Ion Flow in the Bath and Flux Interactions Between Channels

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ABSTRACT   We present an exact solution to the linearized Nernst-Planck-Poisson equation for spherically symmetric current flow. This solution differs from Levitt's solution (Levitt, D. G. 1992. *Biophys. J.*, Eq. A5) by its dependence on an additional parameter, which is equal to the net ion flux for monovalent ion-selective channels. For ion-selective channels, this solution may provide better boundary conditions to modelling the flow in the channel pore itself, although only at low salt concentrations. We use the solution to estimate the effects of flux interaction between closely packed channels.

INTRODUCTION

Modelling the flow of ions through a channel naturally decomposes into two parts:

1) modelling the flow of ions through the pore itself. One approach is through a combination of the Nernst-Planck and Poisson equations (see e.g., Levitt (1991); Chen and Eisenberg (1993)). Another approach is through the Eyring formalism (see Heinemann and Sigworth (1990); Frehland (1978)).

2) modelling the flow of ions in the bath. This is the source of the access resistance (Hille, 1993; Hall, 1975). In the Nernst-Planck approach, the ion flow in the bath provides boundary conditions for the more interesting problem of ion flow in the channel. Levitt (1991) has pointed out that it is essential to have an accurate model of the bath, since the influence of the boundary conditions on the solution to the equations of the flow inside the pore is not yet understood.

In the first part of this work, we present an extension of Levitt's solution to ion flow in the bath (Levitt, 1991, Appendix I). The new feature of our solution is an additional dependence of the access resistance on the ion fluxes.

The above problem leads naturally to another one, namely: What is the access resistance of several closely packed channels (Neumcke, 1975; Laüger, 1976)? An open channel can influence the flux through a neighboring open channel as the access pathways are partly shared. The current through several open channels would not, in this scenario, be an integral multiple of the current through a single open channel. In a previous paper on multichannel recordings of gap junction channels from the earthworm septum (Ramanan and Brink, 1993), we have presented evidence for such nonintegral conductances. Here, we use our model of ion flow in the bath for one channel together with a simple linear flux interaction

hypothesis to estimate the magnitude of diffusion limitations on the conductance of several open packed channels.

RESULTS

Ion flow in the bath

Levitt (1991, Appendix I) has presented a solution to the combined Nernst-Planck and Poisson equations in the bath under certain approximations (see also Laüger (1976)). Here we offer an extension of Levitt's solution. Specifically, we have linearized the combined Poisson and Nernst-Planck equations in accordance with the Debye-Hückel prescription. We present an exact solution to the linearized equation.

It may be noted that the time constant for relaxation to the steady state current after a transition from open to closed conformation is ~0.1 ns (Neumcke, 1975; Laüger, 1976). Here we are interested only in the steady state. We adopt the following model for flow in the baths:

1) The Nernst-Planck equation is:

$$\frac{J_i}{2\pi r^2 D_i} = \frac{dC_i'}{dr} + z_i C_i' \frac{dV'}{dr}. \tag{1}$$

Here the superscript $'$ refers to the bath on the side where the potential is applied, and a similar equation can be written to the bath where the reference electrode is placed, referred to by the superscript $''$. J_i is the flux of the ith ion species (ions/s), with valence z_i and present in concentration C_i (ions/cm^3), with a diffusion coefficient D_i (cm^2/s). V is the potential in dimensionless units of RT/F. Note that V and C_i are dependent (only) on r. In writing Eq. 1, we are thus assuming that the flux is radially directed in the bath. Although convenient for analytical manipulations, this is clearly only a approximation for any real channel where, close to the channel mouth, the pure radial dependence of the potential (and hence the concentration profiles) would be destroyed by image forces from the dielectric and also by any (radially assymetric) fixed charges in the channel. The specific assumptions underlying the radial dependence embodied in Eq. 1 are thus the same as those enumerated in Appendix I of Levitt (1991) (see also

the discussion after Eq. 4 below). Given these assumptions, we write

$$V_i' = \phi_i' + f, \tag{2}$$

where ϕ_i' is the potential at $r = \infty$, i.e., the applied potential, we get upon applying the standard integrating factor,

$$C_i' = C_i'^\infty e^{-z_i f} + \frac{J_i}{2\pi D_i} e^{-z_i f} \int_\infty^r \frac{d\bar{r}}{\bar{r}^2} e^{z_i f}. \tag{3}$$

2) Poisson's equation is:

$$\frac{1}{r^2}\frac{d}{dr}\left[r^2\frac{df}{dr}\right] = -\frac{4\pi F}{\epsilon RT}\rho = -\frac{4\pi e^2}{\epsilon kT}\sum z_i C_i' = -\chi^2\sum z_i C_i' \tag{4}$$

where $\chi^2 = 4\pi e^2/\epsilon kT$. Here, as in Eq. 1, we are assuming that the charge distribution is continuous, and ignoring the effects of finite ion sizes and ion solvation.

