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Letters to the Editor

Comments on: Sodium Fluxes Through the Active Transport Pathway in Toad Bladder

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We should like to offer the following comments on Chen and Walser's very interesting recent paper [1]. Chen and Walser consider the force determining the flux ratio in the pathway for active sodium transport to be the sum of contributions of the trans-membrane electrical potential difference ψ, and the electromotive force of the sodium pump E, which is constant. Kedem and Essig consider the contribution of coupled flows, all of which may vary with potential [3]. The two formulations are compatible, and it can be shown that their combined consideration can lead to meaningful additional information.

Analysis of the equivalent circuit model for active Na transport between identical solutions leads to Chen and Walser's Eq. (10). Dropping subscripts and superscripts,

$$\ln f = -QzF(\psi - E)/\mathrm{R}T. \tag{1}$$

Here z, F, R, and T have their usual significance, f is the flux ratio, and Q is the (constant) ratio of the bulk diffusion coefficient to tracer diffusion coefficient in the active pathway. In their study in toad bladder Chen and Walser found Q in the active pathway to be 2.54 ± 0.34. It was suggested that although the significance of the large value (i.e. $Q > 1$) remains to be determined, coupling between the fluxes of tracer and abundant sodium ions, or between the fluxes of sodium and some other ion seem the most likely possibilities.

The nonequilibrium thermodynamic analysis of isotope fluxes leads to Kedem and Essig's Eqs. (28) and (29). Since for Dominican toad bladders mounted in chambers there has been no evidence of appreciable movement of any species other than Na^+ by way of the active pathway [4, 6], the only coupled flow we consider is that of metabolism. Therefore these equations become

$$JR^*/\mathrm{R}T = \ln f = -(R^*/R)(zF\psi + R_{0r}J_r)/\mathrm{R}T. \tag{2}$$

Here J is the rate of net active Na transport, J_r is the rate of metabolic reaction (e.g. oxygen consumption), and R_{0r} is the phenomenological coefficient relating transport to metablism. R^* and R are the phenomenological exchange resistance and resistance to net flow, respectively; these differ to the extent that there is coupling between the fluxes of tracer and abundant sodium ions ("isotope interaction"). Eqs. (2) have been partially tested in synthetic membranes [5], frog skin [8], and toad bladder [7, 9].

Setting $\psi = E$ makes J and $\ln f = 0$. Eq. (2) then shows that

$$R_{0r} = -zFE/J_{rE}. \tag{3}$$

Introducing Eq. (3) into Eq. (2) gives

$$\ln f = -(R^*/R)\, zF(\psi - (E/J_{rE})J_r)/\mathrm{R}T. \tag{4}$$

Equating the right-hand sides of Eqs. (1) and (4) at $\psi = 0$, we have

$$Q = (R^*/R)(J_{r0}/J_{rE}). \tag{5}$$

Therefore it is seen that Q depends not only on interaction of isotope flows, but also on the rates of metabolism at "level flow" ($\Delta\psi = 0$) and "static head" ($J = 0$) [2].

[Alternatively, Q may be expressed in terms of Kedem and Caplan's parameter q, which quantifies the "degree of coupling" between transport and metabolism ($0 \leqq q^2 \leqq 1$) [2]. Since $J_{rE}/J_{r0} = 1 - q^2$,

$$Q = (R^*/R)/(1 - q^2). \tag{6}]$$

Expressing Q in terms of its components in the above manner indicates the possibility of evaluation of the degree of isotope interaction of the active pathway:

$$R^*/R = Q(J_{rE}/J_{r0}) = -[\mathrm{R}T \ln f/zF(\psi - E)](J_{rE}/J_{r0}). \tag{7}$$

Since J_r has been shown to be a linear function of ψ in both frog skin [8] and toad bladder (M. Lang, S. R. Caplan and A. Essig, *manuscript in preparation*),

$$J_r = J_{r0} + (dJ_r/d\psi)\psi, \tag{8}$$

and

$$J_{rE} = J_{r0} + (dJ_r/d\psi)E. \tag{9}$$

Since $dJ_r/d\psi$ is negative and E positive, J_{rE} is less than J_{r0} and thus R^*/R is less than Q.

In a series of eight studies in the toad bladder we have found $J_{rE}/J_{r0} = 0.324 \pm 0.075$. Combining these data with Chen and Walser's value of

$Q=2.54$ gives a mean value of $R^*/R=0.82$. Thus, rather than being "characteristic of 'single file pore diffusion'", Chen and Walser's value of $Q>1$ may be consistent with negative isotope interaction ($R^*/R<1$), as in "exchange diffusion". Of course, our value of R^*/R cannot be taken too seriously, being based on studies in different laboratories under different conditions. Nevertheless, it is of interest, since it suggests the possibility of evaluating the degree of isotope interaction with sufficient precision to permit useful comparisons in different states of the active transport system. (In the presence of coupling of active Na^+ transport and other flows, as suggested by Chen and Walser for their bladder sacs, the above formulation could be modified to take these additional influences into account.)

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In Reply to: Drs. Essig and Lang

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Essig and Lang's proposal is of considerable interest since it evidently leads to the conclusion that the ratio of tracer diffusion coefficient to bulk diffusion coefficient (Q) is likely to exceed unity in any pathway in which transport is coupled to metabolism. However, there are several elements of uncertainty that remain to be resolved:

1. As these authors note, our formulation is based on E and g_a, the conductance of the active path, being invariant with potential, some evidence of which we presented [1]. If E or g_a varies with potential, our analysis is invalid and our estimate of Q incorrect. The equations they use appear to imply variation of E, g_a, or both with potential.

2. Their assumption that no appreciable movement of any species other than Na^+ occurs through the active path is questionable; biochemically there is no support for such an enzymatic mechanism of sodium transport, and the Koefoed-Johnsen-Ussing model [2] of active Na–K exchange followed by passive K efflux remains to be disproven. Furthermore, we have shown in three separate studies [1, 3, 4] totalling 64 experiments that net sodium flux is only about 80% of short-circuit current in toad bladder sacs. We have recently found that this discrepancy is eliminated if bicarbonate is removed from the bathing media; at the same time, E and g_a are reduced (Chen and Walser, *in preparation*). Hence coupling of Na flux to bicarbonate in the opposite direction apparently occurs, and could thus account for a portion (as yet undetermined) of the deviation of Q from unity.

Despite these reservations, we concur with Essig and Lang's suggestion that coupling of transport to metabolism will generally tend to increase Q, and that with further study useful quantitative deductions may be drawn from such analysis.

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