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Cation Permeation at the Amphibian Motor End-Plate

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Summary. Measurements of acetylcholine-induced single-channel conductance and null potentials at the amphibian motor end-plate in solutions containing Na, K, Li and Cs ions (Gage & Van Helden, 1979; *J. Physiol. (London) (in press)* were analyzed in terms of three models. Two of these models, the "neutral" site channel model and the "charged" site channel model were developed to cater for three cations. Both were shown to be able to explain the dependence of single-channel conductance on membrane potential and gave the following sequences of equilibrium constants and mobilities. $K_{\mathrm{Li}}/K_{\mathrm{Na}}/K_{\mathrm{K}}/K_{\mathrm{Cs}}$ = 7:1.7:1:0.9 and $u_{\mathrm{Cs}}/u_{\mathrm{K}}/u_{\mathrm{Na}}/u_{\mathrm{Li}}$ = 1.4:1:0.58:0.13 at 8 °C. Similar sequences were obtained at 20 °C. Although the neutral model fitted the data for relative conductances in Li-, Cs- and Na-solutions slightly better than the charged model, experiments done in normal [NaCl] and [NaCl]/2 solutions could only be fitted by the neutral model. In contrast, the third model, the Constant Field Equation, was unable to fit the conductance data in any of the above situations. The data available suggests that permeation is through "long" neutral channels, lined with high field-strength negative polar groups and including one or possibly more high resistance barriers for anions.

This paper endeavors to analyze the mechanism of cation permeation at the amphibian motor end-plate. Models which might be consistent with values of single-channel conductance that were obtained in the presence of the four alkali cations, potassium, caesium, sodium and lithium, will be evaluated. Specifically, the influence of cations on acetylcholine null potential (ϵ_0) and single-channel conductance will be taken into account together with the influence of membrane potential on channel conductance. The measurements have been fully described in another paper (Gage & Van Helden, 1979). In addition, measurements of the influence of cation concentration on channel conductance will be used to discriminate between the models.

Eisenman (1968) has broadly characterized cation permeation models into six different groups: two of these either involve a surface being rate-limiting or bare cations moving through a lipid region of membrane. The first possibility is un-

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likely and the second of too small a magnitude to account for conductance in biological membranes. The remaining four possible groups can be divided into two main divisions: those with mobile sites (carriers) and those with fixed sites. Selectivity amongst cations, and between cations and anions, is predicted as a result of interaction of the ions with these sites (for an excellent review on ion selectivity, *see* Diamond and Wright, 1969). It seems unlikely that mobile sites or carriers are involved in end-plate permeability as flux rates calculated from a single-channel conductance of 25 pS would be on the order of 10^7 ions$\cdot$s^{-1} for each mobile site for a voltage of 50 mV. Thus ion permeation at the end-plate is probably through some fixed ionophore or channel which opens in response to the interaction of acetylcholine with a receptor. There are two broad categories for such "fixed site" channels.

1) A "neutral" site channel with negative polar groups lining the inside surface of the channel. This could produce selectivity between cations and anions, and selectivity between different cations.

One extreme limiting case of such a channel would be either the complete absence or only a very low concentration of polar groups lining the channel so that it behaved simply as an aqueous hole through the membrane, in which case ions would traverse the channel with virtually the same partition coefficients and permeabilities proportional to their free solution mobilities.

2) A "charged" site channel with negative monopoles (or their equivalent) lining the inside of the channel. Again this could result in channel selectivity between cations and anions, and between cations.

One could further divide such permeation mechanisms into two further subdivisions (Barry & Diamond, 1971): those in which the channel length is "long" in comparison to the "effective Debye length within the channel"[1] (the so-called "thick" membrane channel) and those in which it is "short" (the so-called "thin" membrane channel). In the case of long channels, macroscopic electroneutrality would be obeyed within the channel. In such a situation there is a very clear distinction between the neutral (polar) channel and the charged channel. In the former case the polar site, though selective for cations, does not have to be occupied by a cation whereas in the latter case the charged site does have to be occupied by a cation to satisfy conditions of macroscopic electroneutrality. This difference results in a very well defined distinction between the neutral and charged

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[1] Normally Debye length is defined assuming an isotropic medium. In referring to the "effective Debye length within the channel", we are considering Debye length in a one-dimensional sense along the length of the channel. In this case the region around an aqueous channel traversing a lipid membrane may to a first approximation be considered to be an insulator so that the Poisson-Boltzman equations can be approximated by a one-dimensional solution along the length of the channel. Because of the molecular dimensions of end-plate channels, the analysis itself and the effective dielectric constant within the channel may well be somewhat contaminated by polar or charged groups lining the channel so that the effective Debye length within it may possibly differ from its value in an isotropic medium.

models as far as their predictions for conductance-concentration curves are concerned. In the case of the neutral channel the ionic conductance should increase linearly with external salt concentration, whereas for the charged channel it should remain essentially independent of external salt concentration provided that the concentration of charged groups lining the channel is not much less than the external salt concentration. In the case of short channels it would be expected that this distinction would not be quite so well defined.

As far as the end-plate channel is concerned, available evidence suggests that it is an aqueous channel of some sort. Provided that the ionic concentration in the channel is no lower (and it could well be higher) than in the bathing solution, the Debye length in the aqueous solution would be less than 1 nm for salt concentrations greater than 100 mM. If the effective Debye length within the channel was of a similar or smaller value, then for a 10-nm thick membrane the channel would tend to behave as a long channel with macroscopic electroneutrality obeyed within it. In this present paper an attempt is made to fit the data recently obtained by Gage and Van Helden (1979) to models of long neutral channels and long charged channels. Experimental results are also compared with the predictions of the constant field (Goldman-Hodgkin-Katz) equation (Goldman, 1943; Hodgkin & Katz, 1949), not because there appears to be any justification for the use of such an equation at the end-plate, but because of the wide use of this equation in the biological literature for describing potentials across membranes. It is not necessary to consider the conductance equations used by Takeuchi and Takeuchi (1960) because in the presence of zero concentrations of some cations on one side of the membrane equilibrium potentials become *infinite*. Although the conductance approach has been widely used in estimates of relative end-plate conductance, it cannot be used here for this reason. The use of independent conductance terms for the individual ions would seem to imply different effective pathways for the ions (*see*, e.g., Jaffe, 1974) and for ions of the same sign these would probably be physically distinct. In the neutral model, however, it will be shown that for a cation and an anion the equations give rise to separate independent terms similar in form to the Takeuchi's equation. It should be stressed that the empirical conductance equation has been very valuable in helping to give some estimate of ionic conductance changes at synapses but that it is really inappropriate for investigating the precise mechanism of ion permeation, especially in the situations described in this paper.

It should also be noted that a diffusional approach has been used in this paper to model permeation. An alternative and somewhat complementary method (*see*, e.g., Läuger, 1973; Hille, 1975) would have been to consider energy profiles and use rate theory. Such an approach has been successfully used for the gramicidin A channel (e.g., Sandblom, Eisenman & Neher, 1977; Eisenman, Sandblom & Neher, 1978; Neher, Sandblom & Eisenman, 1978) which appears to have some similar properties to motor end-plate channels. The rate-theory approach gives rise to fairly complicated expressions for the conductance in asymmetrical solutions and incorporates a large number of parameters in the equations. Qualitatively both approaches should lead to similar conclusions. In fact, as the number of energy barriers increases in the main region over which diffusion is assumed to be important, the rate theory approach will tend to be better approximated by the diffusional approach (for further details, *see* Läuger & Neumcke, 1973). The lat-

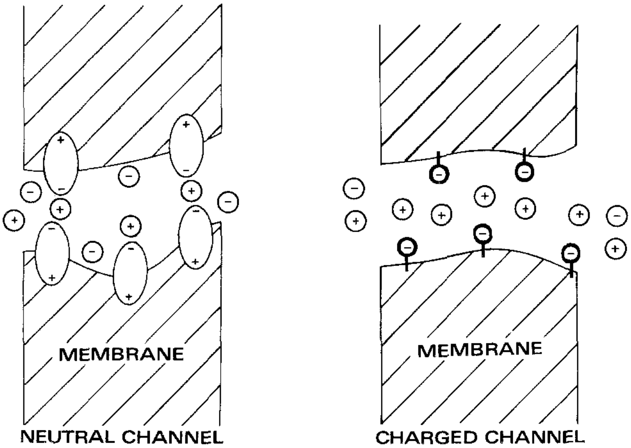

Fig. 1. Schematic diagrams of two types of possible models for the main mechanisms of ion permeation considered in this paper. For diagrammatic purposes only, 5 sites are shown for each channel. In reality there could be a much greater number in each case. The left diagram illustrates one possible model that would give rise to a long neutral-site channel. It has negative polar groups lining the channel which are located in such a way as to include one, possibly two (as shown here) or more high-resistance barriers for anions. For cations, however, the main resistance pathway is within the main part of the channel. Such a channel would be expected to allow anions to enter to balance the cation concentration in it but would not result in them contributing significantly to the channel conductance. The channel would show cation selectivity and would exhibit a conductance which would increase with the external salt concentration. The right diagram illustrates one possible model that would give rise to a long charged-site channel. This is a typical ion-exchange type channel with negative monopoles (or their equivalent) lining the channel which are then balanced by the mobile cations. Except at very high external concentrations, it would be expected that the cation concentration in the channel and hence the channel conductance will tend to remain constant as the external salt concentration is increased

ter is rather easier to visualize and handle mathematically and will be used in this paper.

Preliminary accounts of the model chosen have appeared previously (Barry, Gage & Van Helden, 1978; Barry, 1978).

Theoretical Models

A. Neutral (Polar) Site Channel (Neutral Model)

Figure 1 illustrates in a very schematic way one possible picture of a neutral site channel in a thick membrane. There are negative polar groups (e.g., negative ends of dipoles) lining the channel. This would tend to favor the passage of cations through the channel. The negative polar groups, however, might be located in such a way as to include at least one, possibly two (as in this example) or more, high-resistance barriers for anions. For cations, however, it would be expected that the main resistance pathway would be elsewhere in the channel. Although the permeability of the membrane to anions would be lower than for cations (to an extent depending on the actual location of the sites and geometry of the channel), anions would still be able to enter the channel in order to maintain macroscopic electroneutrality along its length. Two cases involving 3 cations and 1 anion will be discussed: (i) permeant anions and (ii) impermeant anions.