Combining the two Eqs. 3 and 4 leads to a nonlinear integro-differential equation for f. Following the Debye-Hückel prescription, we linearize this equation by expanding all the exponentials to first order in f, i.e., $e^{\pm z f} = 1 \pm z f$. This process of linearizing the exponential is a fundamental dilemma in the theory of electrolytes, see e.g., Robinson and Stokes (1968). For this approximation to hold, nevertheless, f must remain "small" up to the mouth of the channel. Although we do not know if this "smallness" criterion is satisfied beforehand, we can verify that it holds at the end of the calculation. We apply the model only in cases where $f \leq 0.3$ ($\simeq 8$ mV). For monovalent ions, the solution is more accurate as the second order correction itself vanishes. As stated above, even for small f, the effects of finite ion sizes and ion solvation are completely ignored in this formulation (see e.g., Bockris and Reddy (1977)). These effects limit the application of the analysis presented here at high salt concentrations, especially so for nonmonovalent electrolytes.

Define

$$\kappa'^2 = \sum_i \kappa_i'^2 = \chi^2\sum_i z_i^2 C_i'^\infty, \tag{5a}$$

and the dimensionless parameters

$$J = \sum_i J_i = \frac{\chi^2}{\kappa'}\sum_i z_i^2 J_i/2\pi D_i \tag{5b}$$

and

$$I = \frac{\chi^2}{\kappa'}\sum_i z_i J_i/2\pi D_i. \tag{5c}$$

If the diffusion coefficient D_i is the same for all ions, then I is the total current, and, for univalent ions, J is the total flux, both scaled by the factor $\chi^2/(2\pi\kappa' D)$.

The linearized equation now reads:

$$f'' + (2/r)f' = \kappa'^2 f + \kappa' I/r - \kappa' J\left[f/r + \int_\infty^r f\,d\bar{r}/\bar{r}^2\right] \tag{6}$$

where primes denote differentiation *wrt r*, and we have used $\int dy/y^2 = -1/y$. Carrying out an integration by parts reduces the term in square brackets to $\int_\infty^r f'\,d\bar{r}/\bar{r}$. Differentiating once *wrt r* yields a third order differential equation. We reduce this equation to a second-order differential equation, consistent with the fact that there are only two free boundary conditions (say, the applied potential and the electric field at the channel mouth). Changing the independent variable to the dimensionless $y = 2\,\kappa' r$, and defining

$$g = y(df/dy), \tag{7}$$

we get

$$\frac{d^2 g}{dy^2} + \left[-\frac{1}{4} + \frac{0.5J}{y} - \frac{2}{y^2}\right]g = -\frac{0.5I}{y}. \tag{8}$$

Eq. 8 has the same form as Whittaker's equation (Abramowitz and Stegun, p. 505, Eq. 13.1.31). Using Whittaker's functions for the solutions of the homogenous equation, we may apply the method of variation of parameters to find the solution of the inhomogenous equation (see, e.g., Boyce and DiPrima, p. 124). Write

$$g = c_1 M_{0.5J,1.5} + c_2 W_{0.5J,1.5}, \tag{9}$$

where $M = M_{k,\mu}$ and $W = W_{k,\mu}$ are the standard Whittaker functions (Abramowitz and Stegun, p. 505, Eqs. 13.1.32–33). Note that $c_{1,2} = c_{1,2}(y)$ are functions of y. As g satisfies Eq. 8, this sets one constraint to be satisfied between c_1 and c_2. The remaining constraint can be arbitrarily set; choose

$$c_1'M + c_2'W = 0. \tag{10}$$

Substituting Eq. 9 into Eq. 8, carrying out the necessary differentiations, and using the constraint in Eq. 10 yields

$$c_1' = -\frac{I}{2y}\frac{W}{WM' - W'M}, \quad c_2' = \frac{I}{2y}\frac{M}{WM' - W'M}.$$

