LETTER TO THE EDITOR

Electroneutral Na/H Exchange May Depolarize the Membrane Potential in Steady State

Dear Sir:

In our article (Jacob et al., 1984), we showed that electroneutral exchange could affect membrane potential (E_m) in ways that are not intuitively obvious. Scriven and Mundel (1985) have raised a valid concern about one particular aspect, namely our discussion of the effect on E_m of extruding metabolically produced protons by Na/H exchange. In the section of our article entitled "Na/ H Exchange," as they correctly point out, the current arising from proton efflux was unbalanced: this clearly cannot be, since, for an isolated cell in steady state, the net transmembrane current must be zero. In addition, as they also point out, the metabolic production of protons must be accompanied by a metabolic production of anions that have to be accounted for. Thus, the results of that particular section are invalid.

In the following discussion, we correct our model by balancing the currents with the addition of either an anion efflux or a proton influx. Using these models, we derive new equations to replace the invalid equations derived in the section entitled "Na/H Exchange" in our original article. However, we disagree with Scriven and Mundel's statement that we omitted a hyperpolarizing current (I_H). We feel our mistake was to omit a balancing anion efflux or proton influx, both of which carry depolarizing currents. This may explain why our new equations still predict that E_m may be depolarized by the presence of an electroneutral Na/ H exchange.

We start by extending our original model to include an anion efflux that electrically balances the proton efflux. The cell metabolizes a neutral substance (e.g., glucose), which enters passively by Fickian or facilitated diffusion, and the end product is a proton and a particular anion (A, e.g., lactate). The proton is extruded by Na/H exchange and the anion escapes by electrodiffusion. We have:

$$m_{Na} + j_{Na} + x_{Na} = 0; \tag{1}$$

$$m_K + j_K = 0; \tag{2}$$

$$x_H + x_{Na} = 0; \tag{3}$$

$$x_H - j_A = 0, \tag{4}$$

where m_{Na} and m_K are the Na and K pump fluxes, x_{Na} and x_H are the Na and H

fluxes on the Na/H exchange, and j_{Na}, j_K, and j_A are the passive electrodiffusive fluxes for Na, K, and A. By putting $r = -m_{Na}/m_K$, where r is the Na/K pump stoichiometry, we obtain

$$rj_K + j_{Na} - j_A = 0. \tag{5}$$

Using the constant field equation relating flux to E_m (e.g., Schultz, 1980), we obtain

$$E_m = \frac{RT}{F} \ln\left[\frac{rP_K K_o + P_{Na} Na_o + P_A A_i}{rP_K K_i + P_{Na} Na_i + P_A A_o}\right], \tag{6}$$

where P_K, P_{Na}, and P_A are the permeabilities to K, Na, and A, and K_o, K_i, Na_o, Na_i, A_o, and A_i are the extra and intracellular ionic concentrations of K, Na, and A. R, T, and F have their usual meaning. This equation is formally the same as the Mullins-Noda equation (Mullins and Noda, 1963), with the inclusion of anion terms. Since the anion, A, is diffusing out of the cell, its reversal potential will have to be positive to E_m. Thus, the inclusion of the anion terms in Eq. 6 is a depolarizing influence.

The same argument applies if the role of Na/H exchange is to extrude protons that have leaked in by passive diffusion. The possibility of significant inward proton leaks and of the control of pH$_i$ by gradient-coupled transport processes in the face of such leaks has been commented on by several investigators (e.g., Moody, 1981; Boron, 1984; Busa and Nuccitelli, 1984). In this case, Eq. 4 is replaced by

$$x_H + j_H = 0, \tag{7}$$

and we obtain

$$E_m = \frac{RT}{F} \ln\left[\frac{rP_K K_o + P_{Na} Na_o + P_H H_o}{rP_K K_i + P_{Na} Na_i + P_H H_i}\right], \tag{8}$$

where P_H is the proton permeability and H_o and H_i are the extra- and intracellular proton concentrations. As above, the inclusion of the proton terms is a depolarizing influence, as the reversal potential for protons must'be positive to E_m since protons are leaking in (this level of H_i being maintained by Na/H exchange in this model).

In both cases, the corrected model includes an additional depolarizing current, which leads to depolarizing terms in the equation for E_m. The magnitude of the Na/H exchange is not explicit in the final equation. It is, however, implicit in that, without an Na/H exchange (or some other mechanism), these equations would not represent a steady state. In deriving these equations, we equated the Na/H turnover rate with the anion efflux in one case (Eq. 4, leading to the anion terms in Eq. 6) and with the proton influx in the other case (Eq. 7, leading to the proton terms in Eq. 8). In other words, the Na/H exchange couples the electrodiffusive anion or proton fluxes into the system of Na and K fluxes. Thus, the depolarization that we predict cannot be viewed as a consequence of Na/H exchange of itself: it is a consequence of the overall balanced system of proton influx (or anion efflux), Na/H exchange, and other Na and K fluxes.

