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SODIUM TRANSPORT AND OXYGEN CONSUMPTION IN TOAD BLADDER

A THERMODYNAMIC APPROACH

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Summary

The relationship between active sodium transport and oxygen consumption was investigated in toad urinary bladder exposed to identical sodium-Ringer's solution at each surface, while controlling the transepithelial electrical potential difference $\Delta\psi$. Rates of sodium transport and oxygen consumption were measured simultaneously, both in the short-circuited state ($\Delta\psi = 0$) and when $\Delta\psi$ was varied. Under short-circuit conditions, when the rates of active sodium transport changed spontaneously or were depressed with amiloride, the ratio of active sodium transport to the estimated suprabasal oxygen consumption Na^+/O_2 was constant for each tissue, but varied among different tissues. Only when $\Delta\psi$ was varied did the ratio Na^+/O_2 change with the rate of active sodium transport; under these circumstances dNa^+/dO_2 was constant but exceeded the ratio measured at short-circuit $[(Na^+/O_2)_{\Delta\psi=0}]$. This suggests that coupling between transport and metabolism is incomplete. The results are analyzed according to the principles of nonequilibrium thermodynamics, and interpreted in terms of a simple model of the transepithelial sodium transport system.

Introduction

In studies of epithelial sodium transport much attention has been directed at the Na^+/O_2 ratio relating equivalents of sodium transported to moles of oxygen consumed [1—5].

Early studies led to the concept of a unique stoichiometric ratio, common to all tissues under all conditions [3,5]. Recently Vieira et al. [4], using more precise techniques for measuring the rate of O_2 consumption in frog skins, found that the Na^+/O_2 ratio may vary from skin to skin. However, the ratio Na^+/O_2 was constant in each tissue under short-circuit conditions, whether the

rate of active sodium transport varied spontaneously or as a result of the administration of antidiuretic hormone or ouabain. In studies of toad urinary bladders Nellans and Finn reached a different conclusion and have reported that under all conditions the Na^+/O_2 ratio increased from near zero levels at low rates of transport to a maximum as rates of transport increased [2]. In arriving at this conclusion data from many tissues studied under different conditions were grouped together.

Extensive analysis of the behavior of individual tissues may in principle lead to different conclusions than those drawn from the combined data of many tissues. Furthermore, thermodynamic analysis indicates the important influence of the trans-membrane electrical potential difference on the Na^+/O_2 ratio [6]. The experiments presented in this paper are analyzed with attention to these considerations. While our data are for the most part consistent with those of Nellans and Finn, our analysis leads to different conclusions. The results can be interpreted in terms of simple models of the transepithelial sodium transport mechanism.

Materials and Methods

The experiments were performed on isolated urinary bladders of *Bufo marinus* originating from the Dominican Republic and obtained from National Reagents (Bridgeport, Conn.). Toads, stored at room temperature, were double-pithed and the excised bladders mounted in modified Ussing-Zerahn Lucite chambers of 7.1 cm^2 cross-sectional area. Both the mucosal and serosal surfaces were bathed by glucose-Ringer's solution consisting of 10 mM glucose, 115.9 Na^+, 2.5 K^+, 1.8 Ca^{2+}, 117.8 Cl^- and 2.4 HCO_3^- mequiv./l (pH 7.6, 233 mosM/kg H_2O), containing 40 mg/l gentamicin sulfate (Schering) to retard bacterial growth. The solution was passed through a 0.22 micron filtering unit before use.

The electrical potential difference $\Delta\psi(\Psi_{serosal} - \Psi_{mucosal})$ was regulated with an automatic voltage clamp, and the current I was recorded continuously. Oxygen consumption was measured polarographically with Clark electrodes (Yellow Springs Instrument Company, Yellow Springs, Ohio) in a closed system as previously described [4,7]. After an initial equilibration period of 2.5 h, the potential difference across the tissue was clamped sequentially at $\Delta\psi = 0$, ± 40, ± 80 mV for periods of 6 min each. Measurements were made during the final two minutes of each period, during which interval the tissues were in a steady state as indicated by near-constancy of the current and the rate of O_2 consumption. Following two such series, amiloride (a gift of Merck, Sharp and Dohme, N.J.) was added to the mucosal solution to a concentration of $5 \cdot 10^{-7}$ M and then 10 min later $1 \cdot 10^{-5}$ M in order to depress the short-circuit current I_0. At each steady state of current, oxygen consumption was again measured.

