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Letters to the Editors

Metabolic Cost of Sodium Transport and the Degree of Coupling of Transport and Metabolism in Toad Urinary Bladder

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We should like to offer the following comments on Labarca, Canessa, and Leaf's recent paper, in which they conclude that the metabolic cost of sodium transport is independent of the transepithelial electrical potential difference $\Delta\psi$ over a range of ± 50 mV [3]. With simultaneous and continuous measurements of the rates of active Na transport (in our terms J^a_{Na}) and suprabasal CO_2 production $J^{sb}_{CO_2}$, it was found that changes in $\Delta\psi$ caused time-dependent changes in both flows lasting 20 to 40 min. Whereas with 10 min perturbations $J^a_{\mathrm{Na}}/J^{sb}_{CO_2}$ seemed to depend on $\Delta\psi$, under steady-state conditions following longer perturbations $J^a_{\mathrm{Na}}/J^{sb}_{CO_2}$ was statistically the same at $+50$ mV (serosa positive) and -50 mV (serosa negative). This was taken to demonstrate the "complete coupling of Na transport to a supply of metabolic energy... over the range of $\Delta\psi$ studied."

This conclusion is in seeming conflict with our recent finding that, when the rate of oxidative metabolism J_{O_2} was measured with Clark oxygen electrodes 4–6 min after perturbation of $\Delta\psi$, $J^a_{\mathrm{Na}}/J^{sb}_{O_2}$ did indeed vary with $\Delta\psi$, perturbed over a range of ± 80 mV, which was considered to indicate "incompleteness of coupling" [4, 5]. Three considerations may resolve this apparent contradiction:

1) Even with incomplete coupling, the relative rates of transport and metabolism may be near constant over an appreciable range.

Presuming the validity of a linear thermodynamic (NET) formulation, if membranes are exposed to identical solutions at each surface, the ratio of the rates of transport and suprabasal metabolic reaction will be given by [2]:

$$J^a_{\mathrm{Na}}/J^{sb}_r = Z[q + Z(-F\Delta\psi/A)]/[1 + qZ(-F\Delta\psi/A)]. \qquad (1)$$

Here F is the Faraday constant and A is the affinity or, under usual experimental conditions, the negative Gibbs free energy, of the metabolic reaction driving transport; q and Z are dimensionless functions of the phenomenological coefficients of the linear formalism [2, 1]. The degree of coupling q lies in the range $0<q\leqq 1$. It can be seen that only for $q=1$ (complete coupling) is the ratio of flows independent of $\Delta\psi$, being then given by $J_{\mathrm{Na}}^{a}/J_{r}^{sb}\equiv Z$.

For an incompletely coupled system we can use Eq. (1) to estimate the variation in the ratio to be expected over any range of $\Delta\psi$, by assuming the mean parameters found by Lang *et al.* in 11 hemibladders, *viz.* $q=0.86$; $Z=\sqrt{L_{\mathrm{Na}}^{a}/L_{r}}=16.8$; and $A=57.1\ \mathrm{kcal}\cdot\mathrm{mole}^{-1}\ \mathrm{O}_2$ (Tables 2–4 in [5]). Substituting these values in Eq. (1) gives values of $J_{\mathrm{Na}}^{a}/J_{\mathrm{O}_2}^{sb}$ at $\Delta\psi=-50$ and $+50$ mV of 15.6 and 12.4, respectively, i.e., a decrease of 21% over a range of 100 mV. Thus, although the formalism predicts a lower ratio at +50 than at −50 mV, it is seen that the predicted difference is small, and likely to be obscured by experimental variability. On the other hand, for $\Delta\psi=-80$ and $+80$ mV, as in the studies of Lang *et al.* [4, 5], Eq. (1) predicts values of $J_{\mathrm{Na}}^{a}/J_{\mathrm{O}_2}^{sb}=16.1$ and 10.0, respectively, i.e., a decline of 38%. It can be anticipated that a change of this magnitude could be demonstrated experimentally with techniques at hand. A still more sensitive, and more pertinent, test would be provided by examination of the "physiological" range, $0<\Delta\psi<E_{\mathrm{Na}}$. As $\Delta\psi$ approaches E_{Na}, J_{Na}^{a} approaches zero, whereas for an incompletely coupled system J_{r}^{sb} remains finite, so that in this range $J_{\mathrm{Na}}^{a}/J_{r}^{sb}$ falls rapidly towards zero. It should be emphasized that the application of Eq. (1) is restricted to steady states, and requires that the internal parameters Z, q, and A have the same values at all settings of $\Delta\psi$ employed.

2) In the studies of Lang *et al.*, the demonstration of near-constancy of J_{Na}^{a} and $J_{\mathrm{O}_2}^{sb}$ and of linear relationships between J_{Na}^{a}, $J_{\mathrm{O}_2}^{sb}$, and $\Delta\psi$ 4–6 min after symmetrical perturbations of $\Delta\psi$ indicated the existence of a quasi-steady state, characterized by near-constant values of the phenomenological coefficients and A over the range and duration of perturbations employed. We feel that prior to the steady states considered by Labarca *et al.* at 20–40 min their system must have evolved through a series of such quasi-steady states.