In practice, it is debatable whether an anion such as lactate, produced in anaerobic metabolism, crosses the cell membrane as an anion or in a neutral form (either by cotransport with a proton or by diffusion in its undissociated form). For example, the fraction crossing in a neutral form in muscle depends on the type of muscle and the extracellular conditions (Roos, 1975; Mainwood and Worsley-Brown, 1975; Seo, 1984), whereas in red blood cells, lactate crosses entirely as a neutral form (Deuticke et al., 1982). Our model does not apply when lactate crosses as a neutral form since protons go with it.

The original article was intended as an extension of the Mullins-Noda equation and not as a comprehensive analysis of E_m for all cells. Our article contained simple models and many complications can be added. For example, we tacitly assumed that the intracellular ionic concentrations would not change. Small changes in Na_i or H_i will not significantly alter E_m. However, of particular concern is the possibility that an increase in K_i might be a necessary consequence of an increased Na/K pump rate; if so, any depolarization predicted by Eq. 8 might be diminished or even outweighed by the hyperpolarizing influence of an increased K_i. The relative effects of the proton terms and changes in K_i may be determined by assuming that $P_{Na}Na_i$ and P_HH_i are both $\ll P_KK_i$, so that Eq. 8 becomes

$$E'_m = \frac{RT}{F}[\ln(rP_KK_o + P_{Na}Na_o + P_HH_o) - \ln(rP_KK'_i)], \tag{9}$$

where the primes indicate values in the presence of Na/H exchange. The membrane potential in the absence of Na/H exchange is approximated by

$$E_m = \frac{RT}{F}[\ln(rP_KK_o + P_{Na}Na_o) - \ln(rP_KK_i)]. \tag{10}$$

Thus, the change in E_m on introducing a proton leak and Na/H exchange is

$$E_m - E'_m = \frac{RT}{F}\left[\ln\left(\frac{K'_i}{K_i}\right) - \ln\left(1 + \frac{P_HH_o}{rP_KK_o + P_{Na}Na_o}\right)\right]. \tag{11}$$

The first term in Eq. 11 causes a hyperpolarization if $K'_i > K_i$ and the second term causes a depolarization. Thus, if $K'_i > K_i$, then the direction of change in E_m will depend on the relative magnitudes of the two terms. Note that even though $H_o \ll K_o$, the second term is likely to be significant since P_H can be $\gg P_K$. Estimates of P_H for lipid bilayers range from 3×10^{-3} to 3×10^{-9} cm·s^{-1}, and biological membranes appear to have permeabilities in the region of 10^{-3} cm·s^{-1} (Deamer, 1982): typical P_K values are in the range of 10^{-6}–10^{-7} cm·s^{-1}. Indeed, since we are considering an Na/H exchange that is extruding protons that have passively diffused into the cell, we are explicitly considering the case where P_HH_o is significant in Eq. 11 or, for that matter, in Eq. 8.

An increase in K_i is not an inevitable consequence of an increased Na/K pump rate. For the pump rate to increase, there must be a rise in Na_i to stimulate the pump (assuming there is no up-regulation of the number of pump sites), but the only necessary consequence regarding K is an accompanying increase in the passive K efflux to maintain steady state. This increase can be effected in two

ways: either K_i can increase or E_m can depolarize to increase the driving force $(E_K - E_m)$ for K efflux. Since we have assumed a constant K_i, our model predicts a depolarization. The assumption of a constant K_i may be a good approximation to reality since it is not possible for both Na_i and K_i to increase if the cell is to maintain osmotic equilibrium. One might expect, therefore, that an increased pump rate may be associated with a decrease in K_i as water is taken up with the Na. Such an effect has been measured in cardiac muscle by Cohen et al. (1982), albeit in non–steady state: they noted a small decrease in intracellular K activity when the Na/K pump rate was increased as a result of a rapid stimulation of tissue.

In retrospect, the example we chose to demonstrate the effect of Na/H exchange on E_m has many complications. A better example, as discussed above, would have been the one in which Na/H exchange extrudes protons that have leaked in. With the restrictive assumption of constant internal ionic concentrations, we maintain that the effect of introducing an Na/H exchange could be, in the steady state, a depolarization.

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