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THE THERMODYNAMIC DEGREE OF COUPLING BETWEEN METABOLISM AND SODIUM TRANSPORT IN FROG SKIN

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Summary

The tightness of coupling between two processes is advantageously evaluated by the thermodynamic degree of coupling q, varying in absolute value from zero for uncoupled processes to unity for processes which are related stoichiometrically. Two methods for the determination of q in the active pathway in frog skin have been developed, employing amiloride to abolish active sodium transport. The values of q in 6 frog skins varied, but were always less than unity (mean 0.79 ± 0.06 S.E. according to one method, 0.78 ± 0.06 S.E. according to the other). This indicates that metabolism and sodium transport are incompletely coupled in this tissue even when passive transepithelial leakage pathways are taken into account.

The conversion of metabolic energy to electrochemical potential energy in active transport is complex, and there is no a priori reason to assume fixed stoichiometry [1,2]. Indeed the question of tightness of coupling is fundamental to our understanding of the efficiency and the mode of regulation of active transport.

The analysis of these matters is facilitated by consideration of the thermodynamic degree of coupling q defined by Kedem and Caplan [3]. This parameter is based on the phenomenological description of coupled processes, which for active transport may be written [2]

$$\begin{aligned} J_+ &= L_+X_+ + L_{+r}A \\ J_r &= L_{+r}X_+ + L_rA \end{aligned} \qquad (1)$$

where J_+ and J_r represent net transepithelial sodium flux and rate of metabolism (here measured by the rate of suprabasal oxygen consumption), respectively. X_+ represents the electrochemical potential difference of sodium ion across

the membrane, A represents the "affinity", or negative Gibbs free energy, of the metabolic process driving transport, and the L's are phenomenological coefficients. Then q is defined by the following combination of the phenomenological coefficients:

$$q = L_{+r}/\sqrt{L_+ L_r} \tag{2}$$

The quantity q^2 varies from zero for completely uncoupled processes to unity for complete coupling ("stoichiometry"). Studies in various epithelia have demonstrated linearity of both J_+ and J_r in X_+ over ranges of physiological interest, indicating constancy of the phenomenological coefficients and A on brief perturbation of X_+ [4—7]. This supports the validity of the linear formalism and enables us to determine q from the relation

$$q^2 = 1 - [(J_r)_{J_+ = 0}/(J_r)_{X_+ = 0}] \qquad \text{(const. } A\text{)} \tag{3}$$

Here the rate of suprabasal oxygen consumption J_r is evaluated at two extreme states of the system: "level flow", when no electrochemical potential gradient exists across the membrane, and "static head", when the net flux of sodium is zero. (For completely coupled processes at static head J_r=0.)

Two methods were developed for the determination of q in frog skin, both employing the diuretic amiloride. Frog abdominal skin was mounted in modified Ussing chambers with identical solutions bathing the two sides, permitting the regulation of current I by voltage clamping and the monitoring of the rate of oxygen consumption with oxygen electrodes [8]. Under these conditions $X_+ = -F\Delta\psi$, where F is Faraday's constant and $\Delta\psi$ the electrical potential difference.

Method 1. The current-voltage relation is determined prior to and following the abolition of active sodium conductance with 10^{-5} or 10^{-4}M

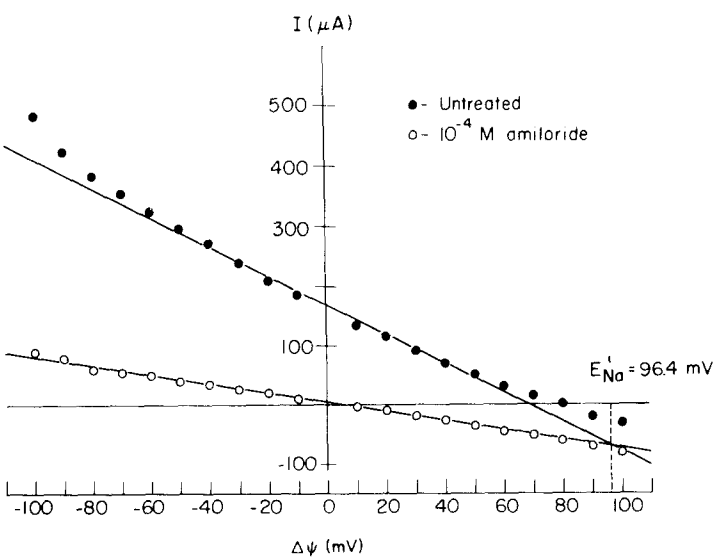

Fig. 1. Dependence of current I on the transepithelial electrical potential difference $\Delta\psi$. ●—●, untreated skin; o—o, the same skin treated with 10^{-4} M amiloride. For both lines the correlation coefficient r=0.90 (P<0.01). The point of intersection of the two lines gives E'_{Na} = 96.4 mV.