3) Perturbations of $\Delta\psi$ for extended periods may appreciably alter the kinetic and energetic parameters of the system, which may be recognized by highly non-linear relationships between J_{Na}^{a}, J_{r}^{sb}, and $\Delta\psi$ (*see* Labarca *et al.*'s Table 3). These findings are reminiscent of the "memory effect" observed earlier in frog skin, where prolonged large perturbations of $\Delta\psi$ resulted in marked effects on both the current and

rate of oxygen consumption on return to the short-circuit reference state [6]. Given such findings, it is to be expected that certain of the phenomenological coefficients, and possibly the affinity, of the NET formulation will vary with prolonged perturbations of $\Delta\psi$. However, in the use of Eq. (1) to predict the value of $J_{\mathrm{Na}}^{a}/J_{r}^{sb}$ at a given value of $\Delta\psi$, it is necessary to employ the values of Z, q, and A appropriate for the particular quasi-steady state under consideration. Accordingly, while Labarca *et al.*'s study evaluates the "metabolic cost of sodium transport" under the conditions considered, it cannot evaluate the degree of coupling between active Na transport and metabolism for an incompletely coupled system, because in prolonged perturbations the tissue parameters change with the passage of time. Under these circumstances $J_{\mathrm{Na}}^{a}/J_{r}^{sb}$ may appear near-constant, but observations of early quasi-steady states indicate that there is no fixed stoichiometry, and hence coupling is incomplete.

Clearly, these various dynamic effects point to significant regulatory phenomena deserving of thorough study. Furthermore, they indicate the need for care in the design and interpretation of experiments which aim at the quantification of kinetic and energetic parameters, lest experimental perturbations alter the very parameters which it is desired to characterize.

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Reply to: Metabolic Cost of Sodium Transport and the Degree of Coupling of Transport and Metabolism in Toad Urinary Bladder

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We appreciate the opportunity to respond to the letter by Essig and associates with the following points:

1. The difference between the findings and interpretations made by Essig *et al.* and by us seems to be based on our different perception of the meaning of "steady state". Our view is that the parameter of metabolism that is measured, either of oxygen consumption or CO_2 production, $J_{CO_2}^{sb}$, must relate directly at the time measured with the rate of sodium transport, J_{Na}. Thus, only with the ratio $J_{Na}/J_{CO_2}^{sb}$ invariant with time can one assume that the metabolic rate expressed by $J_{CO_2}^{sb}$ is, in fact, a measure of the energy required for the observed rate of sodium transport, J_{Na}. We found that only after some 20 min following a step change in transepithelial electrical potential, $\Delta\psi$, was this condition achieved. Therefore, our findings of the same value of $J_{Na}/J_{CO_2}^{sb}$ at +50 mV as at −50 mV is based on measurements made after 20 min following a perturbation of $\Delta\psi$.

Since the rate of oxygen consumption or of CO_2 production is related to the free energy utilized by a cell in sodium transport only through a complex series of chemical metabolic reactions, there may be at one instant in time no relation between energy utilized for transport and the rate of oxygen consumption at that moment. A well-known example is the oxygen debt incurred during a 100 yard dash. The fact that the runner may not breathe during that quick vigorous action does not mean that his muscles did not require energy to run. Almost any ratio of muscular work to oxygen consumption could be obtained during the period of the sprint. Only if one integrated for the total sprint the work done with the oxygen consumption during the run and following it until the lactate, etc., which accumulated during the exertion had been oxidized and levels of high energy intermediates returned to control levels in his muscles, could one obtain the correct ratio of work done to oxygen consumption.

Similarly, when we tried to assess $J_{Na}/J_{CO_2}^{sb}$ over periods of 15 min or less following a large change in $\Delta\psi$, we obtained ratios that varied in a manner similar to those reported by Lang *et al.* (2). However, by comparing $J_{Na}/J_{CO_2}^{sb}$ calculated from the total sodium transport and suprabasal CO_2 produced during the entire 60-min period of voltage clamping with the last 10 min of the hour essentially identical values were obtained (1).

The nonsteady state of the first 20 min following a change of $\Delta\psi$ seems to be compensated for over the following 40 min.

2. We do not think that the error in our measurements would have masked the change in $J_{\mathrm{Na}}/J^{sb}_{\mathrm{CO}_2}$ with $\Delta\psi$ which they claim we should have seen. Thus in a recent series of measurements on 11 toad bladders (Labarca, P., *unpublished data*) $J_{\mathrm{Na}}/J^{sb}_{\mathrm{CO}_2}$ was found to have the following values on varying the magnitude of $\Delta\psi$ from +70 mV to 0 and then back to +70 mV in 10-mV steps.

$\bar{X}$	24.6	24.9	25.8	24.4	23.5	23.0	23.6	23.2	22.1	21.5	22.7	22.8	23.6	24.9	25.4
±SEM	2.2	2.1	2.2	2.1	2.0	1.9	2.2	2.0	2.3	1.7	2.2	1.9	2.7	2.9	5.0

It is evident that no downward trend in the ratio occurred as +70 mV was approached as seen by the first and last figure of this series compared with the value of 23.2 found at $\Delta\psi = 0$.

3. It would have been more prudent on our part, we agree, had we avoided the terminology "complete coupling" to characterize our finding of a constant stoichiometric ratio for $J_{\mathrm{Na}}/J^{sb}_{\mathrm{CO}_2}$ over the range of $\Delta\psi$ tested. A constant but incomplete coupling could account for our findings and measurements at more positive values of $\Delta\psi$ should be made to substantiate the claim of "complete" coupling of sodium transport to metabolism.

4. We agree with Essig *et al.* that experimental protocol must be carefully considered. We tried many different periods of observation, sequences of potential change, etc., and would agree that the conditions under which observations are made may affect the parameters measured. However, we do think our data satisfied the essential requirement of having been obtained during a steady state, whereas we question whether their protocol meets this condition.

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