

Commentary

Charge Movement in the Sodium Channel

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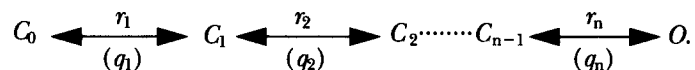
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In this issue of *The Journal of General Physiology*, Hirschberg, Rovner, Lieberman, and Patlak (1995) present a new estimate of the charge displacement that occurs in the activation of voltage-gated sodium channels. This kind of estimate is not new—Hodgkin and Huxley (1952) pointed out that the movement of at least six elementary charges (e_0) must somehow accompany the activation of the sodium conductance. The surprise from the new result of Hirschberg et al. (1995) is that the estimated charge movement, $\sim 12 e_0$, is very much larger than previously thought, presenting some puzzles about the identity of the mobile charges.

Recent studies have presented two kinds of evidence for charge movements of $\sim 12 e_0$, in voltage-gated *Shaker* potassium channels. One set of evidence comes from quantitative measurements of gating currents. In these experiments the charge movement is measured as a displacement current, induced by membrane potential changes, that flows even when the ionic current through the channels is blocked by toxins or by the elimination of permeant ions. To normalize the measurement to charge movement in a single channel, the number of channels is estimated either through analysis of the ionic currents (e.g., before blocking the channels; Schoppa et al., 1992) or through quantitative toxin binding (Aggarwal and MacKinnon, 1994). From these measurements the total charge movement per channel is estimated to be 12 to 14 elementary charges.

Voltage-sensitivity Measurements

The other line of evidence involves the measurement of the voltage sensitivity of channel activation, as Hodgkin and Huxley (1952) did. The merits and limitations of this approach have been known for some time (Almers, 1978), but are summarized briefly here. Consider a channel which exists in a particular closed state C_0 at the most negative membrane potentials, is driven into the (single) open state O at the the most positive potentials, and can exist in a number of intermediate states along the way:



S C H E M E I

In this scheme we assume that each transition involves some charge rearrangement in the channel protein, such that an external voltage-clamp system (e.g., a patch-clamp apparatus) would detect a charge displacement whenever a transition occurs. Thus, the transition $C_0 \leftrightarrow C_1$ is accompanied by a charge movement of magnitude q_1 and has an equilibrium constant (at zero membrane potential) of r_1 . From the definition of electrostatic energy and from the Boltzmann distribution we know that, at equilibrium, the relative probability of a channel being in C_1 rather than in C_0 will depend on the membrane potential E according to

$$\frac{\text{Prob}\{C_1\}}{\text{Prob}\{C_0\}} = r_1 e^{q_1 E/kT}. \quad (1)$$

The ratio of probabilities of occupancy in each pair of states will satisfy a similar equation. With the further consideration that the sum of all the occupancy probabilities must sum to unity, one can obtain the probability p_o of being in the open state as

$$p_o \equiv \text{Prob}\{O\} = \frac{r_1 r_2 \dots r_n e^{(q_1 + q_2 + \dots + q_n)E/kT}}{1 + r_1 e^{q_1 E/kT} + r_1 r_2 e^{(q_1 + q_2)E/kT} + \dots + r_1 r_2 \dots r_n e^{(q_1 + q_2 + \dots + q_n)E/kT}} \quad (2)$$

This complicated expression becomes simple when E takes a large negative value. In that case all the terms in the denominator vanish except the 1; this corresponds to the situation in which the occupancy of all states except C_0 is negligible. Then the open probability approaches

$$p_o \approx R e^{-QE/kT}, \quad (3)$$

an exponential function of E whose steepness depends on Q , the sum of all the charge movements. Here R is the product of all the equilibrium constants at zero membrane potential.

This behavior suggests a way in which the total charge movement in a channel can be estimated: measure the open probability as a function of E , at sufficiently negative values of E . How far negative is enough? This question can unfortunately only be answered a posteriori, when the gating mechanism is well enough characterized to allow the evaluation of Eq. 2. In practice, the best one can do is to measure the open probability down to E values as negative as possible, and obtain an estimate of the apparent total charge by inverting Eq. 3,

$$Q_{\text{app}} = kT \frac{d \ln p_o}{dE}. \quad (4)$$

Q_{app} is always a lower bound for the true total charge Q , approaching it in the limit of very negative E .