Measurement of suprabasal oxygen consumption

The rate of suprabasal oxygen consumption J_r^{sb} is defined as that part of the total rate of oxygen consumption J_r related to transepithelial active sodium transport. The rate of basal oxygen consumption J_r^b is related to all other activ-

ities. In accordance with previous observations in frog skin, there is a linear relationship between J_{r0} (J_r at $\Delta\psi = 0$) and I_0, whether these parameters vary spontaneously or as a result of the administration of amiloride. The intercept at zero short-circuit current is therefore conveniently taken as the rate of basal oxygen consumption J_r^b and

$$J_r^{sb} = J_r - J_r^b \tag{1}$$

Amiloride was used to determine J_r^b because it is easily administered and rapidly effective and presumably has no direct effect on the sodium pump. Furthermore, since the bath electrolyte concentrations are not altered there is no change in the electrochemical potential difference of sodium across the membrane. As previously with ouabain in frog skin [4], when active sodium transport is largely abolished by amiloride there is no demonstrable dependence of J_r on $\Delta\psi$. The implications of the use of amiloride to evaluate J_r^b will be discussed below.

Conductances

It is considered that ions cross the membrane by one of two parallel pathways (Fig. 1) [8]. The first pathway, limited only to sodium ions, is freely accessible to the sodium pump. (This pathway may incorporate varying degrees of "serosal leak.") The second pathway permits the passive movement of any ion through regions not directly accessible to the sodium pump. (This pathway is presumably largely extracellular.) The total conductance κ is the sum of the conductances of the two pathways, respectively κ^a and κ^p.

The conductance κ was evaluated by setting $\Delta\psi$ alternately at ±10 mV for

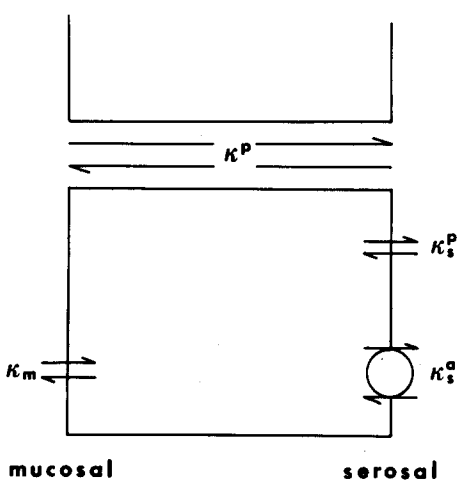

Fig. 1. Model of transepithelial sodium transport systems based on Fig. 3 of [6]. Sodium may cross the membrane via either an "active" or "passive" pathway, of conductances κ^a and κ^p respectively. Under usual operating conditions sodium enters the active pathway by way of a passive permeability barrier of conductance κ_m at the mucosal surface, and leaves by way of the sodium active transport mechanism of conductance κ_s^a at the serosal surface. There may be some degree of back-leakage through a serosal leak of conductance κ_s^p. The total conductance $\kappa = \kappa^a + \kappa^p$. The active conductance κ^a is given by $1/\kappa^a = 1/\kappa_s + 1/\kappa_m$, where $\kappa_s = \kappa_s^a + \kappa_s^p$. Thus $\kappa^a = \kappa_m(\kappa_s^a + \kappa_s^p)/(\kappa_m + \kappa_s^a + \kappa_s^p)$. In the presence of sufficient amiloride to eliminate sodium movement across the mucosal surface, $\kappa_m = 0$, $\kappa^a = 0$, and $\kappa = \kappa^p$.

5 s and applying Ohm's law, since the current-voltage relationship has been shown to be linear with brief small perturbations of $\Delta\psi$ [8].

Passive conductance

The passive conductance κ^p was evaluated by the use of amiloride. Since amiloride depresses the short-circuit current by selectively blocking the active conductance,

$$\kappa^p = (\kappa)_{I_0=0} \tag{2}$$

The administration of $1 \cdot 10^{-5}$ M amiloride to the mucosal solution did not eliminate I_0 completely, but lowered it to 1.7 ± 0.6% (n = 11) of the control level within 5 min. Since the nature of the steady state κ-I_0 relationship during this period was not precisely known κ^p was estimated by extrapolation of the straight line connecting two points on the κ-I_0 plot, one obtained in the control state, the other when amiloride had depressed I_0 to some 2% of control level. Since the wave form of the current-voltage relationship on perturbation of $\Delta\psi$ was square in the presence of $1 \cdot 10^{-5}$ M amiloride no error was introduced by calculating κ from Ohm's law immediately after perturbation of $\Delta\psi$. Estimates of κ^p by this technique differ insignificantly from those derived from the measurement of tracer $^{22}Na^+$ backflux [9].