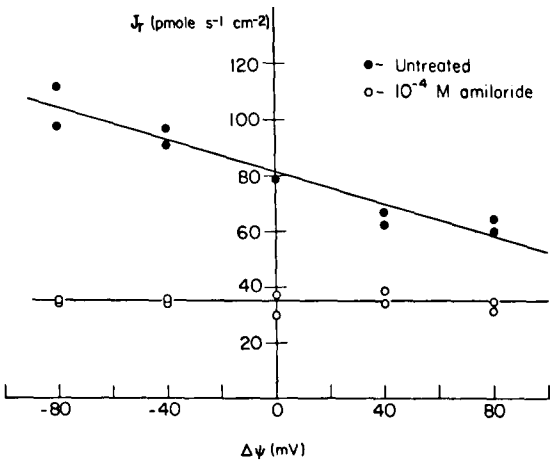

Fig. 2. Dependence of the rate of oxygen consumption J_r on the transepithelial electrical potential difference $\Delta\psi$ (in the same skin as in Fig. 1). ●—●, untreated skin. Correlation coefficient r=0.84 ($P < 0.05$). ○—○, the same skin treated with 10^{-4} M amiloride. r = 0.09 (not significant). Suprabasal oxygen consumption J_r^{sb} is taken as that over and above the level measured in the presence of amiloride. J_r^{sb} ($\Delta\psi$=0)= 46.6 pmol·s^{-1}·cm^{-2}; J_r^{sb} ($\Delta\psi = E'_{Na}$) = 20.7 pmol·s^{-1}·cm^{-2}.

amiloride added to the solution bathing the outer surface. $\Delta\psi$ was perturbed symmetrically for 15 s in the range 0 to ±100 mV. If indeed amiloride abolishes active sodium conductance [9], for a linear system the intersection of these curves gives the value of $\Delta\psi$ adequate to abolish active sodium transport, i.e. the "electromotive force of sodium transport" E_{Na} [10]. We shall call the intersection of linear regions of the current-voltage curves E'_{Na}. J_r was determined from the rate of decline of oxygen tension 3—6 minutes after perturbation of $\Delta\psi$ [4]. Having demonstrated linear dependence of J_r on $\Delta\psi$ in the absence of amiloride, evaluation of J_r at $\Delta\psi$ = 0 and, by extrapolation, at $\Delta\psi = E'_{Na}$ permits the calculation of q according to Equation 3 *.

Figs. 1 and 2 show, in a representative skin, I vs. $\Delta\psi$ and J_r vs. $\Delta\psi$, respectively, in both cases before and after treatment with amiloride. The lack of dependence of J_r on $\Delta\psi$ in the presence of amiloride indicates the abolition of net active sodium transport. The mean value (± S.E.) of E'_{Na} calculated by this means in 6 skins was 83.8 ± 7.0 mV, and the mean value of q was 0.79 ± 0.06.

Method 2. An alternative approach again employs amiloride to eliminate active conductance κ^a. This permits the evaluation of the passive conductance κ^p from the residual conductance, and the calculation of E'_{Na} from the relation [11]

$$E'_{Na} = I_0/\kappa^a = I_0/(\kappa - \kappa^p) \quad (4)$$

Here κ was measured by perturbing the potential by ±10 mV for 15 s prior

*The evaluation of q on the basis of Equation 1 characterizes the system in the linear range, here ±50—60 mV. The use of Equation 3 involving the mathematical construct "E'_{Na}" is a matter of convenience. The same result can be obtained from Equation 1 in the linear range in which $\Delta\psi < E'_{Na}$. For systems with linearity over a sufficiently large range, $E'_{Na} \equiv E_{Na}$.

to the administration of amiloride. The quantity q may then be determined as previously from the $J_r - \Delta\psi$ relationships as in Fig. 2. The mean value of E'_{Na} calculated by this means in the same 6 skins as used in Method 1 was 80.4 ± 4.8 mV, and the mean value of q was 0.78 ± 0.06.

The validity of the above techniques requires that amiloride abolish active conductance without affecting κp. The evidence for the abolition of active conductance was the reduction of I_0 to a mean of 4.5 ± 1.4% of control level (n=6); in addition, as mentioned, J_r becomes independent of $\Delta\psi$. Lack of an effect on κp was demonstrated in 7 additional studies employing 10^{-4} M amiloride by a mean out→in tracer chloride permeability of 95 ± 9% of control level in association with a depression of I_0 to 2.6 ± 0.4% of control level.

The values of E'_{Na} obtained here (63—110 mV) are similar to values of E_{Na} reported by others [10,12,13]. The good agreement between the two methods for evaluation of E'_{Na} and q suggests the self-consistency of the approaches employed. Admittedly there is an element of arbitrariness, in that Figs. 1 and 2 represent quasi-steady states achieved after different periods of perturbation of $\Delta\psi$; however, experiments employing more similar perturbation periods gave still lower values of q. Of course our ability to evaluate q accurately assumes the reliability of our estimates of basal and thus suprabasal oxygen consumption based on the use of amiloride. While this issue requires further study, various considerations indicate that this method is indeed appropriate (Lang, Caplan and Essig, submitted for publication).

Although the present results are preliminary, they indicate incomplete coupling of active transport and metabolism, passive transepithelial leakage pathways having been taken into account. Clearly our findings do not permit an unambiguous mechanistic interpretation; however, they indicate a constraint which must be satisfied by an appropriate model.

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