The practical problem is, how to measure p_o at very negative voltages where p_o becomes vanishingly small. In a recent study, Zagotta, Hoshi, Dittman, and Aldrich (1994) used macroscopic *Shaker* currents, measured in multichannel patches, to es-

timate p_o down to voltages where its value was $\sim 10^{-3}$. The Q_{app} value was $\sim 10 e_0$, but extrapolations of the Q_{app} vs p_o relationship yielded estimates of Q in the range of 12 to 16 e_0 for that channel, consistent with the charge movement measurements mentioned above.

A New Measurement in Sodium Channels

Hirschberg et al. (1995) now report their elegant experiments on sodium channels which extend measurements of p_o to the remarkably low value of 10^{-7} . Instead of using macroscopic current recordings, these workers employed single-channel recordings, making use of the well-known fact that the ratio of channel open time to total observation time yields an estimate of p_o . Being able to measure p_o to much lower values makes it more likely that an estimate of Q_{app} will approach the true value of Q .

The experimental system used by Hirschberg et al. (1995) was optimized in several ways for the detection of rare channel-opening events. First, the recordings were made from patches containing many sodium channels, increasing the frequency of observation of the rare events. Second, the channels that were expressed (μ I rat skeletal muscle sodium channels) were mutated to remove the fast-inactivation process, so that channel events could be observed during chronic depolarizations lasting several minutes. Third, an automated detection scheme was used to pick the "needles" ($\sim 100 \mu$ s channel openings) out of the many megabytes of digitized recordings. As long as they can be detected, brief open times are advantageous, since for a given p_o value, short open times imply a relatively high event rate, and therefore good counting statistics.

Too Much Gating Charge?

The value of Q_{app} obtained for these channels is $\sim 12 e_0$. At first sight this result is pleasing, because it matches well the estimates of Q that have been obtained in potassium channels, which are thought to be structurally similar. There are however two reasons that have led people to expect that in sodium channels the total charge movement associated with channel activation should be somewhat smaller.

The first reason comes from a comparison of the S4 sequences. The S4 region is the favorite candidate for the "voltage sensor," having a number of basic residues in what appears to be a membrane-spanning segment. Assuming these residues are charged, they could be the charges which move in the membrane field. Evidence that they may in fact form the mobile gating charge comes from charge-change experiments, starting with the pioneering studies of Stühmer et al. (1989) and Papazian et al. (1991); see Sigworth (1994) for a review. Further evidence comes from a recent study demonstrating voltage-dependent accessibility of an S4 residue to a membrane-impermeant sulfhydryl reagent (Yang and Horn, 1995). In *Shaker* channels, there are seven basic residues in the S4 of each subunit of the tetrameric channel; in most mammalian sodium channels however there are four, five, six, and eight basic residues, respectively, in the four domains of the α subunit (Goldin, 1995). With fewer basic residues overall, one expects that sodium channels might have less charge movement.

The second reason to expect less activation charge movement in sodium channels comes from the suspicion that one of the S4 regions—the one in domain IV—might have little to do with activation at all, but instead control a voltage-dependent step in inactivation. Support for this view comes from a study of mutations in the domain IV S4 region which affect the voltage dependence of inactivation but not the activation process (Chahine et al., 1994). Thus, if only three of the four S4 regions contribute to the activation gating charge movement, these being the ones with relatively few basic residues, then how can the sodium channel have a charge movement as large as that of the tetrameric *Shaker* channel?

Finally, it should be kept in mind that all measurements of gating charge are approximate and rely on various assumptions. The “limiting slope” measurement of Q_{app} by Hirschberg et al. (1995) places only a lower bound on the true value. Although it is tempting to think that in these novel experiments the asymptotic value is finally being reached, we cannot know this for sure. The alternative measurements of charge, using gating currents, could also turn out to underestimate the total charge. Although they can cover a wider range of potentials than the limiting-slope measurements, they can miss charge movements occurring at very negative potentials.

Experiments are nevertheless underway in several laboratories to measure gating charge movements in channels in which S4 residues have been mutated. Such measurements may put the S4 hypothesis on firmer footing, and perhaps show whether only a few of the basic residues are actually the mobile ones. Regardless of the results of these studies, however, the charge movement of at least $3 e_0$ per *Shaker* subunit or sodium channel domain remains a remarkably large quantity. Its existence requires conformational changes such that three or more charges are transported from a place where they experience the intracellular potential to another place where they experience the extracellular potential; alternatively, a larger number of charges could be transported a smaller distance. It will be very interesting to see what sort of conformational changes these are.

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