Case (i) Permeant Anions. It can be shown (*see Appendix A*) that if the anions have a finite permeability, then the current, I, through the membrane is given by:

$$I = B_1 \left\{ \left[\frac{\xi U'' - U'}{\xi C'' - C'} \right] [\epsilon - \epsilon^*] + u_3[\epsilon - \epsilon_3] \right\} \tag{1}$$

where

$$B_1 = \frac{F^2}{d\gamma} K_3 A_f \left[\frac{a_3'}{\theta'} - \frac{a_3''}{\theta''} \right] \Big/ \ln \left(\frac{a_3'' \, \theta'}{a_3' \, \theta''} \right) \tag{2}$$

$$U' = u_1 K_1 a_1' + u_2 K_2 a_2' + u_4 K_4 a_4' \tag{3}$$

$$U'' = u_1 K_1 a_1'' + u_2 K_2 a_2'' + u_4 K_4 a_4'' \tag{4}$$

$$C' = K_1 a_1' + K_2 a_2' + K_4 a_4' \tag{5}$$

$$C'' = K_1 a_1'' + K_2 a_2'' + K_4 a_4'' \tag{6}$$

$$\epsilon^* = \frac{RT}{F} \ln (C'/C'') \tag{7}$$

$$\epsilon_3 = -\frac{RT}{F} \ln (a_3'/a_3'') \tag{8}$$

$$\theta' = [K_3 a_3'/(K_1 a_1' + K_2 a_2' + K_4 a_4')]^{1/2} \tag{9}$$

$$\theta'' = [K_3 a_3''/(K_1 a_1'' + K_2 a_2'' + K_4 a_4'')]^{1/2} \tag{10}$$

and

$$\xi = e^{\epsilon F/RT} \tag{11}$$

where ϵ is the membrane potential (inside with respect to outside); R, T, and F have their usual significance; subscripts 1, 2 and 4 refer to cations and 3 to the anion (3 was originally chosen to signify the anion to bring the equations into line with other permeation treatments dealing with two cations and an anion, e.g., Barry and Diamond, 1971); superscripts $'$ and $''$ refer to the outside and inside solutions, respectively; a represents the activity of the ion; and u and K denote the mobility within the channel and the partition coefficient of an ion. Both u and K are considered to be constant throughout the channel and γ, the activity coefficient for each of the ions, is also considered to be the same for each ion (generalized Guggenheim assumption. *See* Barry & Diamond, 1970, p. 104) and constant within the channel. It is also assumed that the ionic concentrations within the channel are significantly less than the concentration of polar sites for the external concentrations considered, so that saturation effects are not significant. The synaptic null potential (i.e., zero current potential) ϵ_0, will be solved by a numerical iterative procedure because of the simultaneous presence of both exp (ϵ) and ϵ terms in the equation. Conductance, G, will simply be given by

$$G = I/(\epsilon - \epsilon_0).$$

For a single cation (1) and anion (3), Eq. (1) simplifies to a Takeuchi type equation of the form

$$I = B_1[u_1(\epsilon - \epsilon_1) + u_3(\epsilon - \epsilon_3)]$$

where ϵ_1 and ϵ_3 are the equilibrium potentials for cation (1) and anion (3), respectively.

Case (ii) Impermeant Anions. If anion permeability is effectively zero, then the last term $u_3(\epsilon - \epsilon_3)$ drops out of Eq. (1) and the current will simply be given by:

$$I = B_1 \frac{[\xi U'' - U'][\epsilon - \epsilon^*]}{[\xi C'' - C']} \tag{12}$$

where the terms are the same as before and are defined by Eqs. (2)-(8). Now, however, the null potential, ϵ_0, will be given by:

$$\epsilon_0 = \frac{RT}{F} \ln \left[\frac{u_1K_1a_1' + u_2K_2a_2' + u_4K_4a_4'}{u_1K_1a_1'' + u_2K_2a_2'' + u_4K_4a_4''} \right] \tag{13}$$

which it should be noted is in the same form as the Constant Field Equation for the potential. It is not dependent, however, on any assumptions about the field. Indeed, for the neutral model the concentration varies linearly along the channel and the potential logarithmically along it. As Eq. (12) indicates the expression for the current is quite different from that predicted by the Constant Field Equation.

B. Charged Site Channel (Charged Model)

Conti and Eisenman (1965) have derived equations for a channel with a number of fixed negative sites. Such a channel (*see* Fig. 1) would now exclude anions and would tend to maintain a constant total concentration of cations within it in order to preserve macroscopic electroneutrality. Re-deriving the equations of Conti and Eisenman (1965) to cater for three cations, and to bring the equations into line with the definitions and symbols used for the neutral site channel equations (*see Appendix B*), the new equations become (setting $z = +1$, $n = 1$ in Eqs. (B24)-(B31)).

$$I = B_2 \left[\frac{\xi U'' - U'}{\xi C'' - C'} \right] (\epsilon - \epsilon^*) \tag{14}$$

where

$$B_2 \equiv \frac{F^2 \cdot C_0}{d} A_f \tag{15}$$

$$\xi \equiv \exp(\epsilon F/RT) \tag{16}$$

$$C' \equiv a_1' + K_{21}a_2' + K_{41}a_4' \tag{17}$$

$$C'' \equiv a_1'' + K_{21}a_2'' + K_{41}a_4'' \tag{18}$$

$$U' \equiv u_1a_1' + u_2K_{21}a_2' + u_4K_{41}a_4' \tag{19}$$

$$U'' \equiv u_1a_1'' + u_2K_{21}a_2'' + u_4K_{41}a_4'' \tag{20}$$

$$\epsilon^* \equiv \frac{RT}{F} \ln [C'/C'']. \tag{21}$$

It should be noted that Eq. (14) is identical in form to Eq. (1) for the neutral model with zero anion permeability, differing only in the definition of B_2. B_2 is a constant parameter, independent of external solution composition and dependent only on the concentration of charged negative sites, C_0, within the membrane. It merely behaves as a constant scaling factor in this equation and will not affect relative conductance values. This implies that under the conditions of zero anion permeability both equations will predict an identical voltage sensitivity for the conductance.

Again as in the neutral model the conductance G will be given by:

$$G = I/(\epsilon - \epsilon_0). \tag{22}$$

The nonideality factor, n, which relates the activity of the ions in the membrane to their concentration (*see Appendix B*) and determines cooperative effects between ions at the sites, has been assumed to be equal to 1.0, as in the ideal case.

Setting $n = 1.0$ in Eq. (B32), the null potential again simplifies to:

$$\epsilon_0 = \frac{RT}{F} \ln \left[\frac{u_1K_1a_1' + u_2K_2a_2' + u_4K_4a_4'}{u_1K_1a_1'' + u_2K_2a_2'' + u_4K_4a_4''} \right] \tag{23}$$

writing K_2/K_1 and K_4/K_1 instead of K_{21} and K_{41}, respectively.

It should be noted that this is now in the same form as the equations for the null potential for both the neutral model and also the Constant Field Equation.

C. Generalized Constant Field Equation

Following Lassignal and Martin (1977), the generalized Constant Field (or Goldman-Hodgkin-Katz) Equation can be used to calculate the membrane conductance and null potential for any number of ions of any valency. Although there is no strict justification for using such an equation for end-plate channels, the wide use of the ordinary Constant Field Equation in the biological literature and the versatility of the generalized equation made it worthwhile seeing if in fact this equation did fit any of the data. In this case the ionic current, I_m, due to the m^{th} ion will be given by:

$$I_m = \frac{z_m^2 F^2 \epsilon P_m}{RT} \left[\frac{a_m' - a_m'' \exp(z_m F\epsilon/RT)}{1 - \exp(z_m F\epsilon/RT)} \right] \tag{24}$$

where z_m and P_m are the valencies and permeabilities of that ion. The total membrane current, I, would then be given by summing over all the ionic currents to give:

$$I = \Sigma_m I_m. \tag{25}$$

The null potential can now be obtained by using a numerical iterative procedure to find that value of ϵ for which $I = 0$.

When all valencies have the same absolute magnitude, Eqs. (25) and (24) reduce to the standard Constant Field (Goldman-Hodgkin-Katz) Equation, e.g., for the three permeant monovalent cations alone.

$$\epsilon_0 = \frac{RT}{F} \ln \left[\frac{P_1 a_1' + P_2 a_2' + P_4 a_4'}{P_1 a_1'' + P_2 a_2'' + P_4 a_4''} \right] \tag{26}$$

It should be noted that the mobilities and equilibrium constants do not enter explicitly into Eqs. (25) or (26). They can be implicitly catered for by writing

$$P_m = u_m K_m. \tag{27}$$

This, however, means that for a given value of P_m, different combinations of u_m and K_m will lead to precisely the same values of I_m.

Comparison of Experimental Measurements with Theoretical Predictions

A. Methods

In this section the experimental data obtained by Gage and Van Helden (1979) will be compared with the theoretical models outlined in the earlier section: the neutral (polar) site channel (neutral model), the charged site channel (charged model), and the predictions of the Constant Field Equation. In each case the currents were computed using an 11 digit precision Compucorp 425 (configuration 2:2) programmable calculator (Computer Design Corporation, Los Angeles), and where necessary the null potential was calculated using a Newton-Raphson iterative procedure (e.g., Ralston, 1965, p. 332) to find the potential ϵ at which the total current I was zero.

Table 1. Main ionic composition of external solutions and assumed internal composition in mM

Ion Solution	Outside concentration[a] (a')					Inside concentration[b] (a'')				
	Li	Na	K	Cs	Cl	Li	Na	K	Cs	Cl
Li-solution	115	5.4	2.5	—	121.1	—	10	140	—	3.3
Na-solution	—	120.4	2.5	—	121.1	—	10	140	—	3.3
Cs-solution	—	5.4	2.5	115	121.1	—	10	140	—	3.3
K-solution	—	5.4	117.5	—	121.1	—	10	140	—	10
[NaCl]/2[c]	—	60.5	4.6	—	65.7	—	10	140	—	3.3

[a] These solutions also contained 1.8 mM Ca^{++} and 3 mM phosphate buffer.

[b] These were the estimated concentrations of the internal cell composition.

[c] Osmolality was maintained equal to the Na-solution by the addition of mannitol.