Now ($WM' - W'M$) is the Wronskian. Using either Abel's identity or Eq. 13.1.22 in Abramowitz and Stegun (1972), we find the Wronskian to be $\Gamma(4)/\Gamma(2 - 0.5J)$, where Γ is the standard gamma function (Abramowitz and Stegun, p. 255, Eq. 6.1.1). Integrating for the c values gives:

$$c_1 = -0.5I\frac{\Gamma(2 - 0.5J)}{\Gamma(4)}\int_\alpha^y (dz/z)W,$$

$$c_2 = -0.5I\frac{\Gamma(2 - 0.5J)}{\Gamma(4)}\int_\beta^y (dz/z)M,$$

where α and β can be arbitrarily chosen. Changing α and β merely changes the coefficients of the homogenous solution. The solution for g reads:

$$g = AW - 0.5I\frac{\Gamma(2 - 0.5J)}{\Gamma(4)}$$
$$\cdot\left[M\int_\alpha^y (dz/z)W - W\int_\beta^y (dz/z)M\right],$$

where we have dropped the M term in the homogenous solution as it diverges as $y \to \infty$ (Abramowitz and Stegun, Eq. 13.5.1). This implies that α must be chosen such that $Mc_1 \to 0$ as $y \to \infty$. Setting α to ∞ gives the correct asymptotic behavior (use the limit $J = 0$ in the above expression, and the simplified forms for M and W at $J = 0$ given in the end of the next paragraph).

Using Eq. 7, and integrating gives the final solution for f:

$$f(y) = f_{\text{homog}} + f_{\text{inhomog}}$$

$$= A \int_\infty^y Y\,dz - 0.5I\frac{\Gamma(2 - 0.5J)}{\Gamma(4)} \tag{11}$$

$$\int_\infty^y dz\left[X(z) \int_\infty^y dz'Y(z') - Y(z) \int_1^y dz'X(z') \right],$$

where A is arbitrary, and we have set β to 1. Here X and Y are defined by $X = M_{0.5J,1.5}/y$, $Y = W_{0.5J,1.5}/y$. The constant A can be determined if the electric field at the mouth of the channel is known. The concentration of the ions $C_i(r)$ can now also be found from Eq. 3. In practice, we prefer to avoid the double integral corresponding to the inhomogenous solution in Eq. 11. In the Appendix, we derive a perturbation expansion in J of the inhomogenous solution, which is the one used in the actual calculations. This approximation is suitable for nonselective channels, such as the gap junction channel, where the net ion flux through the channel is small ($J < 0.1$). When $J = 0$, the Whittaker functions take the following form: $M = 12[\cosh(y/2) - (2/y)\sinh(y/2)]$, $W = (1 + 2/y) \exp(-y/2)$ (Abramowitz and Stegun, Eqs. 13.6.3, 13.6.21, 10.2.13, 10.2.17). The expression (Eq. 11) then reduces to Levitt's solution (1991, Appendix I, Eq. A5):

$$f = \frac{2A}{y} e^{-0.5y} - \frac{2I}{y}.$$

The interior of the channel itself is modelled by the equation (Laüger, 1976),

$$J_i = \bar{P}_i \frac{\sinh(0.5\,zu/B)}{0.5\,zu/B} \frac{0.5\,zu}{\sinh(0.5\,zu)}$$

$$\cdot \; [C_i'(R)\exp(0.5\,zu) - C_i''(R)\exp(-0.5\,zu)]. \tag{12}$$

where u is the dimensionless voltage across the channel (units of RT/F), B is the number of equi-height barriers, and $\bar{P}_i$ is the permeability of the channel to the ith ion species. In deriving Eq. 12, it is assumed that (a) ion-ion interactions in the channel can be ignored and (b) the channel can be represented as a sequence of binding sites and activation-energy barriers. Assumption a would be violated, e.g., at high salt concentrations, while assumption b would not hold, e.g., in the presence of fixed charges in the channel that cause long-range fields. A general discussion of the limitations of the reaction rate theory underlying Eq. 12 as applied to channel permeation can be found in Levitt (1982). We also tacitly assume that the binding sites or fixed charges in the channel do not have influence in the bulk solution, i.e., that Eqs. 11 and 12 which describe the bulk flow and channel flow re-

spectively, are independent of each other. For determining the constant A in Eq. 11, we assume that the electric field is constant along the length of the channel (length of the pore $+ 2 \times$ radius of mouth); this gives the field at the mouth of the channel. In a more sophisticated treatment of the channel (e.g., Levitt, 1991), the equations describing the channel interior would have the electric field at the mouth as a boundary condition. These equations, together with Eq. 11, would then self-consistently fix the field at the mouth, and thus the constant A in Eq. 11. We have chosen Eq. 12 primarily for its modelling simplicity, and also because it may be appropriate to a large poorly selective channel such as the gap junction channel. Eqs. 11 and 12 provide a complete description of the channel and its exterior; the current through the channel can be determined by iterating these equations.

