LETTER TO THE EDITOR

The Relationship between Predicted Current and Internal State Transitions of a Single-File Model for Ion Transport across Membranes

Dear Sir:

I believe the measured time-varying K^+ current under voltage clamp which would be predicted by the model of Kohler (1) in a recent issue of this journal is not quite as the author presented it. The fundamental issue is that Dr. Kohler calculated the simulated measured current as being only due to the state transitions representing K^+ crossing the extracellular end of pores free of GP^{++}, whereas, in fact, all of the transitions representing charge movement will contribute to the measured current. Those representing K^+ movement will contribute to the ionic current, while those representing GP^{++} movement will contribute to the gating current.

One way of seeing how to take all the transitions properly into account is to start with the equation of continuity and one of Maxwell's equations, to wit (in MKS units):

$$\nabla \cdot \mathbf{J} = -\frac{\partial \rho}{\partial t} \tag{1}$$

and

$$\nabla \cdot \mathbf{D} = \rho, \tag{2}$$

where: $\mathbf{J}$ = current density, ρ = charge density, and $\mathbf{D}$ = electric displacement.

For a space clamped membrane, Eqs. 1 and 2 can be written in just one dimension (normal to the membrane surface), and become

$$\frac{\partial J}{\partial x} = \frac{\partial \rho}{\partial t} \tag{3}$$

and

$$\frac{\partial}{\partial X}\left(\epsilon \frac{\partial V}{\partial X}\right) = -\rho, \tag{4}$$

where ϵ is the electric permittivity (equal to the electric permittivity of free space times the dielectric constant) and, V = voltage.

Now, suppose the membrane is made of n slabs of thickness dx where ndx is the total membrane thickness, and we approximate the membrane volume charge density by a series of surface charge densities at the slab interfaces (denoted by $\sigma_{0,1}, \sigma_{1,2}, \sigma_{2,3}, \cdots \sigma_{n-1,n}, \sigma_{n,n+1}$). We take the 0, 1 interface to be the extracellular surface of the membrane and the n, $n+1$ interfaces to be the intracellular surface of the membrane.

Then, from Eq. 3:

$$\begin{aligned}
\frac{\partial \sigma_{0,1}}{\partial t} &= -j_1 - j_{\text{inj}} \\
\frac{\partial \sigma_{1,2}}{\partial t} &= j_1 - j_2 \\
&\vdots \\
\frac{\partial \sigma_{n,n+1}}{\partial t} &= j_n + j_{\text{inj}},
\end{aligned} \tag{5}$$

where j_i = current in ith slab (inward positive) due to movement of ions and membrane charged groups and j_{inj} = current injected across the membrane (inward positive).

From Eq. 4, the voltage drop across each of the slabs will be given by

$$V_{1,2} - V_{0,1} = \frac{-dx}{2\epsilon_1}(\sigma_{0,1} - \sigma_{1,2} - \sigma_{2,3} - \cdots \sigma_{n,n+1})$$

$$V_{2,3} - V_{1,2} = \frac{-dx}{2\epsilon_2}(\sigma_{0,1} + \sigma_{1,2} - \sigma_{2,3} - \cdots \sigma_{n,n+1})$$

$$\vdots$$

$$V_{n,n+1} - V_{n-1,n} = \frac{-dx}{2\epsilon_n}(\sigma_{0,1} + \sigma_{1,2} + \sigma_{2,3} + \cdots \sigma_{n-1,n} - \sigma_{n,n+1}), \tag{6}$$

where ϵ_i = the electric permittivity in the ith slab. The membrane potential difference is given by

$$\Delta V = V_{n,n+1} - V_{0,1}, \tag{7}$$

combining Eqs. 7 and 6:

$$\begin{aligned}\Delta V = -\frac{dx}{2}\Bigg[&\sigma_{0,1}\left(\frac{1}{\epsilon_1} + \frac{1}{\epsilon_2} + \frac{1}{\epsilon_3} + \cdots + \frac{1}{\epsilon_n}\right)\\ &+ \sigma_{0,2}\left(-\frac{1}{\epsilon_1} + \frac{1}{\epsilon_2} + \frac{1}{\epsilon_3} + \cdots + \frac{1}{\epsilon_n}\right)\\ &+ \sigma_{0,3}\left(-\frac{1}{\epsilon_1} + \frac{1}{\epsilon_2} + \frac{1}{\epsilon_3} + \cdots + \frac{1}{\epsilon_n}\right)\\ &\vdots\\ &+ \sigma_{n,n+1}\left(\frac{1}{\epsilon_1} + \frac{1}{\epsilon_2} + \frac{1}{\epsilon_3} + \cdots - \frac{1}{\epsilon_{n-1}} + \frac{1}{\epsilon_n}\right)\Bigg],\end{aligned} \tag{8}$$

combining Eqs. 8 and 5:

$$\frac{\partial(\Delta V)}{\partial t} = dx\left[j_{inj}\left(\frac{1}{\epsilon_1} + \frac{1}{\epsilon_2} + \cdots + \frac{1}{\epsilon_n}\right) + \frac{j_1}{\epsilon_1} + \frac{j_2}{\epsilon_2} + \frac{j_3}{\epsilon_3} + \cdots + \frac{j_n}{\epsilon_n}\right]. \tag{9}$$

