only tentative because there are too many possibilities for the intermediate steps. The simple assumption that all these unknown transitions are equal was used by White and Bezanilla (1985) to model the results of ionic and gating currents and single-channel recordings. The agreement is good except for the detailed time course of the K current, which rises sharper in the experimental recording

than in the predicted current. It is clear that a perfect agreement may not be expected if one considers that all the intermediate transitions were assumed the same instead of being fitted to the experimental curves, but this disagreement may be very useful in the search for the details of K channel activation. One possible explanation could be the accumulation of K in the periaxonal space, which distorts the time course of the current and prevents observation of the time course of the fraction of open channels. This hypothesis was explored by White and Bezanilla (1985) and found not to account for the differences. Another possibility, not explored in detail, is an influence of the speeding up of the kinetics when the external potassium concentration is increased (Stuhmer, 1980). As a consequence of potassium accumulation during a depolarizing pulse, changes in the opening rate constants may affect the time course of the K current. In fact, Llano and Bezanilla (1985) found that the time course of the average current recorded with only eight channels in the patch rises more slowly and less abruptly; under the conditions used there was no K accumulation, and presumably the time course of the average current reveals the actual time course of the fraction of open channels. Still another possibility is that the macroscopic current is produced by the contribution of more than one type of channel, as will be discussed below.

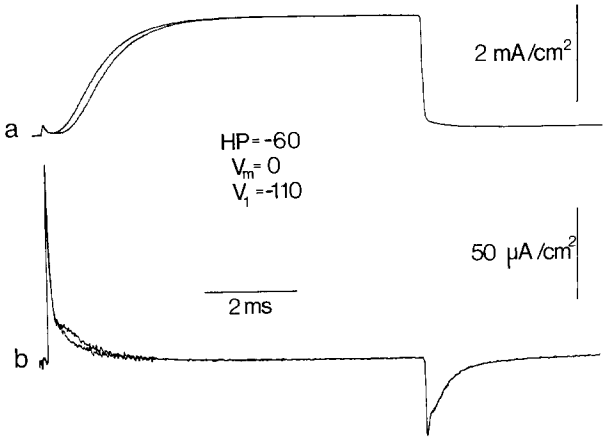

Fig. 6. Cole-Moore shift of ionic and gating currents. (*a*) Traces show superimposed the currents obtained without (leftmost trace) and with a negative prepulse (rightmost trace). V_1 is the value of the prepulse. (*b*) Traces are gating currents recorded with the same pulse protocol after potassium removal. Notice that the prepulse produced a time shift and at the same time an increase in the total charge (area under the gating current) transported

More than One Type of Delayed Rectifier Channel

Using the cut-open axon technique, Llano and Bezanilla (1985) have found at least two types of potassium channels, in the squid axon. One of the channels shows the properties of the channel described by Conti and Neher (1980) and has open times expected from the main features of the macroscopic currents. The second type of channel shows longer open times, and it does not open for every depolarizing pulse. Furthermore, this second type of channel has about twice the single-channel conductance as the other channel. There is not enough data to decide the relative proportion of the two types of channel, but presumably the slow channel will have a smaller contribution than the fast channel in the total current because it opens infrequently. The existence of two kinds of K channels may explain some of the difficulties in the model prediction as explained in the previous paragraph because that model considered an homogeneous population of channels (White & Bezanilla, 1985).

ATP Modulates the K Conductance in Squid

Recently it has been reported that ATP changes the K conductance in the squid axon. The effect of ATP seems to be in both the resting K conductance (Gadsby, DeWeer & Rakowski, 1985) and the delayed rectifier (Bezanilla et al., 1985).

If a squid axon is dialyzed with a solution free of ATP, the potassium currents decline as the ATP is washed out from the inside of the axon. The currents can be recovered by dialyzing with ATP and Mg^{++}; both are required. Non hydrolyzable ATP analogs do not recover the current (Bezanilla et al., 1985). These results suggest that the effect of ATP is mediated through a phosphorylating step. Several other channels have been found to be phosphorylated (*see* Levitan, Lemos & Novak-Hofer, 1983), and it is not surprising that this effect has not been found in squid before since most of the experiments designed to study K conductance are normally done in absence of ATP.

The effect of ATP is to increase the total conductance for depolarizations above −35 mV and decrease it for depolarizations smaller than −35 mV. Furthermore, the ON kinetics is slowed down by ATP while the OFF kinetics is speeded up (Bezanilla et al., 1985). These results are not consistent

with an increase in the K channel population or an increase in the single-channel conductance. Recent experiments (F. Bezanilla, C. Caputo and R. DiPolo, *unpublished*) have revealed that the effect of ATP is to shift the voltage dependence of both the activation and the slow inactivation of the potassium conductance to more positive potentials. The shift of the inactivation explains the apparent increase of the maximum conductance when the holding potential is −60 mV because at this potential there is slow inactivation in absence of ATP, and when the phosphorylation removes it the maximum conductance increases. The decrease of the conductance at potentials more negative than −35 mV is explained by the shift of the activation process bt ATP. The shifts are specific to the potassium channel because no effect was detected on the sodium conductance. The effects of ATP on the potassium channel may be part of a regulatory mechanism for the K conductance in the axon and it may provide an opportunity for the isolation and purification of the delayed rectifier channel.

Concluding Remarks

The activation of voltage-dependent channels appears to be the result of conformational changes of the channels modulated by the membrane potential controlling the position of charged groups in the macromolecule. As a result of a membrane potential change, the rearrangement of charged groups presumably changes the physical state of the channel, which transitions from a closed to an open state. During this rearrangement the movement of the charged groups will produce a current detected as gating current, and the channel will evolve through several closed states before it opens. When it opens, several million ions will pass per second and a small current of a few picoamperes will be detected as a single channel current. The contribution of several thousands or millions of these channels will be recorded as a macroscopic current. We have reviewed briefly the properties of these three type of currents for the sodium and potassium channels. The general picture that emerges is a large number of physical states, most of them in closed conformation, with transitions between states modelled as first order using energy profiles modulated by the membrane field. The number of physical states that are required to account for the currents may seem overwhelming, but it is not surprising from a proteic structure with molecular weight of more than 200,000 such as the sodium channel. The new techniques of molecular biology, high resolution microscopy, and biochemistry are providing more details of the structure of the channel. These results, combined with the electrical measurements of channels *in vivo* and reconstituted in bilayers and aided by molecular dynamics simulations will make possible the correlation of the movement of the charge, as detected by the gating current, with the actual molecular groups and the subsequent molecular changes that lead to channel opening. Only then will we have a better understanding of voltage gating.

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