Two hypotheses reexamined: gating currents and the number of mobile ions in the Na^+ channel

Dear Sir:

In this letter, I discuss two concepts related to the ion flow through Na^+ channels. The first is that of hypothetical "gating particles", which are assumed to move within the membrane. The second concept is that Na^+ ions move across the membrane essentially as rapidly as they would move in free solution under the same potential gradient, despite the labyrinthine nature of the channels, and the potential barriers they encounter in passage. The first concept is hypothetical; the second appears to be highly unlikely. Here I propose alternative concepts based on electrochemical theory, and consistent with the structure and function of Na^+ channels, as we presently understand them.

In 1973, Armstrong and Bezanilla (1) recorded a brief and very small difference between currents resulting from equal amplitude positive and negative voltage clamp steps applied across squid axon, in the absence of permeant cation species in both the internal and external media. They associated this "gating current" with the motion of "gating particles" within the membrane, as proposed by Hodgkin and Huxley (2) as a possible explanation of the high voltage sensitivity of the Na^+ channel. However, because electrodiffusion theory predicts that the motion of cations within the channel under similar conditions would exhibit similar currents (3), the observation of such currents was therefore not per se evidence of the existence of "gating particles." Recognizing the importance of the electrodiffusion component, Armstrong (4) later suggested additional criteria for the existence of gating currents, especially their persistence when all cations had been eliminated from the channel by their replacement in the bathing solutions, and therefore presumably from the channels, by ions usually considered to be impermeable: e.g., Tris for Na^+, and TMA for K^+. The molecular composition (if not the detailed structure) of channels (5), and the analysis of the stochastics of processes involved in gating (6) have now provided an alternative explanation of voltage sensitivity without "gating particles" or "gating currents." Yet they continue to be used as a basis for the analysis of experimental data (7).

Before the Na^+ channel was sequenced by Noda et al. (5), it was widely accepted that only a few ions reside in the Na^+ channel, perhaps two or three. This number was apparently based on the analysis of Caterall (8), who concluded that because $>10^7$ ions per second may pass through one Na^+ channel, each ion must pass in $<10^{-7}$ s, a rate approaching electrodiffusion in free solution, and implying straight-line flow and only weak interaction with the channel. Based on this hypothesis, the conductance of the Na^+ channel could be explained with only two or three Na^+ ions in a channel. According to this view, which was widely accepted, the number of Na^+ ions within a channel would be too few to support the electrodiffusion hypothesis (3). The work of Noda et al. shows that the channel protein is made up of 1,820 amino acid residues; 221 are the acidic residues aspartate and glutamate, which when ionized are negatively charged; and 142 are the basic residues lysine and arginine, which are positive. The hydrophilic nature of these residues results in an aqueous phase within the channel. Although the aspartic and glutamic acids themselves would be almost 100% ionized at pH 7, the fraction of their residues ionized in the channel protein may be considerably less, depending upon their local environment in the protein. If we assume the positive residues inhibit the ionization of an equal number of negative residues, there would be ≈ 80 negatively charged residues in the channel. Because the net charge within the channel must be close to zero, there would then be an equal number of univalent cations in the channel, presumably mostly Na^+ under normal conditions. Chloride ions in the channel could increase the number, and other factors could possibly reduce it, but it appears improbable that the number is less than ~ 40. Because the effective ionic mobility within the channel required to explain the channel conductance is inversely proportional to the number of mobile ions moving within the channel, the high rate of Na^+ ions moving through the channel suggests the presence of many Na^+ ions within the channel, rather than few. Other experimental evidence lends further support to the electrodiffusion view: the observed reduction of the gating currents with a lowering of pH (8) is consistent with the lowering of the number of ionized negative residues, and thus the number of mobile cations within the channel.

