

Commentary

Counting Charges

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Voltage-gated ion channels of excitable tissue have evolved an exquisite sensitivity to membrane potential. The open probability of a typical potassium or sodium channel, for example, may be increased by two orders of magnitude in response to a depolarization of less than 10 mV (Zagotta et al., 1994; Hirschberg et al., 1995). As recognized by Hodgkin and Huxley (1952), this responsiveness to changes of membrane potential is a consequence of the movement of charges during the conformational changes known as gating (see arguments in Sigworth, 1994). Charge transfer must occur across the membrane electric field during gating, and, in principal, it is possible to estimate the total number of elementary electronic charges (e_0) completely transferred across the electric field to account for the voltage dependence of gating observed experimentally.

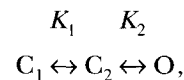
We now know that ion channels are transmembrane proteins and that the charges which move during gating are likely to be comprised mainly of the amino acids of the so-called α subunits of sodium, potassium, and calcium channels. The usual assumption is that charge movement during gating is due to a rearrangement of the sidechains of highly charged amino acids, such as arginine, lysine, aspartate, or glutamate. However, it is worth considering that significant charge also resides in the atoms of neutral amino acids (Creighton, 1993).

As a first step in understanding the molecular basis of voltage dependence and at identifying the important players in voltage sensitivity, it is necessary to know just how many charges move through the electric field as a channel undergoes a transformation from a resting closed state to an open configuration. This turns out to be a relatively trivial task for a channel with exactly two gating states: closed and open. For this two-state channel gating is determined by two voltage-dependent rate constants: one for opening and the other for closing the channel. If these rate constants each depend exponentially on membrane potential, the relationship between steady-state open probability (P_o) and voltage (V) will have the form of a Boltzmann function with a midpoint on the voltage axis of V_{mid} and a voltage-dependent equilibrium constant $K(V)$,

$$P_o(V) = 1/[1 + K(V)],$$

where $K(V) = \exp[Q(V - V_{mid})/kT]$, Q is the number of charges [units: coulombs], and $kT = 2.44$ kJ/mol. In this formalism Q is the weighted sum of all the charges that move during the gating transition in a single channel. The weighting factor is the effective fraction of the electric field through which each charge moves. At a specified temperature the steepness of the curve generated by a Boltzmann function is solely a function of Q . Therefore a fit of the experimental P_o - V relationship (or the macroscopic conductance-voltage relationship) provides a direct estimate of Q , the number of charges per channel.

The rub is that the real channels we all study always have more than two gating states. There are typically more than two closed states and sometimes more than one open state as well. The consequence of this complication can be seen by examining the simplest multi-state model,



where each of the gating transitions is represented by a separate voltage-dependent equilibrium constant, $K_1(V)$ for the closed-closed transition, and $K_2(V)$ for the closed-open transition, each defined as above. The steady-state properties of this gating scheme are determined by the midpoints of the two transitions, V_{mid1} and V_{mid2} , and the charge moved in each step, according to:

$$P_o(V) = 1/[1 + K_2 \cdot (K_1 + 1)].$$

For illustration assume that a total of $Q = 6 e_0$ moves for the conformational transitions between C_1 and O , $3 e_0$ in each step. Fig. 1 *A* shows the P_o - V relationship (*solid line*) for such a channel in which $V_{mid1} = -85$ mV and $V_{mid2} = -50$ mV. This relationship is approximately Boltzmann, but the best-fit estimate for Q is $3.1 e_0$, considerably less than the total charge moved, $6 e_0$.

Because each transition in the above three-state scheme is voltage dependent, we might naturally consider measuring the gating current underlying these two transitions in our quest for the elusive value of Q . Gating current is a consequence of the movement of charges through the membrane electric field during a gating conformational change (Armstrong, 1981; Bezanilla and Stefani, 1994; Sigworth, 1994). The integral of

