Voltage-dependent gating of sodium channels: towards an integrated approach

Richard W. Aldrich

Many recent developments in electrophysiological and molecular techniques have pointed the way for an interdisciplinary approach to understanding the molecular mechanisms of voltage-dependent sodium channel gating. Gating current measurement and single channel recording are powerful tools for elucidating the differences in charge distribution of the channel protein in various conformational states. The successful reconstitution of purified channel proteins and the elucidation of the primary structure of the channel will now allow these powerful techniques to be used on defined molecular systems, leading to a greater understanding of the voltage-driven conformational changes that underly the nerve impulse.

The molecular machinery for the generation and propagation of nerve impulses is the voltage-dependent sodium channel. This integral membrane protein changes conformation in response to changes in membrane potential and thereby controls the flux of sodium ions down their electrochemical gradient into the cell. The resultant depolarization completes the positive feedback by opening more channels. This basic mechanism of impulse generation was discovered by Hodgkin and Huxley over 30 years ago. Since then a major goal of membrane physiology has been the elucidation of the molecular mechanism of voltage-gated conformational changes in this protein.

Study of sodium channel gating has progressed rapidly in the last few years, due to the application of new electrophysiological and biochemical techniques. The kinetic behavior of single channel molecules has been studied with patch clamp methods and planar bilayer methods. The small currents that flow as a result of the conformational changes themselves (gating currents) have been recorded, and have given information about the voltage dependence of transitions between closed states of the channel.

A number of natural high-affinity toxins exist that bind to the channel, either blocking it or altering its gating mechanism. These agents have allowed the biochemical isolation and molecular cloning of the sodium channel molecule and the determination of its primary structure. The combination of these biochemical and electrophysiological techniques now allows us to observe, at fast time resolution, the behavior of single molecules under conditions in which the primary structure can be manipulated and the properties of ion channels by measuring the currents that flow through them under voltage clamp conditions. Voltage dependence of transition rates measured from voltage clamp experiments can be used to infer charge distribution changes in the channel protein as it moves between various conformational states. The goal of electrophysiological studies of channel gating is to find the number of kinetic states of the channel and the voltage dependence of the transition rates between these states. This functional approach to channel gating mechanisms is necessary to test models based on structural information and is the important assay system for functional alterations of the channel by protein modification or site directed mutagenesis.

Fig. 1 shows typical sodium channel currents obtained during a voltage clamp experiment on bovine chromaffin cells. After a voltage step the sodium current turns on sigmoidally, rises to a peak and then decays exponentially. As the voltage is increased the kinetics of the current become faster. The kinetics can be described by the equation:

\[ I(t,v) = N \times p_{o}(t,v), \]

where \( I(t,v) \) is the membrane current expressed as a function of voltage and time, \( N \) is the number of channels in the membrane, \( i \) is the current flowing
through a single channel and \( P_{c}(t, v) \) is the probability of a channel being open. The time course of the current at these various voltages gives a record of the timecourse of \( P_{c} \). This gives the probability (as a function of time) that the channel is in an open state, allowing passage of ions across the membrane. The probability is time-dependent because of the time required for the channel to pass between various conformational states. The kinetics of the probability transient can be used to construct a kinetic state diagram of the channel. From this diagram and the kinetics of the current at various membrane voltages, the voltage dependence of the rates of transition between the various states can be inferred.

The transient behavior of the sodium current requires three types of kinetic states\(^1\): (1) Resting states (R), which are populated at hyperpolarized voltages and do not allow ions to pass; (2) Open states (O), which allow sodium ions to pass into the cell; and (3) Inactivated states (I), which are closed to ion flow and cannot be opened by further depolarization. The sigmoid turn-on kinetics further indicate that there are a number of resting states. Hodgkin and Huxley developed a kinetic model for channel gating that includes explicit voltage dependences for each of these transition rate constants between these types of states. Their model can be formulated as a diagram of the possible transitions between the channel's different conformational states:

\[
R_2 \rightarrow R_1 \rightarrow R_0 \rightarrow O \\
I_2 \rightarrow I_1 \rightarrow I_0 \rightarrow I_1
\]

with each of the transition rates explicitly depending on the membrane voltage. The kinetics of the macroscopic currents, as in Fig. 1, reflect all of the rate constants in the diagram. Upon a step voltage change the channels move from resting states to the open state and then to the inactivated states as the current declines. The Hodgkin–Huxley model is a milestone in membrane biophysics. It accurately predicts the kinetics of sodium currents under voltage clamp and the shape and conduction velocity of the nerve impulse. In addition it has provided a conceptual framework for

work concerned with the mechanism of voltage-dependent channel gating.

The molecular basis of voltage-dependent gating

Membrane potential influences channel opening by producing rearrangements in the distribution of charges on the protein molecule, i.e., changes in conformation. Each distinct conformation (or arrangement of atoms) of a protein has a particular energy associated with it. The energy of a channel depends upon the ionic interactions between charged residues and surrounding molecules, van der Waals forces, hydrophobic interactions, etc. The conformations that minimize the sum of energy from these various factors are most favored, and transitions between states with small energy differences occur most rapidly. The probability \( P_i \) of the molecule being in a conformation state \( i \) depends exponentially on the energy of that state \( E_i \), according to the Boltzmann distribution:

\[
P_i = \frac{e^{-E_i/kT}}{\sum_j e^{-E_j/kT}}
\]

Thus lower energy states are most often occupied whereas higher energy states occur more rarely. A channel with two equal energy states will have equal probability of being in one or the other. The energetics of transitions between states are often summarized by an energy diagram, as shown in Fig. 2.

The energy minima, or wells, correspond to defined states of the molecule and the maximum, or barrier, represents the energy of the transition state between the two states. The rates of transition between the states depend upon the difference in energy between the occupied state and the transition state (the barrier between the two states). The energy of a protein molecule fluctuates due to thermal bombardment by its surroundings. When the channel molecule acquires enough thermal energy to equal the barrier height a transition can occur. A high energy difference leads to a slow transition rate. \( R_{th} \), which depends on the height of the energy barrier in the following way:

\[
R_{th} = A e^{-U_{th}/kT}
\]

where \( U_{th} \) is the height of the barrier between state 0 and state 1, and A is the effective vibration frequency, with units of time\(^{-1}\). This rate theory equation relates the kinetics of transitions between states to energy differences. The diagram shows only two states of the molecule, whereas a real channel would have many more states. Transitions between each of these states can be treated in the same way.

Transition rates are voltage dependent because of the voltage-dependent component of the energy difference. Each channel conformation has a given distribution of charged particles. Because these charges are arranged in the electric field across the membrane, their positions in the field contribute to the energy of the molecule. As the membrane potential changes, the charges redistribute to new conformations, with strong fields favoring more polarized conformations. This redistribution can occur by a number of different atomic movements including rotation of fixed dipoles within the protein, movement of charged particles through the membrane, or conformation-dependent changes in dissociation constants of charged groups.

Transitions between states that have a larger redistribution of charge in the direction of the field will be more voltage dependent.

The voltage-dependent component of the barrier height can be incorporated into the rate equation:

\[
R = A e^{-B - \Delta \mu M/VkT}
\]

where B is the barrier height at zero membrane voltage. V is the voltage difference across the membrane \( M \) is the membrane thickness. and \( \Delta \mu \) is the difference in the effective dipole moment between the occupied state and the transition state. Because of these theoretical considerations, voltage dependence of transition rates measured from voltage clamp experiments can be used to infer charge distribution changes or effective dipole moment changes of the channel protein. This information allows us to make predictions about the
through a single channel and $P_n(t, v)$ is the probability of a channel being open. The time course of the current at these various voltages gives a record of the timecourse of $P_n$. This gives the probability (as a function of time) that the channel is in an open state, allowing passage of ions across the membrane. The probability is time-dependent because of the time required for the channel to pass between various conformational states. The kinetics of the probability transient can be used to construct a kinetic state diagram of the channel. From this diagram and the kinetics of the current at various membrane voltages, the voltage dependence of the rates of transition between the various states can be inferred.