Measurement of the rate of active sodium transport

It has been found that for Dominican toad bladders mounted in chambers the only significant transepithelial active ionic transport is that of sodium [10—12]. Accordingly the rate of active sodium transport is given by

$$J^a_{Na} = (1/F)\,(I + \kappa^p \Delta\psi) \tag{3}$$

where here κ^p was determined at the conclusion of the experiment. Saito and Essig [13] and Hong, C.D. (personal communication) have shown that in carefully mounted toad bladders κ^p remains constant for some 2—8 h. In the present study κ^p was independent of $\Delta\psi$ over a range of ±80 mV: in six hemibladders current-voltage relationships were linear after the near elimination of short-circuit current with amiloride. The mean linear correlation coefficient was 0.997 ± 0.002.

It should be noted that for the calculation of J^a_{Na} it is irrelevant to what extent κ^p represents actual physiological passive conductance or that induced by edge damage. (In the membranes employed, mean κ^a = 0.231 ± 0.028 (S.E.) $m\Omega^{-1} \cdot cm^{-2}$ (n = 11); the range was 0.134 to 0.407 $m\Omega^{-1} \cdot cm^{-2}$. The fraction of total conductance by way of the active pathway (κ^a/κ) was 0.57 ± 0.04 (S.E.); the range was 0.38 to 0.75.)

Analysis of data

Standard statistical procedures were used throughout [14]. Results are presented as mean values ± standard errors of the mean (S.E.), except for the data in Fig. 3 in which the mean and 95% confidence intervals of the data are shown. Differences in slopes dJ_{r0}/dI_0 were analyzed by Student's t-test (Fig. 3). The Wilcoxon signed rank test was used to compare J^a_{Na0}/J^{sb}_{r0} and dJ^a_{Na}/dJ^{sb}_r (Table I).

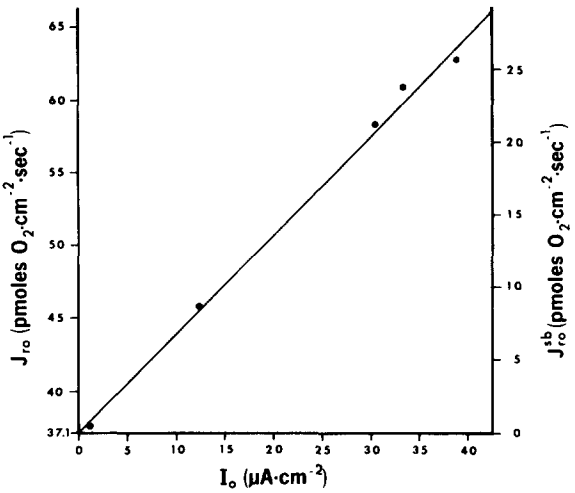

Fig. 2. Example of the relationship between J_{r0} and I_0. I_0 was allowed to vary spontaneously (above 30 $\mu A \cdot cm^{-2}$) or depressed with amiloride (below 39 $\mu A \cdot cm^{-2}$). Slope equals 0.672 pmol $O_2 \cdot \mu A^{-1} \cdot s^{-1}$. $J^{sb}_{r0} = J_{r0} - J^{b}_{r}$, where $J^{b}_{r} = (J_{r0})_{I_0=0} = 37.1$ pmol $\cdot O_2 \cdot s^{-2} \cdot cm^{-1}$. Data are from tissue 6.

Results

Relationship between J^{a}_{Na} and J_r in the short-circuited state

When the potential difference across a hemibladder was clamped at $\Delta\psi = 0$, the short-circuit current I_0 was seen to vary spontaneously. After 1.5—2.5 h of experimental observations I_0 was depressed by the addition of amiloride to the mucosal bathing solution. Concentrations of $5 \cdot 10^{-7}$ M and $1 \cdot 10^{-5}$ M reduced the current to about 50 and 2% of control values, respectively. This

TABLE I

Apparent stoichiometric ratios when $\Delta\psi = 0$ (J^{a}_{Na0}/J^{sb}_{r0}) and when $\Delta\psi$ is varied (dJ^{a}_{Na}/dJ^{sb}_{r}). Values of $\Delta\psi$ employed were 0, ±40, ±80 mV.