In this method a reasonable, though somewhat arbitrary, trial value of ϵ, ϵ_1, was first substituted into the appropriate equation to give the current $I(\epsilon_1)$. A small arbitrary correction, $\delta\epsilon_1$, was then added to ϵ_1 to give a new trial value ϵ_2 (i.e., $\epsilon_2 = \epsilon_1 + \delta\epsilon_1$). A new value of current $I(\epsilon_2)$ was then obtained. After the first two trials a new predicted correction $\delta\epsilon_2$ could be estimated from the two previous currents (in the first case $I(\epsilon_1)$ and $I(\epsilon_2)$) and the correction (in this case $\delta\epsilon_1$). This procedure for predicting the new correction was continued until a satisfactory solution was obtained. In general for the $(n+1)^{\text{th}}$ trial, the new correction, $\delta\epsilon_{n+1}$, to the potential is related to the old correction, $\delta\epsilon_n$, by

$$\delta\epsilon_{n+1} = \frac{I(\epsilon_{n+1})\delta\epsilon_n}{I(\epsilon_n) - I(\epsilon_{n+1})} \tag{28}$$

and the new value of ϵ, ϵ_{n+2} is related to the old value ϵ_{n+1} by

$$\epsilon_{n+2} = \epsilon_{n+1} + \delta\epsilon_{n+1}. \tag{29}$$

Iterations were terminated when either $|I(\epsilon_n)|$ or $|\delta\epsilon/\epsilon_n|$ was less than 10^{-10}.

The value of the conductance, G, was determined both experimentally and theoretically by:

$$G(\epsilon) = I(\epsilon)/(\epsilon - \epsilon_0) \tag{30}$$

where ϵ_0 is the value of the null potential in each case.

In order to predict values of the conductances, it was necessary to make certain assumptions about the ionic composition of the cell. In Na-, Li- and Cs-solutions (*see* Table 1 for full ionic composition in each case) the internal ionic composition of the cells was considered to be 140 mM K^+ and 10 mM Na^+ (*see*, e.g., Katz, 1966). Chloride was considered to be in equilibrium with the normal resting membrane potential (Hodgkin & Horowicz, 1959). For a −90 mV potential this gives an internal chloride concentration of 3.3 mM. In a K-solution (with KCl substituted for NaCl) the cell membrane potentials were rather depolarized and a value of 10 mM was assumed for internal chloride concentration. It should be noted, however, that the precise value of the internal chloride concentration changes only the value of the constant B_1 in the neutral model equations and hence had no affect on values of relative conductances, provided there were no changes in its value during an experiment. These assumed ionic concentrations together with the composition of the external solutions are given in Table 1.

Error corrections to compensate for normalization procedures, to compensate for errors in measurements in Na-solution to which the other measurements were normalized, were estimated as follows. If σ_{Li} and σ_{Na} were the SEM's of the experimentally measured conductances in Li- and Na-solutions, or the error contributions estimated from the SEM's of the null potentials, then the compensated error in the conductance measured in the Li-solution, $\bar{\sigma}_{\mathrm{Li}}$ would be calculated from

$$\bar{\sigma}_{\mathrm{Li}} = G_{\mathrm{Li}}\,((\sigma_{\mathrm{Li}}/G_{\mathrm{Li}})^2 + (\sigma_{\mathrm{Na}}/G_{\mathrm{Na}})^2)^{1/2}$$

where G_{Li} and G_{Na} are the conductances in Li- and Na-solutions, respectively. If, alternatively, δG_{Li} and δG_{Na} were the error contributions estimated from the curves in Figs. 3 and 2 (i.e., in $K_{\mathrm{Na}}/K_{\mathrm{K}}$ or $K_{\mathrm{Li}}/K_{\mathrm{K}}$) then the error contribution to the conductance in the Li-solution, compensating for the normalization procedure, $\overline{\delta G}_{\mathrm{Li}}$, would be given by

$$\overline{\delta G}_{\mathrm{Li}} = G_{\mathrm{Li}}((\delta G_{\mathrm{Li}}/G_{\mathrm{Li}}) + (\delta G_{\mathrm{Na}}/G_{\mathrm{Na}})).$$

Experimental methods used for measuring ionic conductances as a function of external concentration were the same as those used by Gage & Van Helden (1979) in obtaining the other data used in this paper. For full details, *see* Gage, McBurney & Van Helden (1978).

B. Ionic Conductances as a Function of Membrane Potential

Many of the conductance measurements of Gage and Van Helden (1979) included measurements on the same fiber at a number of different membrane potentials. These measurements were used and normalized to the conductance at −50 mV (in the case of Li-, Na- and Cs-solutions or −30 mV in K-solution) for each fiber and the results then averaged for each solution. This normalization procedure helped to compensate for any cell-to-cell variations in the absolute value of conductance. From the average values of ϵ_0, obtained over a large number of different cells for each of the solutions, the values of the relative permeability terms

$$\frac{P_m}{P_{\mathrm{K}}} = \frac{u_m K_m}{u_{\mathrm{K}} K_{\mathrm{K}}}$$

were obtained using Eqs. (13), (23) or (26) to give an explicit value or Eqs. (1) or (25) to obtain the value using numerical methods. Using this value of the relative ionic permeability, different values of relative equilibrium constant, K_m/K_{K}, were tried for each of the models in turn to see whether the voltage dependence of conductance could be explained by the models and to determine the values of the equilibrium constants so obtained. Since all the solutions contained a small amount of sodium, the Na-solution results were analyzed first and estimates of $K_{\mathrm{Na}}/K_{\mathrm{K}}$ and $u_{\mathrm{Na}}/u_{\mathrm{K}}$ used to correct for the small contribution of sodium in determining parameters in Li- and Cs-solutions.

Figure 2 shows the normalized experimental results obtained for NaCl com-

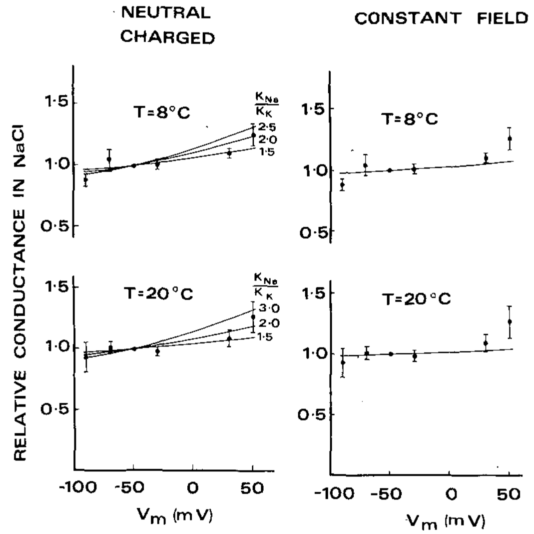

Fig. 2. Experimental values of the relative single-channel conductance in Na-solutions, measured as a function of membrane voltage, V_m, and compared with the theoretical predictions of the three models. It should be noted that the voltage-sensitivity predictions are identical for both the charged and neutral models. Both the charged and neutral models fit the data for a reasonably high value of K_{Na}/K_K, the Na/K, equilibrium constant of the sites, with the overall permeabilities being estimated from the null potentials (Table 2). Again it can be seen that, especially at higher depolarizing potentials, the constant Field Equation predictions are at variance with the experimental results. These values were normalized to the values at −50 mV. For solution composition *see* Table 1. The experimental values of the elementary conductance (points with SEM bars) are those obtained by Gage and Van Helden (1979) by power spectral density analysis of electrical noise during the iontophoretic application of acetylcholine at the end-plate

pared with the curves predicted for the neutral and charged models and the constant Field equation. In each case both the theoretical and experimental points were normalized to give a relative conductance of 1.0 at a membrane potential of −50 mV. As can be seen, there is an increase in channel conductance as the membrane is depolarized. Both the neutral and charged models predicted identical results as indicated by Eqs. (12) and (14) and fit the data reasonably well. At both 8 and 20°C the equilibrium constant K_{Na}/K_K is about 1.7 ± 0.2. At 8°C the null potential of about −5.3 ± 0.6 mV (*see* Table 2) gave for both models a permeability ratio P_{Na}/P_K of about 0.98 ± 0.03 and hence a mobility ratio u_{Na}/u_K of about 0.58 ± 0.08. At 20°C (*see* Table 2) similar results and parameters were obtained. As can be seen from the Constant Field Equation predictions [Eqs. (24) and (26)], the curves are independent of values of K_{Na}/K_K for a given P_{Na}/P_K, and hence, as seen in Fig. 2, this equation cannot explain the voltage dependence of the conductance in these Na-solutions.

Figure 3 shows a similar set of normalized experimental results obtained in Li-solution compared with the three sets of predicted curves of relative conductance *vs.* membrane potential. These were again normalized as for the Na-solution (Fig.

Table 2. Permeation parameters[a] calculated from measurements of null potential, ϵ_0 (Table 2 of Gage & Van Helden, 1979) and from curves of relative conductance *vs.* membrane potential shown in Figs. 2–4

M	Li	Na	Cs
	Temp. = 8°C		
P_M/P_K[b]	0.90 ± .03	0.98 ± .03	1.24 ± .03
K_M/K_K[c]	7 ± 1	1.7 ± .2	0.9 ± .2
u_M/u_K[d]	0.13 ± .02	0.58 ± .08	1.4 ± .4
ϵ_0 (Exp.)	−7.3 ± .7 mV	−5.3 ± .6 mV	0.1 ± .5 mV
	Temp. = 20°C		
P_M/P_K[b]	0.96 ± .05	1.08 ± .02	1.23 ± .05
K_M/K_K[c]	7 ± 2	1.7 ± .2	1.0 ± .5
u_M/u_K[d]	0.14 ± .05	0.64 ± .10	1.2 ± .8
ϵ_0 (Exp.)	−6.1 ± 1.1 mV	−3.2 ± .4 mV	−0.1 ± .9 mV

[a] The parameters were calculated using the neutral and charged models, the two models giving identical predictions for null potentials and voltage sensitivity.

[b] The errors shown for the permeabilities are those calculated from the SEM of the null potential, ϵ_0.

[c] The errors shown for the equilibrium constants are those estimated from the curves in Figs. 2–4.

[d] The errors shown for the mobilities take into account both these sources of error.