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$$R_{II} = A e^{-E_{II}/kT}$$

where $E_{II}$ is the height of the barrier between state $O$ and state $I$, and $A$ is the effective vibration frequency, with units of time. This rate theory equation relates the kinetics of transitions between states to energy differences. The diagram shows only two states of the molecule, whereas a real channel would have many more states. Transitions between each of these states can be treated in the same way.

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Because of these theoretical considerations, voltage dependence of transition rates measured from voltage clamp experiments can be used to infer charge distribution changes or effective dipole moment changes of the channel protein. This information allows us to make predictions about the
structure of the channel protein in its various conformational states and to understand the physical basis of channel gating.

By examining the voltage dependence of the peak current, Hodgkin and Huxley calculated that the probability that a channel would open changed e-fold in 4 mV. This corresponds to a redistribution of charge equivalent to a net movement of six charges across the membrane in order for a single channel to open. This amount of charge moving across the membrane would cause a small but appreciable electric current to flow in the outward direction as channels move from resting to open states. These tiny currents, called gating currents, were first reported by Armstrong and Benzanilla. They are remarkable in that they allow direct measurement of the charge that moves across the cell membrane as the channel proteins undergo conformational changes.

Since their discovery, gating currents have been intensively studied by a number of laboratories. Because they are very small compared to the current carried by sodium ions through open channels, great care must be taken to isolate them from the ionic currents flowing through open channels and from the current flowing through the membrane capacitance during step changes in voltage. In general, ionic currents can be limited by ion substitution and/or pharmacological block of the channels, and linear capacitative currents can be eliminated by a subtraction procedure. When this separation is achieved, the kinetics of the gating currents can be studied and compared to those of ionic currents in order to further refine kinetic models. Like macroscopic current kinetics, the kinetics of gating currents are governed by the entire set of transition rates for the channel. However, they emphasize transitions that are the fastest and most voltage dependent. Consequently their kinetics can be examined to differentiate among various models that predict identical ionic current kinetics.

The voltage dependence of transitions between closed states can be studied by measuring the charge (integral of current) that moves in voltage regions where the channels do not open. An important finding of gating current experiments is that there is no discernible gating current component that corresponds to inactivation. Instead, the charge that moves during opening (or activation) transitions is immobilized, with a time course similar to inactivation of ionic currents. This finding indicates that there is no charge redistribution in the direction of the field corresponding to transitions to inactivated states, and that the voltage dependence of the time course of current decline (see Fig. 2) is a result of the voltage dependence of the opening transitions (R→O). In terms of the state diagram shown above, this means that the vertical transitions are not voltage dependent and that their rates are not equal.

Single channel recording

Single channel recording techniques offer a different view of gating mechanisms in that they allow various subsets of the transition rates to be isolated, whereas macroscopic and gating current measurements reflect all of the possible transitions. For example, the mean duration of a channel opening depends only on the rate constants of transitions from the open state. The small currents flowing through single open sodium channels were first described by Sigworth and Neher. They are on the order of 1 pA in magnitude. This corresponds to a net flux of 6000

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Fig. 3 Three possibilities for sodium channel gating that predict identical macroscopic sodium currents but different single channel behaviour.
ions ms⁻¹ that the channel remains open. The ability to measure the individual openings and closing of the channels has increased our power to measure rates of transitions into and out of the open state. The times of opening and closing transitions can be measured directly. Distributions of the time spent in the open state, the elapsed time from the voltage step to the channel opening (first latency) and the probability that a channel will reopen after it has closed, can be compared with distributions that are predicted from various kinetic models. Fig. 3 shows how three different kinetic schemes that predict identical macroscopic currents can be differentiated with the use of single channel recording.

