LETTER TO THE EDITOR

Comments on "Some Unexpected Consequences of a Simple Physical Mechanism for Voltage-Dependent Gating in Biological Membranes":

Dear Sir,

In a recent paper, Finkelstein and Peskin (1) have called attention to a long-standing *apparent* anomaly in the response of membranes to a small change in the trans-membrane voltage. The authors state the problem, and the "unexpected consequences of their proposed "simple physical mechanism" in their Introduction:

"The vexing problem is to account plausibly and physically for the steepness of the conductance-voltage relationship.... this formally requires the transfer of several charges from one side of the membrane to the other.... Small movements within the bilayer ... only move charge through a fraction of the total membrane potential.... We take here a different tack ... Gating charges on the channel walls that move a short distance across a closed gate move through the entire transmembrane potential. The analysis of such systems reveals unexpected consequences and properties...."

This apparent anomaly was discussed in very similar terms, over fifteen years ago, and resolved when the equations for the electrochemical flow of ions across the membrane were solved with Poisson's equation for the change in electric field; that is, without the assumption of microscopic electroneutrality (the constant field approximation) (2, 3).[1] The Introduction of reference 3 states in part,

"When a depolarizing voltage step is applied to a voltage clamped axon ... the conductivity changes much more rapidly than can be explained by simple theory.... A logical difficulty in interpreting the phenomena is that the voltage step V is the voltage change across the entire membrane.... One should then ... consider only that portion of the applied voltage step that appears at [the gating] boundary ... only a fraction of the applied step, V.... But a marked change occurs if one considers the effect of the boundary layer on ion flow, and the concomitant effect on the distribution of the electric field through the membrane."

This paper went on to analyze the gating effect of Ca^{++} ions, showing that the voltage change across the gating region, i.e., the boundary, or double layer could be even greater than that of the stimulus. Later papers (4, 5, 6) extended the calculations in various ways, all demonstrating the salient point that the voltage change across the gating region would be at least of the order of the applied stimulus, irrespective of the details of the model. It was later shown that when the effects of stochastic fluctuations are properly considered, the voltage change can in fact be many times greater than the stimulus (7, 8).

It is thus apparent that the application of the proper physiochemical equations had long since resolved the "vexing problem" cited by the authors. Their model, however, appears to be novel in its details, assuming a gate in which the ratio of open to closed dwell times is independent of position of the gating charges: here, the orientation of dipoles.

This is an interesting idea, and while it does not conform to the usual concept of the basis for conformational gating, it is useful to consider this novel approach to the problem. I therefore believe it is worthwhile to point out several questionable points in the authors' analysis. Because of the difficulty I at least have in determining their quantitative effect on the performance of their model, I believe it would be useful for the authors to comment on their significance.

Questionable Aspects of the Model: In the Introduction, p. 549: "Energetically, the walls of the channel are a much more favorable location for the gating charges than in the interior of the bilayer, because of the high dielectric constant of the aqueous channel relative to the lipid bilayer." Similarly, in the Discussion on p. 553: "The presence of gating charges within or near the polar, high dielectric-constant medium of the channel is physically reasonable and preempts the energetic problem associated with their being in the low dielectric-constant medium of the lipid bilayer."

While the authors do not elaborate on "the energetic problem," the structure they propose—charges on low dielectric constant channel walls, with a high dielectric constant medium in the channel—would tend to concentrate the electric field of the gating dipoles within the channel, where it would be intercepted by the gating "shutter."

These statements are, however, in direct conflict with the long-recognized properties of water in the close vicinity of ions or charged groups and surfaces; that is, in exactly the milieu which the authors assume to be the nature of the channel, and which is also the presently accepted view (9). The proximity of the water molecules to charges, positive or negative, tends to hold their dipoles in fixed orientation, and results in a lowering of the dielectric constant ϵ from about 78 in free water to the range of 5–10 in the vicinity of an ion or charged group (10). This would not be much greater than the ϵ of lipids (3.5 to 5). This is, however, irrelevant, since it is known that the channel is through a protein (9), as in fact stated by the authors. Proteins have a much higher ϵ, which may be as high as 40 (11, 12).[2] Since this is the reverse of the authors' assumption, it is important to know how this affects their model's performance; if my understanding is correct, it would appear to be of importance.

The authors analyze their model in terms of the channel being a "classical conductor"; this approximation is made throughout their paper, including the section on quantitative behavior, and

[1] So far as I am aware, these equations had not been previously solved for arbitrary diffusion parameters and boundary conditions.

[2] The dielectric constant will not necessarily be the same throughout the protein molecule, but should be high in the regions of ionized polar groups, which would be hydrophilic, and thus tend to face into the (low dielectric constant) aqueous channel.

the presumably rigorous analysis in the Appendix. This assumption leads to the conclusion, as shown in their Appendix, that $\rho = 0$ in the steady state, ρ being the (microscopic) net local charge density. Such an approximation is indeed useful for certain didactic purposes (see, for example, Fig. 1 of reference 8), its applicability to each phase of the analysis must be carefully examined to avoid possibly "approximating out" crucial phenomena.[3]

Although the authors do not explicitly say so, they realize that the channel must significantly depart from a classical conductor at the closed gate, but implicitly minimize the importance of the departure by assuming that the Debye length within the channel is small compared to the length of the channel (footnote 1). From this they concluded that "it is a good approximation to place the entire voltage drop across the gate." The Debye length is useful in the theory dilute electrolytes, giving the approximate distance in which the electric field of an ion is effectively neutralized by counterions. But while the Debye length within the channel may be <1Å, the charge separation between an ion and counterion must total at least one ionic diameter, ~5Å. There will then be a large electric field over at least this distance on both sides of, as well as across, the suggested "shutter," so that in the authors' model the gating charges must actually move through a substantial fraction of the membrane thickness, even if the shutter itself were to be of negligible thickness.

The present view of the structure of channels channels such as the Na^+ channel is that they contain a large excess of negatively charged groups in their walls, whose overall charge is substantially balanced by the permeable positive counterions. Therefore, if the properties of the channel are substantially uniform across the membrane, the net charge per unit length will be substantially zero except in the vicinity of the closed gate; thus to this extent the assumption that $\rho = 0$ is an acceptable approximation. This may not, however, apply when considering the charge gradient *across* the membrane: the negative charges are in the wall, while the positive charges are in the aqueous channel. There is thus certainly a substantial charge gradient normal to the channel walls, with a resulting potential gradient. It appears that this factor may affect the authors' results for lateral movement of the channel walls, as analyzed in their Appendix.

[3]This approximation was implicitly assumed by Finkelstein and Mauro in Appendix I to their paper "Equivalent circuits as related to ionic systems," 1963 *Biophys. J.* 3:215–237. This resulted in the electric field being constant over the whole regime, up to the interfacial boundary. This analysis was widely accepted, and masked the very phenomena described in the present paper.

Finally, the model does not of itself solve the problem of the steepness of the conductance change in some excitable membranes, still requiring the motion of many electron charges, albeit over only a fraction of the full membrane thickness. However, the change in the potential distribution within the channel with open and closed gates, and the dependence on the channel conductance α, of the time required for establishment of the new steady state, which they discuss (p. 551), are exactly the factors which may lead to such high membrane sensitivity (7, 8). It would thus be of interest for the authors to apply the Fokker-Planck analysis to their model.

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