I now calculate the probability that univalent cations, whether Na^+ or other ions, would be eliminated from the channel by using nominally impermeant cations in the bathing solutions. Based on the molecular weight of the polypeptide molecule constituting the Na^+ channel (5), and assuming $\approx 30\%$ of its volume consists of water (10), the volume of the channel would be ≈ 80 nm^3. For simplicity, the channel will be assumed to be spherical in form; its radius r_0 would then be ≈ 2.7 nm. The ionized negative residues, both those associated with an Na^+ ion, and those that are free of any cation, will be assumed to be uniformly distributed within this volume. The electrostatic problem may then be solved as that of a symmetrically charged sphere; the electrical potential difference V between the center of the sphere and its surface (the bathing solution), is then

$$V = q/\epsilon_0 \epsilon_r r_0, \tag{1}$$

where q is the net charge within the sphere, ϵ_0 is the absolute dielectric constant, 8.85×10^{-12} fd-m^{-1}, and ϵ_r is the relative dielectric constant of the hydrated protein medium within the sphere, ≈ 20 (11–13). Using the above figures, the value of V would be 0.34 V per unit charge. The potential difference would be larger for a charge located at a site closer to the mouth of the channel; for example, at 5 Å from the mouth (i.e., the surface of the sphere), the potential difference would be ≈ 3.5 V.

Many univalent cation species, which may be present in either bath, will be able to enter the channel, and to be mobile

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within it, even though their conductance, that is, its ability to pass through the channel, may be far less than that of the Na^+ ion; I will refer to such cations as being permeant, rather than permeable.

The effect of low concentrations of permeant or permeable ion may be calculated from the equilibrium relationship between C_{ext}, their concentration in one or both of the bathing solutions, and C_{ch}, their concentration within the channel:

$$C_{ch} = C_{ext} \exp(-V \cdot F/RT). \quad (2)$$

Based on the assumptions discussed above, we then obtain for the ratio C_{ext}/C_{ch} as a function of n, the number of ionized negative residues in excess of the number of cations present in the channel,

n	V	C_{ext}/C_{ch}
1	0.34	2×10^{-6}
3	1.0	10^{-17}
10	3.4	10^{-57}
15	5.1	10^{-85}

C_{ext}/C_{ch} is the maximum molar concentration of univalent cations, permeant or permeable, in baths which would allow the "washing out" of the cations within the channels to a one molar concentration (i.e., ~15 cations per channel), for n unneutralized negative residues. A different number of cations within the channel would not affect the C_{ext}/C_{int} ratio, but would imply a proportional change in the value of C_{ext}. If the effective radius of the channel protein is larger than the assumed 2.7 nm: e.g., 5 nm, the ratios becomes $\approx 10^{-3}$, 10^{-9}, 10^{-31}, and 10^{-46}. Thus, to "wash out" more than two or three ions from a channel would require the permeant ion concentration in the baths to be less than 10^{-12} M; to eliminate a significant fraction, e.g., a third of the ions, the purity would have to be far greater. These calculations are, if anything, conservative in their estimate of the allowable permeant ion concentration. The purity of the reagents used in the baths has not (so far as I am aware) been discussed, and it is doubtful that the required purity is achievable.

Electrodiffusion theory shows that the change in the local electric field required to open (or close) a channel's gate must be accompanied by a redistribution of the ions within the channel, especially in the vicinity of the gate (6, 14, 15). This redistribution will result in a brief pulse of current (2), consistent with the observed "gating currents," as stated above; the calculations presented above show that although the flow of current through the channel would be substantially eliminated by replacing ions in the baths with impermeant species, the so-called "gating current" component would not be significantly modified.

The channel gates carry an electric charge (16), and thus the motion of the gates when opening or closing will add an additional component to the observed currents. However, because these charges are located in the side-chains of amino acid residues, the electric field will deflect them a very small distance, probably not more than 1 Å (17), a current equivalent to the transport of only a few hundredths of an electron charge across the channel; it is, however, possible that this strain may induce a delayed, but considerably larger, allosteric change in the protein configuration.

The concept of "gating particles" began with Hodgkin and Huxley (2) in an effort to explain the large voltage sensitivity of the flow of Na^+ through the membrane, many years before the existence of ion channels was recognized. Because the physical nature of the channel has been determined (5), and the nature of gating elucidated by single-channel recordings (18), it has been shown that electrodiffusion theory can quantitatively explain the experimental observations (6).[1] Not only has the existence of gating particles not been demonstrated, but the hypothesis has become unnecessary to the explanation of the functioning of Na^+ and other channels.

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[1]Figs. 11 and 12 of this reference are relevant. In the legend to Fig. 10, "Dashed line" should be "Dotted line," and "Lower solid-line curve" should be "Dashed line curve." There are a number of other typographical errors in this paper; the author will send a correction sheet, along with a reprint, on request.

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Franklin F. Offner

Biomedical Engineering Department

Northwestern University

Evanston, Illinois 60208