2) to give a value of 1.0 at a membrane potential of −50 mV. As can be seen from the curves both at 8 and 20°C, there is now an even greater increase in conductance (than in Na-solution) as the membrane is depolarized. Both the neutral and charged models fitted the experimental data well. For both models at 8°C this requires an equilibrium constant K_{Li}/K_K of about 7 ± 1. At 8°C (*see* Table 2) the experimental null potential of about −7.3 ± 0.7 mV gives for each of the models [Eqs. (13), (23) and (26)] a permeability ratio, P_{Li}/P_K, of 0.90 ± 0.3, thus implying that for both neutral and charged models the mobility ratio in the channel u_{Li}/u_K is about 0.13 ± .02. At 20°C (*see* Table 2) again similar results and parameters were obtained. As can again be seen from the Constant Field predictions [Eqs. (24) and (26)], for given P_{Li}/P_K the curves are independent of values of K_{Li}/K_K, and hence as seen in Fig. 3 the Constant Field Equation can in no way explain the large voltage dependence of the conductance in Li-solution also.

The appropriate curves for Cs-solution are shown in Fig. 4, again comparing experimental results with the sets of predicted curves of relative conductance *vs.* membrane potential for the three models. These were again normalized to give a value of 1.0 at a membrane potential of −50 mV. Now it appears that either the relative conductance remains approximately constant or else decreases slightly as the membrane potential is depolarized. At 8°C especially there seems to be a slight decrease, and both the neutral and charged models which fit the data would indicate an equilibrium constant, K_{Cs}/K_K, of about 0.9 ± 0.2. Again the Constant Field Equation does not fit the data. The null potential (*see* Table 2) of about +0.1 ± 0.5 mV gives a permeability ratio P_{Cs}/P_K, of 1.24 ± .03 and hence a

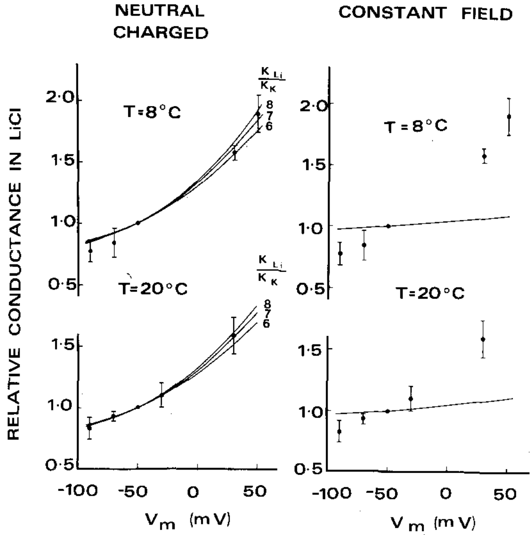

Fig. 3. Experimental values of the relative single-channel conductance in Li-solution, measured as a function of membrane voltage, V_m, and compared with the theoretical predictions of the three models. The experimental and other details are the same as for Fig. 2. Again it should be noted that the voltage sensitivity predictions are identical for both the charged and neutral models. It can be shown that the theoretically predicted curves fit the data well in the case of both the neutral and charged models for fairly high values of K_{Li}/K_K, the Li/K equilibrium constant of the sites, but are at complete variance with the predictions of the Constant Field Equation

mobility ratio, u_{Cs}/u_K of about 1.4 ± 0.4. At 20°C (*see* Table 2) again similar results and parameters were obtained for the neutral and charged models. However, with the large standard errors indicated in Fig. 4, the experimental points are not significantly different from the Constant Field predictions at this temperature.

Similar voltage-sensitivity experiments were carried out in K-solution. However, because of the high resting permeability of the cell membrane for both K^+ and Cl^- ions, there were both experimental problems and difficulties in interpretation of the results. As has been clearly demonstrated (Hodgkin & Horowicz, 1959) the influx of KCl when KCl concentration is increased causes the cell to radically change its internal composition, particularly as far as Cl^- is concerned. It can also cause the cell to undergo changes in volume. As indicated in Fig. 5, there does seem to be some increase in channel conductance with membrane depolarization. The Constant Field Equation itself does predict a slight slope in this direction. Making the rather unreasonable assumption that $[Cl]_i$ remains constant at 10 mM at all potentials, the neutral and charged models predict that there is virtually no increase in conductance. In fact the concentration of $[Cl]_i$ should decrease below 10 mM at very negative potentials and increase above it as the cell is depolarized. This will result in the neutral model now differing from the charged model and predicting an increase in conductance at depolarized potentials. The difficulty in making precise predictions about the slope lies in the uncertainty

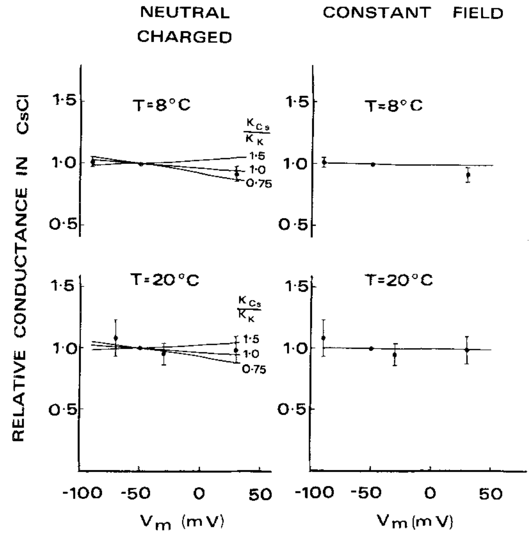

Fig. 4. Experimental values of the relative single-channel conductance in Cs-solution, measured as a function of membrane voltage, V_m, and compared with the theoretical predictions of the three models. Again it should be noted that the voltage-sensitivity predictions are identical for both the charged and the neutral models. The experimental and other details are the same as for Fig. 2. In Cs-solution it can be seen that the experimental values do not distinguish between the three models

about the exact changes in internal chloride concentration and cell volume that would have taken place while the potential was clamped at a particular value before the acetylcholine was iontophoretically applied to the end-plate.

C. A Comparison of Conductances in the Different Solutions

In Table 2 and Figs. 2-5 conductances were all calculated for each solution relative to the value at −50 mV. A further test of the applicability of the three models already used to estimate equilibrium constants from conductance-voltage curves was to take the values of permeabilities obtained from null potentials and the relative partition coefficients or equilibrium constants (Table 2) estimated from those curves, Figs. 2–4, and use them to predict the absolute value of the ionic conductance in each solution. Since each of the models, however, have a constant multiplication factor in their conductance equations, their predictions were normalized to the experimental value of the conductance in Na-solution and predictions were made for both Li- and Cs-solution. Table 3 compares these predicted conductances for the three models with the absolute values of conductance measured experimentally at a potential of −50 mV. The estimated errors in the predicted values take into account the uncertainty in the equilibrium constant estimated from Figs. 2–4 and a smaller error contribution resulting from the uncertainty in ϵ_0 (*see* Table 2). Because of the normalization procedure men-

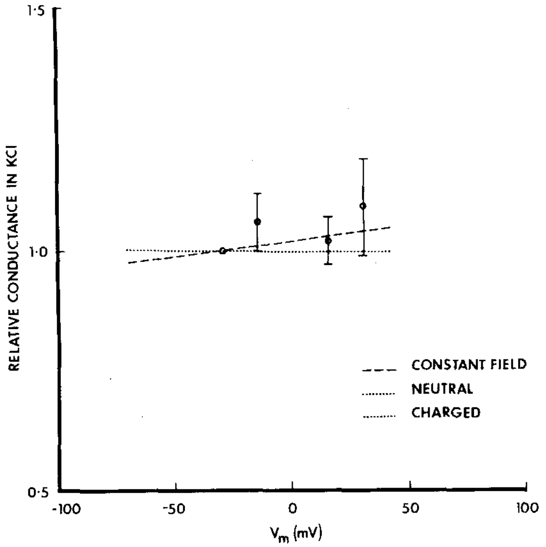

Fig. 5. Experimental values of the relative single-channel conductance at 20 °C in K-solution, measured as a function of membrane voltage, V_m, and compared with the theoretical predictions of the three models. The experimental details are the same as for Fig. 2 except that the conductances were normalized now to the value at -30 instead of -50 mV. An equilibrium constant K_{Na}/K_K coefficient of 1.7 as estimated from Fig. 3 and a permeability ratio P_{Na}/P_K of 1.08 (*see* Table 2) were used in each case. It may be seen that the neutral and charged models once more give identical predictions. In view of the large errors given for the experimental data and possible complications arising in such a high KCl solution, the data do not satisfactorily discriminate between the three models.

tioned above, the errors in Li- and Cs-solution were appropriately compensated for (*see Methods*) to include the error in the conductance in Na-solution.

It can be seen immediately from Table 3 that the Constant Field Equation cannot predict conductances in Li-solution. The charged model is much closer, although the conductance it predicts in Li-solution at 20°C is somewhat lower than the experimental value. The neutral model predictions are, however, fitted even better and agree with experimental values within the errors shown.

D. Ionic Conductances as a Function of External Concentration

Thus far it would appear that, although the neutral model does fit the data well, the charged model provides a fit only marginally worse in some of its predictions. One critical experiment that clearly distinguishes between these two models is the effect of ion concentration on channel conductance. In general it is to be expected that with any fixed charge system membrane conductance should be relatively independent of the external ion concentration because, unless it greatly exceeds the concentration of negative sites within the channel, the concentration of mobile cations there will remain relatively constant during changes

Table 3. Predicted[a] and experimental[b] values of single-channel conductances in different salt solutions in pS

Cation	Li	Na	Cs
	Temp. = 8°C		
Experimental	12.7 ± 1.5	27.9	34.3 ± 1.6
Neutral site	$15.7^{+2.0}_{-1.9}$	27.9	$39.4^{+5.4}_{-5.0}$
Charged site	9.9 ± 1.5	27.9	$47.6^{+12.4}_{-10.8}$
Constant Field	26.5 ± 0.7	27.9	32.3 ± 0.7
	Temp. = 20°C		
Experimental	17.7 ± 1.9	28.9	34.7 ± 2.4
Neutral site	16.0 ± 2.1	28.9	$36.9^{+10.0}_{-7.1}$
Charged site	$10.1^{+3.8}_{-2.5}$	28.9	$43.2^{+26.8}_{-14.6}$
Constant Field	26.8 ± 0.8	28.9	31.3 ± 0.9

[a] The predicted values for both the neutral and charged site models and the constant Field Equation were calculated using the parameters obtained from Table 2, estimated from the voltage sensitivity of conductance. The predicted conductances were all estimated for a membrane voltage of −50 mV and were normalized to the experimental value of the conductance in Na-solution at each temperature. The errors (including both errors in ϵ_0 and estimated errors for the equilibrium constants given in Table 2) in both the Li- and Cs-solutions were appropriately compensated (*see* text) to take into account this normalization procedure.