We apply the results to a model of a gap junction channel. The channel parameters are typical ones for gap junction channels: radius $R = 8$ Å, length of pore $L = 100$ Å (Loewenstein, 1981). The number of equi-height barriers B is fixed to 20, to approximate a large aqueous channel (Laüger, 1976). The solutions on both sides consist of 165 mM CsCl. The diffusion coefficients are $D_{Cs} = D_{Cl} = 2.0 \times 10^{-5}$ cm^2/s. The relative permeabilities of the ions in selectivity experiments is given by $\bar{P}_{Cl} = 0.55\,\bar{P}_{Cs}$ (Brink and Fan, 1989). We have arbitrarily fixed this ratio to be the permeability ratio inside the channel. The absolute permeabilities are unknown, however the single-channel conductance is known to be ~ 100 pS. We have fixed $\bar{P}_{Cs} = 1.0 \times 10^{-13}$ cm^3/s; the resulting single-channel conductance is ≈ 102 pS. The I–V graph for the model is linear for a ± 100-mV variation in holding potential, as also seen in experiment (Brink and Fan, 1989).

We note that the anion to cation permeability ratio inside the channel which is arbitrarily chosen can actually be fixed if the single-channel conductance were known at a variety of salt concentrations. Due to the paucity of experiments at high salt concentrations, and also due to complications engendered by the numerous substates exhibited by this channel (Ramanan and Brink, 1993), we are unable to specify this ratio precisely. We only note that knowledge of this ratio, together with the single-channel conductance, would also determine the actual permeabilities of the ions inside the channel.

Fig. 1 a shows the variation of the potential and concentration of ions on the side of the channel that faces the applied potential of $+100$ mV for a single channel patch. The inset of Fig. 1 a is a demagnification of Fig. 1 a for larger distances, and Fig. 1 b shows the same data in a log-log scale. At great distances from the mouth where the concentration of the ions are equal (inset, Fig. 1, a and b), the drops in ion concentrations and the potential fall off as $1/r$ as expected for spherically symmetric current flow. Near the mouth the concentrations and the potential are primarily determined by the field inside the channel (through the integration constant A in Eq. 11).

As the Eq. 11 describing the flux in the baths is more complicated than Levitt's solution, involving as it does integrating Whittaker functions, it is useful to compare the
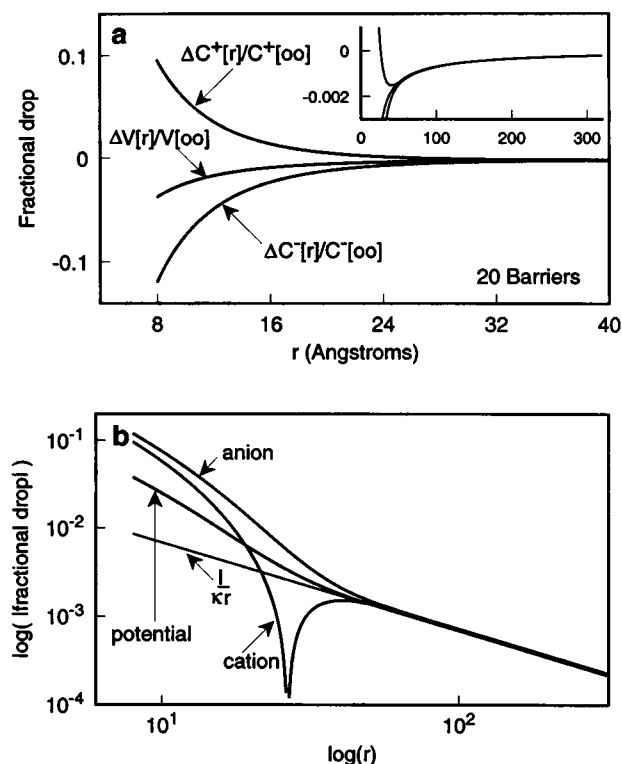
FIGURE 1   (a) Drop in potential and the concentrations of the two ions Cs^+ and Cl^- from infinity to the mouth of a model gap junction like channel held at a potential of 100 mV. The parameters for the model channel are described in the text. The inset of a is an enlargement of the main panel, 20 equi-height barriers are assumed. (b) The same data in a log-log scale. The line $I/(\kappa r)$ is the potential drop expected from a purely resistive access-resistance model. The potential drop predicted by the model agrees with the purely resistive model for distances greater than ~50 Å. At such distances, the concentration drops also fall off as $1/r$. See text for discussion.