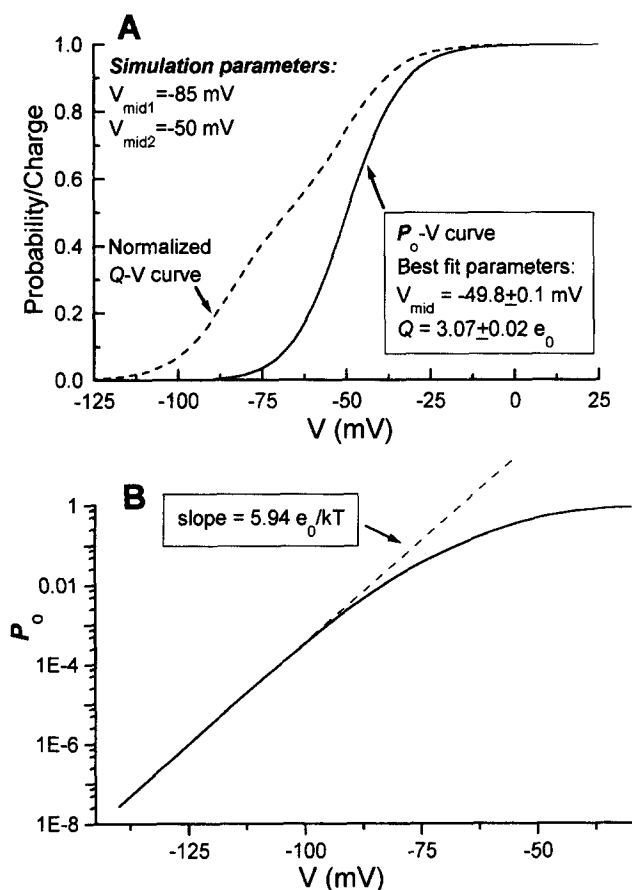


FIGURE 1. (A) Integrated gating current (*dashed line*) and steady state P_o (*solid line*) expected for a linear three-state gating scheme ($C_1 \leftrightarrow C_2 \leftrightarrow O$) in which each transition carries a charge of $3 e_0$; $V_{mid1} = -85$ mV and $V_{mid2} = -50$ mV. The P_o - V curve is fit by a Boltzmann function with the indicated parameters. Note that the estimate of Q is less than the total charge movement of $6 e_0$. (B) Semi-log plot of P_o - V showing the limiting slope (*dashed line*) only reached for voltages more negative than -90 , i.e., where $P_o < 10^{-3}$. The regression line has a slope of Q/kT with $Q = 5.94 \pm 0.01 e_0$.

gating current is a direct measurement of the total amount of charge that moves, for example, in a population of N channels. The scaled integrated gating current for our three-state channel is plotted in Fig. 1 A as a dashed line. Although it provides some information about the voltage dependence of individual gating transitions, this integrated charge cannot by itself provide an estimate of Q , because N cannot be determined from a gating current measurement.

So how can Q be determined? Noceti et al. (1996) in this issue of *The Journal of General Physiology* use two different methods on potassium and calcium channels expressed heterologously in oocytes. The first method makes use of a remarkable simplicity in the P_o - V relationship first realized by Almers (1978). If the gating scheme is a linear sequence of closed states leading to a single open state, and P_o is sufficiently small, the slope

of $\ln(P_o)$ versus V reaches a limiting value that is proportional to Q (Fig. 1 B). The second method, which Noceti and colleagues call variance analysis, involves an estimate of the number of channels, N , in a patch, using nonstationary fluctuation analysis of ionic current (Sigworth, 1980). The total gating charge in the same patch is then measured in the absence of ionic current to obtain the number of charges per channel, Q (Schoppa et al., 1992). Noceti et al. (1996) improve on both of these techniques. For limiting slope they use a slow ramp command voltage to avoid the problems of extracting a small signal from the responses to a series of voltage steps, the typical method for activating voltage-gated channels. This allows them to measure P_o (actually a scaled P_o from total conductance of the membrane) at very low values. For the variance analysis method they measure ionic current and gating current using identical solutions, thus obviating any ambiguity about the effects of the solutions on either type of current. Instead of removing permeant ions or using pore blockers to eliminate the ionic current, Noceti et al. (1996) obtain their gating currents at the reversal potential of the ionic current.

In this study Q is estimated for the same isoforms of voltage-dependent channels by each of these methods, and it is indeed gratifying that the estimates of Q for *Shaker* potassium channels agree ($Q \sim 13 e_0$). The importance of this result is that although each method for estimating Q is imperfect, the imperfections are fundamentally different. For example, the limiting slope analysis is an asymptotic procedure, and there is no guarantee that V is sufficiently negative for any experiment that the limit is achieved. If P_o is not low enough, Q tends to be underestimated. Note, for example, that for the three-state model we have examined the limiting slope is not reached until the membrane potential is more negative than -90 mV, where $P_o < 0.001$ (Fig. 1B). Other ways of underestimating Q from the limiting slope are (a) if the gating scheme is branched, rather than linear, as Noceti et al. (1996) show in an *Appendix*, or (b) if charge moves during transitions between multiple open states. By contrast, the variance analysis method for estimating Q depends critically on an equivalence between the number of channels that open in response to depolarization and the number of channels which generate gating current. If a channel produces gating current, but not ionic current, Q may be overestimated by variance analysis, because N will be underestimated. Similarly Q will tend to be overestimated in a branched kinetic scheme if, for example, charge movement occurs in a branch that has no open states (Noceti et al., 1996). The equivalence of Q values using both methods for *Shaker* potassium channels indicates, as originally suggested by Armstrong and Bezanilla (1973) for sodium channels, that virtually all of

the gating current is coupled with the opening of the channels.