[b] The experimental values of conductance were those obtained by power spectral density analysis [γ(psd), *see* Table 2 of Gage & Van Helden, 1979]. The errors are the normalized SEM (*see* text), and it should be noted that they differ slightly from the raw SEM in that paper.

in extracellular concentrations. In contrast in a neutral site channel, the concentration of mobile cations within the channel would not be held constant by the concentration of polar sites but will vary in proportion to the external concentration of the cations until their concentration within the channel becomes significant with respect to the site concentration and the assumption about the lack of site saturation breaks down.

A simple experiment was undertaken to test the two models. Channel conductance was measured in a solution with normal NaCl concentration and then in a solution with a lower NaCl concentration (NaCl/2), NaCl being replaced osmotically with mannitol (temperature 20°C). The two solutions (*see* Table 1) were adjusted to keep the KCl product constant in order to minimize changes in

Table 4. Experimental values[a] of relative single channel conductance and null potential measured in [NaCl]/2 solutions at 20°C and compared with the predicted values[a] for both the neutral and charged site channel models and the Constant Field Equation

	Conductance [NaCl]	Conductance [NaCl]/2	ϵ_0([NaCl]) (mV)	ϵ_0([NaCl]/2) (mV)
Experimental[b]	1.0	0.61 ± 0.09	$-3.2 \pm .4$	-12.0 ± 1.2
Neutral site[c]	1.0	0.69 ± 0.01	$-3.2 \pm .4$	-19.4 ± 0.4
Charged site[c]	1.0	1.03 ± 0.01	$-3.2 \pm .4$	-19.4 ± 0.4
Constant Field[c]	1.0	0.67 ± 0.00	$-3.2 \pm .4$	-19.4 ± 0.4

[a] The conductance values were measured and calculated relative to those values in normal [NaCl] solution and the composition of it and of the [NaCl]/2 solution made up to hold the KCl product constant and minimize changes in internal cell composition and cell volume is shown in Table 1.

[b] The errors shown for the experimental values were the SEM. As in Table 3, the errors in experimental values were compensated for to take into account the normalization procedure.

[c] The errors shown for the theoretical predictions were estimated from the errors in the permeability ratio (P_{Na}/P_K) estimated from the SEM of the null potentials together with the errors arising from the errors estimated for the equilibrium constants (*see* Table 2).

internal cell composition and cell volume (Hodgkin & Horowicz, 1959). Table 4 compares the experimental measurements with the predictions of the three models. The parameters used in the models were those estimated from experimentally obtained null potentials and curves of conductance *vs* membrane potential (listed in Table 2). It may be seen in Table 4 that the charged model predicts that the relative conductance will increase slightly to about 1.03 ± 0.01 as $[Na]_o$ is lowered, whereas the neutral model predicts a decrease to about 0.69 ± 0.01 of the control value. It is interesting that the Constant Field Equation also predicts a decrease in conductance. The experimentally measured conductance dropped significantly to about 0.61 ± 0.09 of the control value. This certainly seems to agree with the predictions of the neutral model and to be in contrast with the predictions of the charged model. There is one apparent anomaly in Table 4 and that is the predicted values of the null potential, ϵ_0, compared with the experimental value. A possible explanation for this could be the reduction in ionic strength resulting from a simple replacement of NaCl with mannitol. Such a change in ionic strength might be expected to cause less shielding of the polar sites in the channel and hence to increase their effective site strength. This should make them increase their equilibrium selectivity for Na *vs.* K. As would be expected, an increase in K_{Na}/K_K should result in a small drop in u_{Na}/u_K because of the slight reciprocal relationship existing between the mobilities and equilibrium constants (*see Discussion* for further details). Increasing K_{Na}/K_K from 1.7 to 3 and dropping u_{Na}/u_K from 0.637 to 0.5 in fact changes the value of ϵ_0 from −19.4 to −12.3 mV but keeps the relative conductance at 0.69. Using these same parameter values in the charged model would result in the same ϵ_0 change but would drop the relative conductance only to 0.86. In order to drop the relative conductance to an appro-

Table 5. Main ionic composition of external and internal solutions used in predicting conductance and null potential as a function of external NaCl concentration (*see* Figs. 6 and 7)

Solution	Ion	Concentration[a] outside (*a*′) in mM			Concentration[b] inside (*a*″) in mM		
		Na	K	Cl	Na	K	Cl
Na/1		120	2.5	125.1	10	140	3.32
Na/2		60	2.5	65.1	10	140	1.44
Na/4		30	2.5	35.1	10	140	0.701
Na/10		12	2.5	17.1	10	140	0.320
Na/20		6	2.5	11.1	10	140	0.203
Na/40		3	2.5	8.1	10	140	0.146
Na/80		1.5	2.5	6.6	10	140	0.118

[a] These solutions allowed for a constant 2 mM Hepes buffer with 1 mM Na, 1.8 mM $CaCl_2$ and 2.5 mM KCl in each case.

[b] In order to estimate the internal chloride concentration, it was assumed that chloride was in equilibrium wih the membrane potential set by sodium and potassium ions.

priate low value (0.70) for the same change in ϵ_0, the equilibrium constant would have to rise much higher (from 1.7 to 4) and hence u_{Na}/u_K would drop (from 0.637 to 0.375). However, since the concentration of ions within the charged site channel should tend to remain independent of the external solution concentration, it seems harder to visualize how the effects of ionic strength would be "seen" by sites within the channel. Substituting either of the last two sets of parameters into the Constant Field Equation gives the right ϵ_0 but the conductance ratio would be 0.82. As expected, the Constant Field Equation cannot explain these results in terms of changes in equilibrium constants and mobility ratios.

An alternative explanation would be in terms of membrane surface charge and the neutral model. A decrease in ionic strength in the [NaCl]/2 solution would be expected to increase the surface negative charge on the membrane surface. This would then tend to increase the concentration of the Na^+ ions, and correspondingly decrease the concentration of Cl^- ions, in the solution adjacent to the membrane relative to their concentration in the bulk solution. For example, increasing the local concentration of Na^+ ions from 60.5 to 80 mM and decreasing the local concentration of Cl^- from 65.7 to 40 mM results in a more positive null potential of -12.7 mV, and yet the relative conductance still remains low at 0.64. This explanation will not hold in terms of the charged model, since it predicts no effect of external anion concentration on conductance. Again the Constant Field Equation cannot explain these results since, if these above concentrations are substituted into the equations, it predicts the right ϵ_0 but the relative conductance would now be increased to 0.84.

The evidence would seem to suggest therefore that the neutral model fits the data (with perhaps an increase in the K_{Na}/K_K equilibrium constant and slight decrease in mobility ratio) and that the charged model does not. It seems likely,

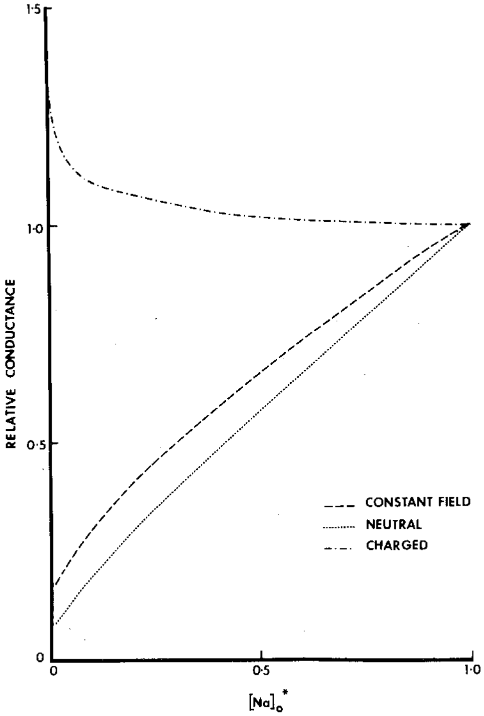

Fig 6. Relative single-channel conductance predicted as a function of the external sodium concentration $[Na]_0^*$ relative to its value of 120 mM in normal Na-solution (*see* Table 5 for details) for the three models. In each case an equilibrium constant, K_{Na}/K_K of 1.7 and a permeability ration of 1.08 (*see* Table 2) and zero chloride mobility were used. It may be seen that the neutral and Constant Field models predict a decrease in conductance as the outside sodium concentration decreases. The nonzero intercept occurs because of the presence of 2.5 mM K^+ in all solutions (*see* Table 5). In contrast an essentially constant and in fact even slightly increasing conductance occurs for the charged model as the sodium concentration decreases. This increase occurs because the less selected but more mobile K^+ ion carries a greater fraction of the current at the lower concentrations

therefore, that end-plate channels behave as channels with negative sites that are neutral (polar) rather than charged. In addition, the decrease in conductance observed with the drop in salt concentration is consistent with the ion concentration in the channels being significantly less than the concentration of polar sites over the range of external salt concentrations considered in this paper.

Conductance and null potential were predicted for both neutral and charged site models, and for interest for the Constant Field Equation also, over a wide range of NaCl concentrations. These calculations assumed constant ionic strength and membrane parameters. The assumed concentrations, both external and internal, used for these calculations are given in Table 5. The permeability parameters used were as in Table 2. Figure 6 shows the relative conductance as a function of the reduction in external sodium concentration, $[Na]_o^*$, expressed as a fraction of the normal sodium concentration, (i.e., $[Na]_o^* = [Na]_o/120$). It may be

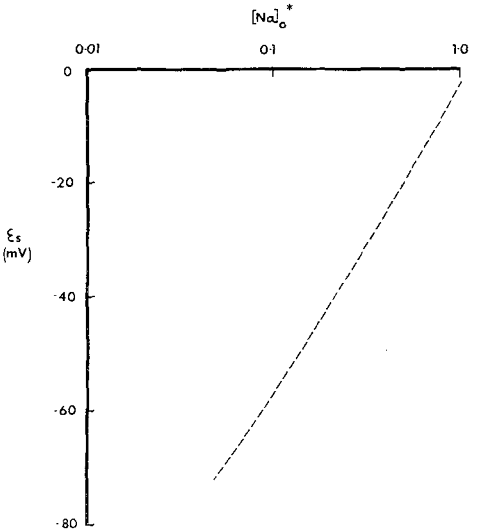

Fig. 7. The null potential predicted as a function of the external sodium concentration $[Na]_o$* relative to its value of 120 mM in normal Na-solution (*see* Table 5) for the three models. The parameters used were the same as for Fig. 6. As is implicit in this figure, the predictions of the three models were identical. This only arises in the case of a zero anion permeability

seen as already predicted in Table 4 that as $[Na]_o$* is reduced the relative conductance actually increases. This is because as $[Na]_o$* decreases relative to $[K]_o$ the more mobile K^+ ions are able to replace the Na^+ ions within the channel. On the other hand, the relative conductance predicted by the neutral model decreases as $[Na]_o$ decreases. As indicated in the figure, the significant conductance at zero sodium concentration arises because of the assumed presence (*see* Table 5) of 2.5 mM K^+ in all solutions. Interestingly, though not surprisingly, the Constant Field Equation also predicts a decrease in conductance with a decrease in $[Na]_o$.