results yielded by the two methods. The potential drop to the mouth of the channel from infinity ($f(R)$, see Eq. 2) provides a useful reference; we have computed this value for Levitt's solution and through the method above with identical parameters, and computed the percent difference δ between the two values of $f(R)$. With the parameters used in Fig. 1, $I = 0.034$ and $J = 0.0096$, and δ is ~0.3%, which is insignificant given the various approximations already involved in deriving the linearized equation. By varying the permeabilities and concentrations of the ions, we have computed δ for a range of I and J. We can summarize our results thus: In the range $J \leq I \leq 0.4$, δ is less than the quantity $100IJ$ (%). For a monovalent salt at 160 mM, $I = 0.4$ is equivalent to a current of ~180 pA. Thus Levitt's solution is within 5% of the solution presented above for concentrations of up to 500 mM of monovalent salts and for imposed potentials of up to 200 mV. For nonmonovalent salts, since the quantity J increases as the square of the valence (see Eq. 5b), δ is greater than for monovalents at a given salt concentration; it may then prove necessary to use the solution given here, or even the complete nonlinearized Eqs. 1 and 4.

At distances of 100 Å, a typical interchannel distance, the inset of Fig. 1 a shows that the concentrations of both ions are reduced from their free concentration. It is this depletion

combined with the reduction of the applied voltage by ~1% that causes the interaction between the fluxes in a closely packed multichannel patch.

Channel access resistance interaction

If two channels are close to each other, then the drops of potential and ion concentrations from infinity to the mouth of each channel will be influenced by the presence of other channels. Stated differently, the access resistances of packed multichannels will not be an integer multiple of that for a single channel (Neumcke, 1975; Laüger, 1976; Weissman, 1978). As an example, we note that gap junction channels are typically found in plaques in close proximity. It is thus possible that the nonunitary multichannel conductances observed in this channel (Ramanan and Brink, 1993) are caused by interaction of the fluxes flowing into neighboring channels. Wilders and Jongsma (1992) have used this idea to illustrate the limitations of the double patch-clamp technique. They conclude that flux interaction can lead to a large underestimate of the number of channels in a gap junction plaque. Our estimate, shown below, is a refinement of their method in that 1) it allows for charge screening, selectivity of the channel and barriers to ion transit through the channel and 2) it uses a more representative model of the bath, as presented above.

The circumstance that we are interested in is a multichannel patch, i.e., an experiment where a single patch contains a number of channels which can be simultaneously open. The membrane size and other membrane parameters are then fixed. Let m be the total number of active channels in this single patch. At a given holding potential, as we look at different times in the current trace from this patch, we will see different number of channels (up to a maximum of m) simultaneously open, as the various channels open and close. An amplitude histogram of the trace from this single patch will then have $(m+1)$ peaks, the peaks corresponding respectively to all channels closed, 1 channel open, 2 channels open, . . . , all m channels open. By reading off the peak maxima (for example), we can find the currents through a lone open channel, and also through 2, 3, . . . , m simultaneously open channels. When only one channel is open, the situation is that considered in the first section of this paper. Now consider the scenario with two channels simultaneously open. Each channel will deplete and accumulate ions in a manner similar to that described in Eqs. 1 and 4. If the channels were far apart, then the current passed by the two simultaneously open channels would simply be two times the current carried by a lone open channel. If the channel were close together, however, the fluxes carried by the two channels would interfere with each other. For a lone open channel, we derived a simplified Eq. 1 by assuming a pure radial dependence for the potential and the concentrations. With two open channels, even ideally there are no preferred radial (i.e., one-dimensional) coordinates, and the situation seems analytically intractable. We hence adopt the following strategy.

For a lone open channel, $f(R)$ represents the voltage drop (see Eq. 2) from infinity to the "mouth" of the channel. Let S be the average interchannel separation, and let n channels be simultaneously open. As noted above, n will vary as we look at different times in the whole trace from the patch, since at different times one, two, ... channels will be simultaneously open. The plan is then: set $f(R) + (n - 1)f(S)$ and $(C_i(R) - C_i'^\infty) + (n - 1)(C_i(S) - C_i'^\infty)$ to be, respectively, the voltage drop and concentration drop from infinity to the mouth of any of the n open channels. We may then iterate with Eqs. 11 and 12 to find the current through any one of the n open channels. Repeating this procedure for $n = 2, 3, \ldots, m$ yields the current through a single open channel when $(n-1)$ of its neighbors are simultaneously open.