If we now let the slabs become infinitesmally thin, Eq. 9 becomes:

$$\frac{\partial(\Delta V)}{\partial t} = j_{inj}\int_0^\delta \frac{dx}{\epsilon} + \int_0^\delta \frac{j}{\epsilon}dx, \tag{10}$$

where δ = membrane thickness.

Eq. 10 is just the generalization to the case where j and ϵ are functions of x of the familiar equation:

$$\frac{\partial(\Delta V)}{\partial t} = \frac{j_{inj} + j_{ionic}}{C_m}. \tag{11}$$

In the voltage clamp case $\partial(\Delta V)/\partial t = 0$. So, for the voltage clamp case Eq. 10 may be written:

$$j_{\text{inj}} = \frac{-\int_0^{\delta} \frac{j}{\epsilon}\,dx}{\int_0^{\delta} \frac{dx}{\epsilon}}. \tag{12}$$

In the situation hypothesized by Kohler (1), ϵ is a constant; in this case Eq. 12 goes to:

$$j_{\text{inj}} = \frac{1}{\delta}\int_0^{\delta} j\,dx. \tag{13}$$

Noting that the measured current is just the negative of the injected current, Eq. 13 becomes:

$$I_{me} = \frac{1}{\delta}\int_0^{\delta} [I_{K^+}(x) + I_{CP^{++}}(x)]\,dx, \tag{14}$$

where I_{me} is the measured current predicted by Kohler's model.

It might be noted that if the gating current were considered due to a system of dipoles, then

$$I_{\text{gating}} = \frac{\partial P(x)}{\partial t},$$

where $p(x)$ = the polarization of the gating system normal to the membrane surface at any depth x. Eq. 14 written specifically for the system of dipoles is then:

$$I_{\text{me,gating}} = \frac{1}{\delta}\int_0^{\delta} \frac{\partial P(x)}{\partial t}\,dx = \frac{1}{\delta}\frac{\partial}{\partial t}\int_0^{\delta} P(x)\,dx = \frac{N_c}{\delta}\frac{\partial P_s}{\partial t},$$

where N_c = channel density and P_s = total dipole moment normal to the membrane surface of a single channel.

This last equation above is just that used in another recent paper in this journal (2) to describe gating currents.

Having arrived at Eq. 14 for the measured current during voltage clamp, the question is: What is the relationship between Eq. 14 and the variables of the Kohler model; i.e., what current measurement does the model actually predict?

In the Kohler model I is not a continuous function of x, but rather is a constant between sites and changes discontinuously at each site. We can designate current in the different regions as: I_{01,K^+} = K^+ current between external surface and site 1, I_{12,K^+} = K^+ current between sites 1 and 2, I_{23,K^+} = K^+ current between sites 2 and 3, I_{3i,K^+} = K^+ current between site 3 and the internal surface, $I_{01,GP^{++}}$ = GP^{++} (gating) current between external surface and site 1, $I_{12,GP^{++}}$ = GP^{++} (gating) current between sites 1 and 2, $I_{23,GP^{++}}$ = GP^{++} (gating) current between sites 2 and 3, $I_{3i,GP^{++}}$ = GP^{++} (gating) current between site 3 and the internal surface.

Putting the above designations into Eq. 8 and using the site spacing and membrane thickness set by Kohler, we arrive at:

$$I_{me} = \frac{1}{100\ \text{Å}}\Big[20\ \text{Å}(I_{01,K^+} + K_{01,GP^{++}}) + 30\ \text{Å}(I_{12,K^+} + I_{12,GP^{++}}) + 30\ \text{Å}(I_{23,K^+} + I_{23,GP^{++}}) + 20\ \text{Å}(I_{3i,K^+} + I_{3i,GP^{++}})\Big]. \tag{15}$$