The neutral, charged, and Constant Field models predict the same value of null potential at each NaCl concentration, and these values are shown in Fig. 7 for all models as a function of Na concentration. It can be seen that the null potential becomes very large and negative as $[Na]_o$ decreases significantly. It approaches a value of −103.6 mV at zero external sodium concentration, slightly more negative than the K^+ equilibrium potential of −102 mV. This occurs because of the assumed presence of 10 mM sodium inside the cell even at zero external sodium concentration.

Discussion

In this paper we have analyzed the applicability of models of ion permeation at the end-plate using measurements of channel conductance obtained in the presence of the four alkali cations: potassium, caesium, sodium, and lithium (Gage &

Van Helden, 1979). The measurements, based on the analysis of electrical noise resulting from the iontophoresis of acetylcholine, provided conductance-voltage data for the four cations at 8°C and for caesium, sodium, and lithium at 20°C. It was found that as the membrane was depolarized the single channel conductance increased in both Na- and Li-solutions, the effect being very much greater in Li-solution. In contrast, in Cs-solution single channel conductance appeared to decrease slightly with membrane depolarization. In this paper, the effect of NaCl concentration on single channel conductance has also been examined.

Each set of data was analyzed in terms of the three models: a neutral (polar) site channel model (neutral model), a charged site channel model (charged model), and the Constant Field Equation. In the first two cases the models were derived (*see* Appendices *A* and *B* for the neutral and charged models, respectively) to allow for three cations (and an anion in the case of the neutral model). In all three models the expression for the membrane potential (by virtue of assuming negligible anion permeation) was identical and both the relative partition coefficients or equilibrium constants (K_{Na}/K_K and K_{Li}/K_K) and the relative mobilities (u_{Na}/u_K) and (u_{Li}/u_K) only appeared together as permeabilities [e.g., $P_{Na}/P_K \equiv (K_{Na}/K_K)\cdot(u_{Na}/u_K)$]. In contrast, both the neutral and charged models take into account competition between cations for sites and so allow for some independence and separation between equilibrium constants and ionic mobilities with regard to their predictions of single-channel conductances.

From the values of the null potentials, relative ionic permeabilities were calculated and these were the same for each model. Incorporating these values of permeability, the three models were initially used to try to explain the voltage-dependence of channel conductance. The results could not be explained by the Constant Field model. In contrast both the neutral and charged models were able to explain the voltage dependence of conductance and both models yielded identical equilibrium constants and relative mobilities (Figs. 2–4 and Table 2). Because of the low voltage-sensitivity of conductance and the size of the experimental errors, the data obtained in Cs-solution did not adequately distinguish between any of the three models. The sequences of equilibrium constants for the neutral and charged models were $K_{Li}/K_{Na}/K_K/K_{Cs} = 7 \pm 1{:}1.7 \pm 0.2{:}1{:}0.9 \pm 0.2$ at 8°C and $7 \pm 2{:}1.7 \pm 0.2{:}1{:}1.0 \pm 0.5$ at 20°C. Correspondingly the sequence of mobilities was reversed, being $u_{Cs}/u_K/u_{Na}/u_{Li} = 1.4 \pm 0.4{:}1{:}0.58 \pm 0.08{:}0.13 \pm 0.02$ at 8°C and $1.2 \pm 0.8{:}1{:}0.64 \pm 0.1{:}0.14 \pm 0.05$ at 20°C. A somewhat similar binding constant sequence was obtained by Neher, Sandblom and Eisenman (1978) for the gramicidin A channel. This was $K_{Li}/K_{Na}/K_K/K_{Cs} = 5.9{:}2.9{:}1.48{:}0.83$ as given by K^h in Table 2 of their paper.

The inverse relationship between ionic mobility and equilibrium constant has been measured in ion-selective glasses, where it is relatively easy to measure the two parameters independently. Eisenmann (1965) found, for example, that for one potassium selective glass, $K_K/K_{Na} \simeq 34$ with u_K/u_{Na} in the glass $\simeq 0.3$. In comparison he found in a different glass values of K_K/K_{Na} and u_K/u_{Na} of 55 and 0.15, respectively. Since a glass is a relatively open system, it does not seem unreasonable that in a more sterically restricted biological channel there should be an even greater proportional reduction in the relative mobility of the cation, which has the greater affinity for the membrane site.

The qualitative physical explanation for the change in conductance with membrane potential is as follows. The conductance of a channel is mainly due to cations. The predominant species of cation in the channel depends on the concentration of each ion in the adjacent solutions and on the membrane potential. Consider, for example, the results of experiments in Li-solution. Outside the cell the predominant ion is Li^+ and inside it is K^+. For large depolarizing potentials the sites inside the channel will tend to be occupied more by K^+ than by Li^+ and the membrane current will be carried predominantly by K^+. Since K^+ ions have a higher mobility (being less selected by the membrane sites than Li^+), the membrane conductance will be higher at more depolarized potentials. Alternatively, for large hyperpolarizing potentials the sites inside the channel will tend to be occupied by Li which will then be carrying the current. Since Li^+ ions have a lower mobility (being more selected by the membrane than K^+), the membrane conductance will be lower than at more depolarized potentials. It can be readily seen by referring to the equations for either the neutral or charged models that in symmetrical salt solutions the conductance becomes independent of membrane potential.

A further test of the applicability of each of the three models involved using the values of permeabilities and equilibrium constants, obtained from null potentials and conductance-voltage curves, to predict the conductances in both Li- and Cs-solution relative to the conductance in Na-solution (measured at the same potential). It immediately became clear that, as expected, the Constant Field Equation was completely unable to predict the LiCl results. In contrast, the neutral model fitted the conductance data for both solutions at both temperatures. The charged model was only marginally worse in fitting the data at 20°C.

The critical experiment to distinguish between the two models involved halving $[NaCl]_o$ and comparing single channel conductance with that in normal $[NaCl]_0$. As may be noted (*see* Table 4), there was a drop in conductance as predicted by the neutral model and not the slight increase in conductance predicted by the charged model. The increase in conductance in the latter case would be expected because of the relative increase in the number of the more mobile K^+ ions in the channel as $[Na]_0$ is decreased, the total number of cations in the channel tending to remain constant. The predictions for the two models rely on the assumption that the permeation parameters (K_{Na}/K_K and u_{Na}/u_K) remained constant as the concentration of NaCl decreased. As noted earlier however, the measured null potential of -12 mV was considerably less than predicted. As suggested earlier also, there were two possible explanations for this result, both resulting from the radical decrease in the ionic strength of the extracellular solution. In one it was suggested that the decrease in ionic strength within the channel may have caused an increase in the site strength of the negative groups in the membrane, because of a decrease in their shielding by the ions. This would be expected to cause an increase in the binding of the smaller Na^+ ion and a corresponding decrease in its mobility through the membrane. An increase in K_{Na}/K_K from 1.7 to 3.0 and a decrease in u_{Na}/u_K from 0.64 to 0.5 gave (using the neutral model) both a drop in conductance and a more positive null potential similar to the values measured experimentally. In the other, it was suggested that the decrease in ionic strength of the extracellular solution would have increased the negative charge on the membrane surface. This would then tend to increase the local concentration of Na^+ ions and decrease the local concentration of Cl^- ions adjacent to the membrane. As shown earlier, this could also result in null potential and conductance values similar to those measured experimentally.

Predictions of changes in conductance for an extended range of decreasing concentrations (assuming constant permeation parameters and hence constant ionic strength) suggest for the neutral model a somewhat linear decrease in conductance, until a limiting conductance is reached because of the presence of K^+ ions. This contrasts with the charged model which predicts a slight continued increase in conductance as the concentration is decreased. As far as the null potential is concerned, all three models predict (provided the permeation parameters and local concentrations remain constant) that the null potential would continue to become more negative as $[Na]_o$ decreases, until the potassium equilibrium potential is reached.

The neutral model, as considered in this paper, was derived using the assumption that the ionic concentrations within the channel were very much less than the concentration of polar sites. If this assumption is dropped and saturation of the sites is allowed then preliminary calculations indicate that, as expected, the single channel conductance will tend to saturate if the external salt concentration becomes high enough. The drop in conductance seen experimentally is consistent with this assumption over the range of external salt concentrations considered in this paper.

It would appear that the neutral site channel model is the most appropriate model of the three for the end-plate channel. As already suggested such a channel would have negative polar groups lining it. A possible physical picture has been presented in Fig. 1. In general terms, all that is needed is a channel into which anions follow cations in order to preserve macroscopic electroneutrality. The negative polar groups would introduce selectivity for the cations, and some or all of them could introduce relatively low mobility for the anions. The conductance of the channel would then essentially depend only on the movement of cations through the channel.

It is possible, however, that a more complicated, composite model could equally well fit the data. There may be regions of neutral sites and regions of charged sites, or there may be uncharged regions and charged regions (with the charged regions being "short" in comparison with the "effective Debye length" in the channel) as has been suggested for ion permeation across "tight junctions" of the gall-bladder (Moreno & Diamond, 1975).

It should be noted that the equilibrium constant sequence corresponds to Eisenman sequence XI (Eisenman, 1965) which depends on very much higher field strength sites than would be apparent by considering the overall permeability sequence alone (i.e., $P_{Cs} > P_K > P_{Na} > P_{Li}$ at 8°C) which is that of sequence I, the lowest site strength sequence. The low temperature dependence of the voltage sensitivity of conductance would be predicted from the neutral model.

The neutral model does not, by itself, predict anything about the lifetimes of end-plate channels. If, however, there is some reciprocal relationship between channel lifetime and channel conductance (Gage & Van Helden, 1979), then by virtue of such a relationship the different channel lifetimes in the different alkali cation solutions may simply be reflecting changes in conductance occurring in a neutral channel.