The essence of the simplification above for treating multichannels is therefore the assumption of linearity in channel flux and field interaction, as represented algebraically by the superposition of voltage and concentration drops. An analogous situation arises in electrostatics in the calculation of the capacitance of two spheres both of radius R placed a distance S apart; a linear approximation is accurate there to third order as long as $R/S < 1/3$ (Mason and Weaver, p. 115). Yet another analogue can be found in the theory of chemoreceptor adsorption (Berg and Purcell (1977), see especially Appendix A); here again the second order effects can be ignored if R is small compared to S. A similar hypothesis was also assumed by Wildsma and Jongers (1992) in their treatment of flux interaction among gap junction channels.

A less serious approximation is that all open channels are at a distance S from one another. While this is (trivially) true for 2 open channels, and may be (nongenerically) true for 3 open channels, it cannot hold geometrically for $n > 3$, where n is the number of simultaneously open channels in a given multichannel patch. This last restriction can be removed if we know the geometrical arrangement of channels in the given patch. In the absence of such information, the parameter S represents the average interchannel separation in the given patch. Even then, the essential nonlinear dependence of Eq. 11 on the radial variable (r or y) implies that the parameter S can be regarded only as an approximate measure of the true average interchannel separation.

Fig. 2 presents the results of calculation of flux interaction between several gap junction channels. The single channel parameters are as in Fig. 1; the average interchannel separation is fixed at 100 Å (Makowski, 1988). Let g_n be the calculated conductance when n channels are simultaneously open, and define $\Delta g_n = ng_1 - g_n$. In the absence of flux interaction, Δg_n is zero for all n. In Fig. 2 a, we have plotted the relative reduction in conductance $\Delta g_n/g_n$ against $(n-1)$, where n is the number of open channels. A total of 10 active channels were assumed, and the number of barriers (the parameter B in Eq. 12) was set to 2. Note that the graph is linear at all potentials. This permits us to present our results in a condensed form based on the following observation: If there is a (pipette) resistance in series, we can show (Ramanan and Brink, 1993) that $\Delta g_n/g_n = (n - 1)/(1 + T)$, where T is the ratio of series conductance to channel con-
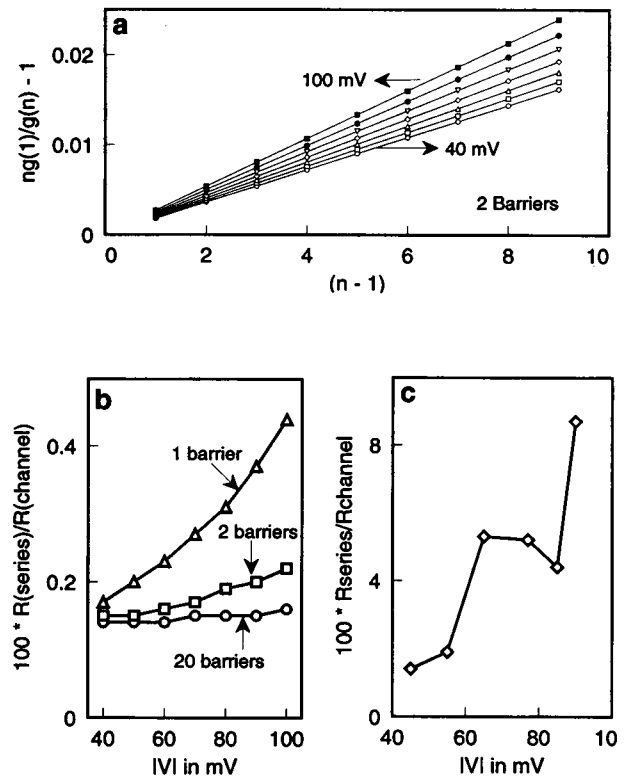

FIGURE 2 (a) Plot of the relative reduction $\Delta g_n/g_n$ in the current per open channel against $(n - 1)$, where n is the number of simultaneously open channels in a single patch. The number n of simultaneously open channels will vary as we look at different times in the trace. Panel a is for a collection of model gap junction channels with parameters described in the text and an average interchannel separation of 100 Å. The slope of the line can be expressed in terms of an equivalent resistance in series with the channel. This equivalent series resistance for the model is shown in b for differing number of equi-height barriers in the channel. (c) Plot similar to b for experimental data from a single multichannel patch at various potentials (see also Ramanan and Brink (1993)). The channels in the patch are gap junction channels found in the earthworm septum. Although the equivalent series resistance in the model (b) increases with voltage in a manner similar to that seen in the experimental data (c), it is too small (by almost an order of magnitude) to explain the data.