The left column of the figure shows a macroscopic sodium current and examples of three types of underlying single channel kinetics that could lead to the overall timecourse of the current. On the right are partial state diagrams showing the relative rates of transition into and out of the open state. Fig. 3(A) shows that the rising phase of the current reflects rapid opening of channels upon a voltage step and the subsequent slow decline reflects the slow closing of these channels. This scheme predicts that the first-latency distribution will consist primarily of openings that occur before the time of peak current and that the open duration distribution will have a mean comparable to the time constant of the decline in current. According to this scheme, a given channel will open only once (on the average) during a depolarizing step.

In Fig. 3(B) the channels open quickly but close preferentially to resting states, from which they can reopen a number of times before they inactivate. This scheme predicts that the first latencies will be short, but that the open durations will be longer than the overall decay in current. It also predicts that a given channel will open a number of times per voltage clamp step.

The scheme in Fig. 3(C) incorporates slow activation and fast inactivation. In this case channels can open for the first time considerably after the time of peak current, the open durations are short, and the channels open an average of one time during a depolarization.

This approach has been used to differentiate between the different schemes for sodium channel gating in cultured mammalian excitable cells. Distributions of first latency, open duration, and number of openings per depolarization were constructed and compared with the time course of the average current from many traces (which is similar to the macroscopic current). It was found that the lower scheme (Fig. 3E) is correct for these channels over a wide voltage range: channel opening is highly voltage dependent and can be rate limiting. Once a channel opens it quickly closes to an inactivated state and does not reopen. Furthermore, experiments performed at different voltages show that the open channel inactivation rate has very little voltage dependence. This is consistent with the gating current results from squid axons and indicates that there is not a significant net rearrangement of charge on the channel protein as this transition occurs.

Somewhat different results are obtained when patch clamp experiments are performed on membrane patches that have been removed from the cell. Under these conditions channels often have longer open times and a greater probability of reopening. This is consistent with a slowing and possibly a change in the voltage dependence of open channel inactivation during patch isolation. The mechanism for this change is not clear.

This view of gating is different to that expected from the Hodgkin-Huxley model in that the slower and more voltage-dependent processes are the opening rather than the inactivating transitions. The assumption that the decay of current reflects the inactivation process would erroneously lead to voltage dependence. The inactivation process being considered and would therefore lead to incorrect predictions of molecular rearrangements during inactivation.

An emerging view of sodium channel gating

The ability to record macroscopic currents, gating currents and single channel activity has contributed enormously to our understanding of the voltage-dependent gating mechanism of sodium channels. We are making progress towards a complete kinetic description of gating. From this and the voltage dependence of all the transition rates, we can determine the difference in charge distribution between the various conformational states of the channel. The next step is to combine this description with information that has been obtained from chemical and physical approaches to the channel structure and to eventually correlate the kinetically defined states of the channel with three-dimensional conformations of the channel protein.

At this time, however, single-channel and gating currents have not been obtained from the same preparation. In addition, the primary sequence of the channel has been determined from eel electroplax, a tissue that has not been well studied electrophysiologically, although recent patch clamp studies of pure electroplax channels reconstituted into lipid bilayers suggest that they behave in a similar way. It is possible that species differences make it misleading to combine results obtained from different preparations. There seem to be differences in subunit composition of sodium channels obtained from eel electroplax, mammalian muscle, and mammalian brain. Furthermore, a number of different types of sodium channels have been described in voltage clamp studies. An additional complication is that the gating behavior of the channels seems to change when a membrane patch is isolated from a cell, making it difficult to predict the behavior of reconstituted proteins from cell-attached patch data. These problems must be worked out. It is clear nonetheless, that the tools are at hand for determining the molecular basis of voltage-dependent gating of sodium channels.

Selected references
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Richard W. Aldrich is at the Department of Neurobiology, Stanford University Medical School, Stanford, CA 94305, USA.