Finally, although the simple neutral model provides an adequate fit to the experimental data available, it may well be necessary to take more account of other factors such as surface fixed charge or short charged regions within the channel if new results cannot be explained by the model. It may then be necessary to use a rate theory approach.

We would like to thank Dr. R. H. Adrian for helpful discussions. The technical assistance of Miss G. P. Dixon and Mrs. C. M. Prescott was also very much appreciated. The work was supported by grants from the Australian Research Grants Committee and the National Health and Medical Research Council.

Appendix A

Ion Permeation through a Long Neutral Site Channel

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This outline derivation follows that of Barry and Diamond (1971). The membrane will be considered as lying in a plane perpendicular to the x axis and extending from the outside interface of the membrane where $x = 0$ to the inside interface where $x = d$. a_i, c_i, u_i, K_i and J_i refer, respectively, to the activity, concentration, mobility, partition coefficient, and flux of ion i. Superscripts $'$ and $''$ refer to the outside and inside solutions, respectively. The standard state chemical potentials of the ith ion in water and in the membrane are written as $\mu_i^o(w)$ and $\mu_i^o(m)$, respectively. The other parameters have their usual significance and have been defined in the text. The case for three univalent cations symbolized by subscripts 1, 2 and 4 and one univalent anion symbolized by subscript 3 (subscript 3 was chosen for the anion to bring the equations in this Appendix into line with those of Barry and Diamond, 1971) is considered.

The two principal assumptions are as follows: (i) That the only sites within the membrane are fixed "neutral" (lacking net charge) negative polar groups, with a site concentration significantly higher than the concentration of ions within the channel. (ii) That the channel length is long in comparison with the Debye length for ions within it so that macroscopic electroneutrality can be assumed within it. Since the channel lacks net charge, this implies that the concentration of mobile cations is balanced by an equal concentration of mobile anions.

The other assumptions are as follows: (iii) That the solutions adjacent to the membrane are perfectly stirred so that unstirred layer effects may be neglected. This is a reasonable assumption for short times, before effects such as transport number effects become important (e.g., Barry & Adrian, 1973). (iv) that the generalized Guggenheim assumption applies, i.e., that at each point in the solution all the ions have equal activity coefficients. As in Barry and Diamond, 1971, and Conti and Eisenman (1965), initially the cooperativity relationship will be assumed so that within the membrane

$$a_i = \gamma c_i^n. \tag{A1}$$

(v) μ_0, γ and u for each are assumed to be constant throughout the channel. (vi) The electrochemical potential, μ, is assumed to be continuous across the membrane solution interface, i.e., there is a negligible interfacial resistance.

If Ψ represents the potential at any point then the total membrane potential ϵ is given by

$$\epsilon = \Psi'' - \Psi' = \Psi'' - \Psi(d) + \Psi(d) - \Psi(0) + \Psi(0) - \Psi' \tag{A2}$$

where $\Psi'' - \Psi(d)$ and $\Psi(0) - \Psi'$ represent the two boundary potentials at the two membrane solution interfaces and $\Psi(d) - \Psi(0)$ represents the diffusion potential along the length of the channel.

Across each boundary (e.g., at the $x = 0$ interface) we have for the ith ion

$$\mu^o_i(w) + RT \ln (a_i') + z_iF\Psi' = \mu_i^o(\mathrm{m}) + RT \ln a_i(0) + z_iF\Psi(0) \tag{A3}$$

where R, T and F have their usual significance and where z_i represents the valency. Rearranging Eq. (A3) and using Eq. (A1), we obtain

$$c_i(0) = (K_ia_i'/\gamma)^{1/n}\cdot \theta' \tag{A4a}$$

for $i = 1$, 2, and 4, and

$$c_3(0) = (K_3\, a_3'/\gamma)^{1/n}/\theta' \tag{A4b}$$

where θ' is defined by:

$$\theta' \equiv \exp\,[-z_iF(\Psi(0) - \Psi')/nRT] \tag{A5}$$

and where the potential-independent partition coefficient (ion-site binding constant) K_i is defined by:

$$K_i = \exp\,[(\mu_i^o(w) - \mu_i^o(m))/nRT]. \tag{A6}$$

By writing down the full set of equations for the four ions and using the electroneutrality condition that

$$c_1(0) + c_2(0) + c_4(0) = c_3(0) \tag{A7}$$

it can be shown that

$$\theta' = [(K_3a_3')^{1/n}/([K_1a_1']^{1/n} + [K_2a_2']^{1/n} + [K_4a_4']^{1/n})]^{1/2}. \tag{A8}$$

Expressions identical to Eqs. (A3) – (A8) also apply for the inside membrane interface for $c_1(d)$, $c_2(d)$, $c_3(d)$ and $c_4(d)$ except that θ', a_1', and a_4' are replaced by θ'', a_1'', a_2'', a_3'' and a_4'', respectively, where

$$\theta'' = [(K_3a_3'')^{1/n}/(K_1a_1'']^{1/n} + [K_2a_2'']^{1/n} + [K_4a_4'']^{1/n})]^{1/2}. \tag{A9}$$

This completes the solution of the boundary conditions in terms of partition coefficients and bathing solution composition. Substituting Eqs. (A8) and (A9) (making use of Eq. (A5) and an equivalent equation for θ'') into Eq. (A2) gives

$$\epsilon = \Psi(d) - \Psi(0) + \frac{nRT}{F} \ln [\theta''/\theta']. \tag{A10}$$

Assuming the validity of the Nernst-Planck flux equations, the flux (positive inwards), $J_i(x)$, of the ith ion at any point x within the channel is given by

$$J_i(x) = -u_i nRT \frac{dc_i(x)}{dx} - u_i z_i c_i(x) F \frac{d\Psi(x)}{dx} \tag{A11}$$

since $d\gamma/dx = 0$ (see assumption *v*). Since at any point in the solution (assumption *ii*)

$$c_1(x) + c_2(x) + c_4(x) = c_3(x) \tag{A12}$$

following Barry and Diamond (1971), these equations may be solved for the four ions. It follows that the concentration profile for anions in the membrane is linear so that

$$c_3(x) = c_3(0) - x[c_3(0) - c_3(d)]/d \tag{A13}$$

and it can readily be shown that the potential gradient is not constant in the membrane and, in fact, that the potential varies logarithmically along the channel. The full set of equations can then be solved in the same manner as that employed by Planck in deriving his expression for the liquid junction potential (*see* MacInnes, 1961, p. 462). Defining

$$U(x) = u_1c_1(x) + u_2c_2(x) + u_4c_4(x) \tag{A14}$$

$$V(x) = u_3c_3(x) \tag{A15}$$

$$\zeta \equiv \exp[\{\Psi(d) - \Psi(0)\} F/nRT] \tag{A16}$$

and

$$\xi = \exp(\epsilon F/nRT). \tag{A17}$$

The general expression for the current, I, (defining an inward current as negative) can be shown to be given by

$$I = \frac{RTF}{d} \frac{[c_3(0) - c_3(d)]}{\ln [c_3(d)/c_3(0)]} \left[\left(\frac{\zeta U(d) - U(0)}{\zeta c_3(d) - c_3(0)}\right)\left(\ln\zeta + \ln\left\{\frac{c_3(d)}{c_3(0)}\right\}\right) - \left(\frac{V(d) - \zeta V(0)}{c_3(d) - \zeta c_3(0)}\right)\left(\ln\left\{\frac{c_3(d)}{c_3(0)}\right\} - \ln\zeta\right)\right]. \tag{A18}$$

This is identical to Eq. (35) of Barry and Diamond (1971) with $U(x)$ as defined by Eq. (A14). From Eqs. (A16), (A17) and (A10)

$$\ln \xi = \ln \zeta + \ln [\theta''/\theta'] \tag{A19}$$

or

$$\xi = \frac{\theta''}{\theta'}\zeta. \tag{A20}$$

Substituting for ξ from Eqs. (A19) and (A20) together with Eqs. (A4a) and (A4b) together with the equivalent set of Eqs. for $c_1(d)$, $c_2(d)$, $c_3(d)$, and $c_4(d)$ it can then be shown, allowing for an effective channel permeation area A_f, *that*

$$I = B_1 \left\{ \left[\frac{\xi U'' - U'}{\xi C'' - C'} \right] [\epsilon - \epsilon^*] + u_3 [\epsilon - \epsilon_3] \right\} \tag{A21}$$

where

$$B_1 = \frac{F^2}{nd}\left(\frac{K_3}{\gamma}\right)^{1/n} A_f \left[\frac{(a_3')^{1/n}}{\theta'} - \frac{(a_3'')^{1/n}}{\theta''}\right] \Big/ \ln\left(\frac{(a_3'')^{1/n}\cdot\theta'}{(a_3')^{1/n}\cdot\theta''}\right) \tag{A22}$$

with

$$U' = u_1(K_1a_1')^{1/n} + u_2(K_2a_2')^{1/n} + u_4(K_4a_4')^{1/n} \tag{A23}$$

$$U'' = u_1(K_1a_1'')^{1/n} + u_2(K_2a_2'')^{1/n} + u_4(K_4a_4'')^{1/n} \tag{A24}$$

$$C' = (K_1a_1')^{1/n} + (K_2a_2')^{1/n} + (K_4a_4')^{1/n} \tag{A25}$$

$$C'' = (K_1a_1'')^{1/n} + (K_2a_2'')^{1/n} + (K_4a_4'')^{1/n} \tag{A26}$$

$$\epsilon^* = \frac{nRT}{F}\ln [C'/C''] \tag{A27}$$

$$\epsilon_3 = -\frac{RT}{F}\ln\left(\frac{a_3'}{a_3''}\right) \tag{A28}$$

and θ', θ'' and ξ are given by Eqs. (A8), (A9) and (A17), respectively.

The null potential, ϵ_o, is given in the case of zero anion permeation by

$$\epsilon_0 = \frac{nRT}{F}\ln\left[\frac{u_1[K_1a_1']^{1/n} + u_2[K_2a_2']^{1/n} + u_4[K_4a_4']^{1/n}}{u_1[K_1a_1'']^{1/n} + u_2[K_2a_2'']^{1/n} + u_4[K_4a_4'']^{1/n}}\cdot\right] \tag{A29}$$

It should be noted that Eq. (A28) is independent of the value of n.