ductance. Although the Δg_n in our model are caused solely by flux interaction, and we do not assume any pipette resistance, the linearity of the graph of $\Delta g_n/g_n$ against $(n-1)$ as shown in Fig. 2 a permits us to rephrase the effects of flux interaction in terms of an equivalent series resistance. Such an equivalent series resistance is plotted in Fig. 2 b for simulations of a patch with 10 open channels and 1, 2, and 20 barriers. Fig. 2 b shows that the equivalent series resistance varies with potential. Such a dependence on the holding potential is also found in experimental data from multichannel recordings from the gap junction channel (Ramanan and Brink, 1993). We have replotted the data from Fig. 7 of this last reference in terms of an equivalent series resistance in Fig. 2 c; this series resistance increases with increasing applied voltage. However, the ratios of equivalent series resistance to channel resistance in the model (0.2–0.5%) are an order of magnitude lower than that in the data (typically

1–6%). Clearly, flux interactions are only a small component of experimentally observed nonunitary multichannel conductances.

With the parameters given, the flux interactions in the model result in a ~2–4% increase reduction in the current when 10 channels are simultaneously open. For a 100-pS channel, this would mean a reduction of the theoretical current from 100 to 96–98 pA with ten simultaneously open channels. This is at the limits of observeability, especially as the accompanying open-channel noise increases with the number of open channels. For gap junction channels, the situation is further complicated in practice by a significant presence of substates (Ramanan and Brink, 1993).

The resistive model of flux interaction (Wilders and Jongsma, 1992) assumes that the access resistance to a single channel increases by $\rho/(2\pi S)$ in the presence of an adjacent open channel, where ρ is the resistivity of the bathing solution and S is the interchannel separation. In our modelling, however, the net change in the channel current produced by superposition of flux interaction from adjacent channels will depend on the sensitivity of the single-channel current to changes in the potential and the ion concentration at the mouth $[\Delta I \simeq \Delta u(dI/du) + \Delta C_i(dI/dC_i)]$ caused by a nearby open channel. This dependence is implicit in Eqs. 11 and 12. Note that the change in the single-channel current due to flux interaction is thus not directly related to the slope of the single channel I–V curve. Thus a change in any of the free single-channel parameters, namely: 1) the relative permeabilities of anion to cation; 2) the channel mouth radius; 3) the number of barriers B, will change the equivalent access resistance in our model (by a factor of 50–200%). However, the access resistance formula $(\rho/2\pi S)$ serves as a good order-of-magnitude estimate. From Fig. 2, the equivalent access resistance (for 20 barriers) is $\simeq 20$ MΩ, as compared to $\rho/(2\pi S)$ ~25 MΩ.

The flux interaction resistance estimate $(\rho/2\pi S)$ will be good at salt concentrations where the Debye length is much smaller than the average interchannel separation S. At low salt concentrations, where the Debye length becomes comparable to S, use of the method presented here would yield more accurate results. This remark is based on the observation that the screening term (the homogenous term in Eq. 11) falls off near-exponentially with a length constant ~ the Debye length. At distances much greater than the Debye length, the inhomogenous term dominates; this term, which falls off as $1/r$, accounts for the $(\rho/2\pi S)$ part of the access resistance. This can be seen in Fig. 1, where the potential and concentration drops from infinity go as $1/r$ for large distances $(\gg \kappa r)$.

CONCLUSION

We have presented an exact solution to the linearized Nernst-Planck-Poisson equation for ion flow in the bath. A new element of the solution is the dependence of the equivalent access resistance on an additional parameter J, which is the ion flux for monovalents. For nonselective or poorly-

selective channels, such as the gap junction, the parameter J (see Eq. 5) is small as the fluxes of anion and cation are oppositely directed. This enabled us to use a perturbation expansion in J for the model of the gap junction channel. However, for ion-selective channels, such as K$^+$ or Na$^+$ channels, the magnitude of the flux J will be comparable to the current I. Even then, the errors in the perturbation expansion Eq. A2 are only of the order of IJ^2, and hence it would be accurate to $\simeq 1\%$ for $I \leq 0.2$. We also note that, in the normal physiological range of concentrations for monovalent salts, Levitt's solution (1991) is very close to the solution given here, and should preferably be used as it computes far more easily.

The parameter J (Eq. 5) varies as the square of the valence of the permeant ion. This indicates that, in modelling the flow of multivalents through ion-selective channels, the parameter J may be as significant as the current I in determining the flow through the bath. However, the limited applicability of the Debye-Hückel approximation for multivalent ionic species may render the use of the nonlinear equation (Eqs. 3 and 4) necessary at high salt concentrations. Even for low multivalent salt concentrations, the perturbation expansion Eq. A2 will no longer be valid for large J, and it may prove necessary to use the full nonperturbative Eq. 11 for deriving the boundary conditions at the channel mouth.