The final step in mapping the internal dynamics of the Kohler model to the measured current it predicts is to count all the transitions which go into the various current components. The value of I_{01,K^+}

for example, will be proportional to all those transitions which involve moving a K^+ from the outside to site 1 minus all those transitions which involve moving a K^+ from site 1 to the outside. (Note: I am using the convention that inward current is positive, opposite to Kohler's.) Analogous logic applies to each of the components. Using Kohler's symbols (his Fig. 3) for each state:

$$\begin{aligned} I_{01,K^+} = NQ_0(&k_{1,4}p_1 + k_{2,6}p_2 + k_{3,7}p_3 + k_{5,8}p_5 + k_{9,13}p_9 \\ &+ k_{10,15}p_{10} + k_{12,18}p_{12} + k_{14,19}p_{14} + k_{21,24}p_{21} - k_{4,1}p_4 \\ &- k_{6,2}p_6 - k_{7,3}p_7 - k_{8,5}p_8 - k_{13,9}p_{13} - k_{15,10}p_{15} \\ &- k_{18,12}p_{18} - k_{19,14}p_{19} - k_{24,21}p_{24}), \end{aligned} \tag{16}$$

$$\begin{aligned} I_{01,GP^{++}} = 2NQ_0(&k_{1,11}p_1 + k_{2,16}p_2 + k_{3,17}p_3 + k_{5,20}p_5 \\ &+ k_{9,22}p_9 + k_{10,23}p_{10} + k_{12,26}p_{12} + k_{14,25}p_{14} + k_{21,27}p_{21} \\ &- k_{11,1}p_{11} - k_{16,2}p_{16} - k_{17,3}p_{17} - k_{20,5}p_{20} - k_{22,9}p_{22} \\ &- k_{23,10}p_{23} - k_{26,12}p_{26} - k_{25,14}p_{25} - k_{27,21}p_{27}), \end{aligned} \tag{17}$$

$$I_{12,K^+} = NQ_0(k_{4,3}p_4 + k_{6,5}p_6 + k_{13,12}p_{13} - k_{3,4}p_3 - k_{5,6}p_5 - k_{12,13}p_{12}, \tag{18}$$

$$I_{12,GP^{++}} = 2NQ_0(k_{11,10}p_{11} + k_{16,14}p_{16} + k_{22,21}p_{22} - k_{10,11}p_{10} - k_{14,16}p_{14} - k_{21,22}p_{21}), \tag{19}$$

$$I_{23,K^+} = NQ_0(k_{3,2}p_3 + k_{7,6}p_7 + k_{17,16}p_{17} - k_{2,3}p_2 - k_{6,7}p_6 - k_{16,17}p_{16}), \tag{20}$$

$$I_{23,GP^{++}} = 2NQ_0(k_{10,9}p_{10} + k_{15,13}p_{15} + k_{23,22}p_{23} - k_{9,10}p_9 - k_{13,15}p_{13} - k_{22,23}p_{22}), \tag{21}$$

$$\begin{aligned} I_{3i,K^+} = NQ_0(&k_{2,1}p_2 + k_{5,3}p_5 + k_{6,4}p_6 + k_{8,7}p_8 + k_{14,10}p_{14} \\ &+ k_{16,11}p_{16} + k_{19,15}p_{19} + k_{20,17}p_{20} + k_{25,23}p_{25} - k_{1,2}p_1 \\ &- k_{3,5}p_3 - k_{4,6}p_4 - k_{7,8}p_7 - k_{10,14}p_{10} - k_{11,16}p_{11} \\ &- k_{15,19}p_{15} - k_{17,20}p_{17} - k_{23,25}p_{23}), \end{aligned} \tag{22}$$

and, finally, because of Kohler's assumption *c:*

$$I_{3i,GP^{++}} = 0. \tag{23}$$

Equations 14–22 then constitute the complete mapping between the internal dynamics of the Kohler model and the K^+ and gating currents predicted by it. Specifically, the potassium current will be given by:

$$I_{K^+} = 0.2(I_{01,K^+} + I_{3i,K^+}) + 0.3(I_{12,K^+} + I_{23,K^+}), \tag{24}$$

and the gating current by:

$$I_{GP^{++}} = 0.2I_{01,GP^{++}} + 0.3(I_{12,GP^{++}} + I_{23,GP^{++}}). \tag{25}$$

The numerical values of the time-varying K^+ currents thus calculated will be at least somewhat different from the simulations shown in Kohler's paper, in which he considered the current due to only a few of the system's transitions. The instantaneous current (Kohler's Fig. 7) seems especially likely to show a significance difference. On the other hand, the stationary currents will still be the same as he showed, since at steady state there will be no net charge movement in the statistical ensemble of pores containing GP^{++}, while in the statistical ensemble of pores free of GP^{++} the current as a function of x will be constant. These points follow from Eq. 2, which reduces to $\partial I/\partial x = 0$ in the time invariant case.

In Kohler's model this means that in the steady state the K^+ fluxes into and out of each internal site will be equal to each other, so that the fluxes across the membrane faces in the steady state case (but only then) will correspond to the measured current.

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