In the more ideal case, assuming no cooperativity effects (n=1) Eqs. (A21)–(A28), reduce to Eqs. (1)–(8) and Eq. (A29) reduces to Eq. (13).

Appendix B

Ion Permeation through a Long Charged Site Channel

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This outline follows that of Conti and Eisenman (1965), extending their treatment to allow for a third cation which, as in *Appendix A,* will be denoted as ion 4. The assumptions are basically the same as for the neutral model in *Appendix A,* except that now assumption *i* states that the only sites within the channel are fixed charged (having net charge) negative sites. Assumption *ii* is the same as before except that now there are only cations in the channel and their concentration balances the concentration of charged groups. Assumption *iv* will be in principle the same as before and again the nonideal cooperativity factor (considered to be constant throughout the channel) will be included so that

$$a_i = \gamma c_i^n. \tag{B1}$$

In addition, equilibrium constants K_{ij} for ions i and j will be defined as

$$\frac{a_i^m}{a_j^m} = K_{ij}\frac{a_i^w}{a_j^w} \tag{B2}$$

where superscripts m and w refer to the membrane and aqueous phases respectively and K_{ij} is algebraically equivalent to the ratio of partition coefficients K_i/K_j defined for the neutral model.

The derivation of Conti and Eisenman allowed for the possibility of a nonuniform spacing of sites so that the concentration of fixed negative charges or sites, C_o, the standard-state chemical potential in the membrane, μ^o, and the activity coefficient, γ, were all functions of x. Extending their treatment to apply to 3 cations considerably complicates the analysis. Assuming a uniform spacing of sites, however, enables a straightforward analysis to be undertaken. It was therefore assumed that C_o, μ^o and γ were all constant and independent of x. The equilibrium constants were also considered to be the same at each interface.

The other definitions and symbols are the same as in *Appendix A.* At any point within the membrane channel:

$$c_1(x) + c_2(x) + c_4(x) = C_o \tag{B3}$$

since

$$\frac{a_2(0)}{a_1(0)} = \frac{c_2(0)^n}{c_1(0)^n} = K_{21}\left(\frac{a_2'}{a_1'}\right) \tag{B4a}$$

and

$$\frac{a_2(d)}{a_1(d)} = \frac{c_2(d)^n}{c_1(d)^n} = K_{21}\left(\frac{a_2''}{a_1''}\right) \tag{B4b}$$

then the mole fractions of the three ions are given at $x = 0$ (just within the channel) by

$$X_1(0) \equiv c_1(0)/C_o = (a_1')^{1/n}/C' \tag{B5}$$

$$X_2(0) \equiv c_2(0)/C_o = (K_{21}a_2')^{1/n}/C' \tag{B6}$$

and

$$X_4(0) \equiv c_4(0)/C_o = (K_{41}a_4')^{1/n}/C' \tag{B7}$$

where

$$C' \equiv (a_1')^{1/n} + (K_{21}a_2')^{1/n} + (K_{41}a_4')^{1/n} \tag{B8}$$

similarly at $x = d$ (just within the channel) a similar set of equations may be given for the three mole fractions $X_1(d)$, $X_2(d)$ and $X_4(d)$ as

$$X_1(d) \equiv c_1(d)/C_o = (a_1'')^{1/n}/C'' \tag{B9}$$

$$X_2(d) \equiv c_2(d)/C_o = (K_{21}a_2'')^{1/n}/C'' \tag{B10}$$

$$X_4(d) \equiv c_4(d)/C_o = (K_{41}a_4'')^{1/n}/C'' \tag{B11}$$

where

$$C'' \equiv (a_1'')^{1/n} + (K_{21}a_2'')^{1/n} + (K_{41}a_4'')^{1/n}. \tag{B12}$$

Writing down the three Nernst-Planck flux equations, dividing each by the ionic mobility, adding the two resulting equations for ions 2 and 4, and remembering Eq. (B3) we obtain

$$\frac{J_1}{u_1} = -nRT\frac{dc_1}{dx} - c_1 \cdot zF\frac{d\Psi}{dx} \tag{B13}$$

and

$$\left(\frac{J_2}{u_2} + \frac{J_4}{u_4}\right) = -nRT\frac{d}{dx}(C_o - c_1) - (C_o - c_1)\,zF\frac{d\Psi}{dx} \tag{B14}$$

where $c_1 = c_1(x)$ the channel concentration of cation 1, where Ψ is the potential and J_1, J_2 and J_4 are the fluxes of ions 1, 2 and 4, respectively. Multiplying Eq. (B13) by $(C_o - c_1)$ and Eq. (B14) by C_o, subtracting and integrating the resultant equation (remembering that $X_1(x) = c_1(x)/C_o$), it may be shown that

$$\ln\left[\frac{\dfrac{J_1}{u_1} - J^+X_1(x)}{\dfrac{J_1}{u_1} - J^+X_1(0)}\right] = \frac{J^+x}{nRT\,C_o} \tag{B15}$$

where

$$J^+ \equiv \frac{J_1}{u_1} + \frac{J_2}{u_2} + \frac{J_4}{u_4} \tag{B16}$$

to define $X_1(x)$ as a function of x. Now multiplying Eq. (B13) by $(J_2/u_2 + J_4/u_4)$ and Eq. (B14) by J_1/u_1, subtracting and integrating the resultant equation, the relationship between $\Psi(x)$ and $X_1(x)$ may be shown to be given by

$$zF(\Psi(x) - \Psi(0)) = -nRT \ln \left[\frac{\dfrac{J_1}{u_1} - J^+X_1(x)}{\dfrac{J_1}{u_1} - J^+X_1(0)} \right] \tag{B17}$$

substituting for Eq. (15) in Eq. (B17) results in

$$\Psi(x) - \Psi(0) = -\frac{J^+}{zFC_o}x. \tag{B18}$$

This implies that within the membrane (between the boundary potentials at each interface) the electric field is constant since J^+ and C_o are constant. This is the same result as given by Conti and Eisenman (1965) for two ions and only occurs as a result of the sites being constant and uniformly distributed.

Substituting $\Delta\Psi/d$ for $d\Psi/dx$ in the Nernst-Planck flux equations, where $\Delta\Psi \equiv \Psi(d) - \Psi(0)$ and d is the membrane thickness, each equation can be integrated separately within the membrane as in the Constant Field derivation to give, for example, for cation 1:

$$J_1 = -\frac{u_1zF\Delta\Psi}{d}\frac{[c_1(0) - c_1(d)e^{zF\Delta\Psi/nRT}]}{[1 - e^{zF\Delta\Psi/nRT}]}. \tag{B19}$$

Similar expressions apply for J_2 and J_4 so that the total current, I, is given by (defining an inward current as negative):

$$\text{i.e.} \qquad I = -zF(J_1 + J_2 + J_4) \tag{B20}$$

$$I = \frac{z^2F^2\Delta\Psi}{d}\left\{\frac{[u_1c_1(d) + u_2c_2(d) + u_4c_4(d)]e^{zF\Delta\Psi/nRT} - [u_1c_1(0) + u_2c_2(0) + u_4c_4(0)]}{[e^{zF\Delta\Psi/nRT} - 1]}\right\}. \tag{B21}$$

In order to re-express Eq. (B21) in terms of the potential difference right across the membrane $(\Psi'' - \Psi')$ and the activities in the external solutions, the conditions for the continuity of the electro-chemical potential across the interfaces at $x = 0$ and $x = d$ must be used. Subtracting the two continuity equations results in

$$\Delta\Psi = \Psi(d) - \Psi(0) = \epsilon + \frac{nRT}{zF}\ln\left[\frac{(a_1'')^{1/n}/c_1(d)}{(a_1')^{1/n}/c_1(0)}\right] \tag{B22}$$

where

$$\epsilon = \Psi'' - \Psi'. \tag{B23}$$

Substituting for $\Delta\Psi$ from Eqs. (B22) and (B23) and using Eqs. (B5)-(B12), Eq.

(B21) becomes:

$$I = B_2\left[\frac{\xi U'' - U'}{\xi C'' - C'}\right](\epsilon - \epsilon^*) \tag{B24}$$

where

$$B_2 = \frac{z^2F^2C_o}{d} \cdot A_f \tag{B25}$$

$$\xi \equiv \exp\,(zF\epsilon/nRT) \tag{B26}$$

$$C' \equiv (a_1')^{1/n} + (K_{21}a_2')^{1/n} + (K_{41}a_4')^{1/n} \tag{B27}$$

$$C'' \equiv (a_1'')^{1/n} + (K_{21}a_2'')^{1/n} + (K_{41}a_4'')^{1/n} \tag{B28}$$

$$U' \equiv u_1(a_1')^{1/n} + u_2(K_{21}a_2')^{1/n} + u_4(K_{41}a_4')^{1/n} \tag{B29}$$

$$U'' \equiv u_1(a_1'')^{1/n} + u_2(K_{21}a_2'')^{1/n} + u_4(K_{41}a_4'')^{1/n} \tag{B30}$$

$$\epsilon^* \equiv \frac{nRT}{zF}\ln\,[C'/C''] \tag{B31}$$

and where A_f represents the effective membrane area per cm^2 available for permeation. Although it was deemed necessary to solve these equations using constant sites with a uniform distribution, if $a_4' = a_4'' = 0$, the equations may be shown to be equivalent to Eq. (71) of Conti and Eisenman (1965), an equation derived without any requirement for constant and uniformly distributed sites. It should be noted that Eq. (B24) is identical to Eq. (A21) in *Appendix A,* with zero anion permeation ($u_3 = 0$), and with B_2 (independent of external concentration and only dependent on site concentration) replacing B_1 in that equation. This implies that both of these equations will predict the same voltage sensitivity but will differ concerning potential-concentration predictions and predictions of conductance in solutions of different salts.

The zero current potential, ϵ_0, is given by

$$\epsilon_0 = \frac{nRT}{zF}\ln\left[\frac{u_1(a_1')^{1/n} + u_2(K_{21}a_2')^{1/n} + u_4(K_{41}a_4')^{1/n}}{u_1(a_1'')^{1/n} + u_2(K_{21}a_2'')^{1/n} + u_4(K_{41}a_4'')^{1/n}}\right] \tag{B32}$$

which is identical to Eq. (A29) for the neutral model if $z = 1$ and K_{21} and K_{41} are replaced by K_2/K_1 and K_4/K_1.

Setting $n = 1$ for the more ideal case with no cooperativity effects, Eqs. (B24)-(B32) reduce to Eqs. (14)-(21) and (23) in the text.

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