Calcium Channel Gating

Besides the self-consistent estimates of Q obtained for potassium channels, Noceti et al. (1996) have helped clarify a mystery that has intrigued aficionados of calcium channel gating for more than a decade. Gating currents for calcium channels are often quite large with respect to the ionic currents in the same cells (e.g., Kostyuk et al., 1981; Bean and Rios, 1989). This would be surprising for any type of channel, because the charge rearrangement within a single protein molecule is likely to produce a small current compared to the 10^6 – 10^8 ions per second that move through an open channel. However the relatively slow kinetics of calcium channel gating lead to the expectation of a small gating current. This can be shown as follows.

Suppose that a voltage-dependent channel has M gating states, any of which may be open or closed. In response to a step of voltage the time-dependent probability of being in state j , $P_j(t)$ will have the form of a weighted sum of $M - 1$ exponential relaxations plus a constant (Colquhoun and Hawkes, 1977). In response to a voltage step the gating current for such a channel is the scalar:

$$I_g(t) = NZP'_j(t) ,$$

where Z is the $1 \times M$ row vector representing the effective charge distribution in each gating state, and $P'_j(t)$ is the time derivative of the $M \times 1$ column vector $P_j(t)$ with elements as defined above (Horn, 1984). Because the derivative of an exponential is an exponential with the same time constant, τ , the gating current must have the same time constants as found in the ionic current. However a time derivative weights each exponential component by $1/\tau$. Therefore the magnitude of the gating current depends not only on the amount of charge movement in individual gating transitions (embedded in Z), but also on the kinetics of the gating [through the derivative $P'_j(t)$]. Thus, a slowly gating channel, such as a calcium channel, will tend to have a smaller absolute magnitude of gating current than a more rapidly gating channel, like a sodium channel.

The most extreme example of a discrepancy between the amplitudes of ionic and gating currents for calcium channels is obtained in skeletal muscle. The origin of a major component of the charge movement in this tissue is a population of dihydropyridine (DHP) receptors, fewer than 5% of which also serve as functional calcium channels (Schwartz et al., 1985; Rios and Brum, 1987; Nakai et al., 1996). If calcium channels can generate gating current without opening, the Q values determined from variance analysis should be anomalously large. This is due to the fact that if some chan-

nels never open, N will tend to be underestimated from fluctuation analysis. In fact Noceti et al. (1996) show that Q estimated from the variance analysis method is larger than that determined by limiting slope for the α_{1E} calcium channel subunit (~ 15 vs. $\sim 9 e_0$). Interestingly, this discrepancy is abolished by coexpressing α_{1E} with the β_{2a} subunit. In the coexpression experiment both Q values approximated the limiting slope value observed in all α_1 subunits studied ($\sim 9 e_0$).

The data of Noceti et al. (1996) strongly suggest the presence of a functionally heterogeneous population of calcium channels, some of which generate gating but not ionic current in response to a depolarization. Such silent channels may underlie the appearance of consecutive clusters of blank or "null" traces observed in single channel recordings in response to a series of depolarizations (Hess et al., 1984). The β_{2a} subunit enhances the coupling between charge movement and channel opening, so that every channel that translocates gating charge is capable of opening. This form of modulation contrasts with that observed with the β_{1a} subunit, which shifts the activation curve without affecting estimates of Q (Noceti et al., 1996). In the extreme case of the DHP receptors of skeletal muscle, the voltage-dependent transitions that generate gating current apparently have a more important physiological role, namely excitation-contraction coupling, than creating a conductance pathway for calcium ions. Perhaps it is no coincidence that the α subunits of these DHP receptors are associated with β_1 , rather than β_{2a} subunits (Ruth et al., 1989). It will be interesting to use these powerful methods to characterize other modulators of calcium channels, such as agonists like BAYK 8644 and cyclic AMP, as well as to explore the molecular basis for charge movement in mutagenesis studies.

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