The net predicted effect of flux interactions is much weaker than is often observed in multichannel recordings from gap junctions. The model of interacting fluxes also shows a dependence of nonunitary multichannel conductances on the applied voltage. Such a dependence, although far greater in magnitude, is seen in the experimental data. It is possible that the channels are closer to each other than assumed (~100 Å) in the calculation of flux interaction, or that their mouths are shaped differently from circular regions. For our calculations, the channels would have to be packed to within 60 Å (center to center) to account for the nonunitary conductances observed in the data. This seems reasonable for, as seen in Fig. 1, it is till distances of ~50 Å that the charge screening effects are in effect at the salt concentrations used in the experiment. However, a packing distance of 60 Å is less by a factor of ~2 than the spacing seen in x-ray diffraction data for crystallized gap junctions, although from a different preparation (liver cells (Makowski et al., 1984)). We note that the flux-interaction model can be independently tested by, e.g., 1) changing the viscosity of the bath solutions by substituting D$_2$O for water or 2) increasing the concentration of the bathing solutions, as these have predictable effects. Ramanan and Brink (1993) have offered other explanations for these nonunitary multichannel conductances, such as long-lived substates. Since the number and probability of occurrence of these substates in the 100-pS channel are highly variable from patch to patch, and often from channel to channel in the same patch, it is hard to isolate the effects of flux-interaction from that of substates. On the whole, it seems likely that flux interaction is only one component of such nonunitary conductances in the 100-pS channel.

If nonintegral conductances are partially due to flux interaction between neighboring channels, the relation between macroscopic properties and single-channel properties may be complicated by factors such as the geometry of channel plaques. The observation of nonintegral multichannel conductances may therefore be another indicator of channel clustering (Wang and Thompson, 1992).

APPENDIX

Assume that the solution g_n for Eq. 11 is known to order J^n. The g_{n+1} satisfies the following equation:

$$\frac{d^2 g_{n+1}}{dy^2} + \left[-\frac{1}{4} - \frac{2}{y^2} \right] g_{n+1} = -\frac{0.5J}{y} g_n. \qquad \text{(A1)}$$

We use this formula to get the inhomogenous term for g correct to $O(J^2)$. The result for f correct to the same order follows after some tedious manipulation. This is given by:

$$f_{\text{inhomog}} = -\frac{2I}{y} - \frac{JI}{y} [\cosh(y/2)E_1(y/2) + \exp(-y/2)\text{Chi}(y/2)], \qquad \text{(A2)}$$

where E_1 and Chi are the exponential integral and hyperbolic cosine integrals, respectively (Abramowitz and Stegun, Chap. 5). Programs for computing these integrals can be found in Moshier (1989, pp. 355 and 370). f_{homog} is itself an integral involving a Whittaker function. This is most conveniently calculated by using the representation given in Abramowitz and Stegun (Eq. 13.2.5). Inserting this gives

$$\Gamma(2 - 0.5J)f_{\text{homog}} = A \int_\infty^y dz \, e^{-0.5z}z \int_0^\infty dt \, e^{-zt} t^{1-0.5J}(1 + t)^{1+0.5J}.$$

Interchanging the order of integration, and integrating wrt z yields

$$f_{\text{homog}} = -A\frac{\exp(-y/2)}{\Gamma(2 - 0.5J)} \int_0^\infty dt \exp(-yt)\frac{t^{1-0.5J}(1 + t)^{1+0.5J}}{(0.5 + t)^2}$$

$$\cdot [1 + (0.5 + t)y], \qquad \text{(A3)}$$

which is a form suited for computation.

The concentrations C_i can be calculated to first order by noting that

$$\frac{C_i'(y)}{C_i'^\infty} = 1 - z_i f(y) - 2\bar{J}_i \left[\frac{\kappa'}{\kappa_i'} \right]^2 \left[\frac{1}{y} - \int_\infty^y \frac{d\bar{y}}{\bar{y}} \frac{df}{dy} \right] + O(f^2) \qquad \text{(A4)}$$

and

$$\int_\infty^y \frac{d\bar{y}}{\bar{y}} \frac{df}{dy} = -\frac{1}{J} \left[4f'' + \frac{8f'}{y} - f - \frac{2I}{y} \right],$$

where the primes are differentiation wrt y.

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