Tissue no.	J^{a}_{Na0}/J^{sb}_{r0} (mol Na^+/mol O_2)	dJ^{a}_{Na}/dJ^{sb}_{r} (mol Na^+/mol O_2)
1	20.4	17.1
2	17.6	21.4
3	16.5	14.1
4	15.7	22.3
5	15.3	12.8
6	15.4	23.0
7	14.8	23.1
8	13.9	24.6
9	13.1	37.8
10	10.6	18.2
11	9.3	22.5
$\bar{x}$ ± S.E.	14.8 ± 0.9	21.5 ± 2.0

$J^{a}_{Na0}/J^{sb}_{r0} < dJ^{a}_{Na}/dJ^{sb}_{r}$, $p < 0.02$

allowed us to observe active sodium transport and the associated oxygen consumption over a wide range.

Vieira et al. showed that when the frog skin was short-circuited the rate of oxygen consumption J_{r0} was linearly related to I_0, whether I_0 varied spontaneously or was altered with vasopressin or ouabain [4]. We have found the same relationship to be true for toad bladder, where amiloride was used to suppress I_0. An example is shown in Fig. 2. Individual slopes for eleven hemibladders were evaluated by linear regression analyses of data obtained in the absence and presence of amiloride. The mean correlation coefficient r equaled 0.976 ± 0.008.

This linearity facilitates the analysis of the relationship between active Na^+ transport and metabolism. The intercept $(J_{r0})_{I_0=0}$ is an estimate of basal oxygen consumption (i.e. that unassociated with transepithelial active Na^+ transport), J_r^b, while the inverse slope is proportional to an apparent stoichiometric ratio relating active Na^+ transport and suprabasal oxygen consumption

$$Na^+/O_{2(\Delta\psi=0)} = dJ^a_{Na0}/dJ_{r0} \equiv J^a_{Na0}/J^{sb}_{r0} = (1/F)I_0/J^{sb}_{r0} \quad (4)$$

Estimates of J^a_{Na0}/J^{sb}_{r0} for eleven hemibladders are listed in Table I. There is no unique stoichiometric ratio common to all tissues, since many of the slopes of the J_{r0} vs. I_0 relationship are significantly different from one another (Fig. 3). For exemple, dJ_{r0}/dI_0 for tissue 6, shown in Fig. 2 is different from that of tissues 1, 10 and 11 ($p < 0.01$). (Note that since I_0 and J_{r0} are measured in the same tissues, dJ_{r0}/dI_0 is independent of tissue area.)

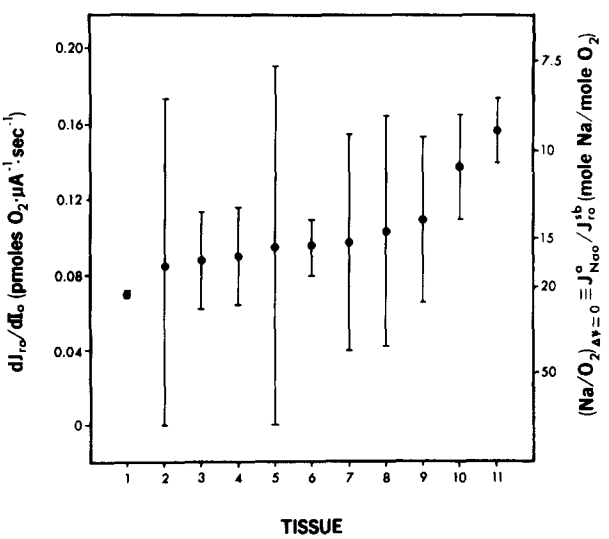

Fig. 3. Comparison of the slopes dJ_{r0}/dI_0 in eleven hemibladders. The 95% confidence intervals of the slopes dJ_{r0}/dI_0 are shown. When analyzed by the t-test, the following pairs were significantly different: $p < 0.05$: 1,8; 1,3; 3,10; 5,10; 7,10; $p < 0.025$: 1,9; 4,10; 9,11; $p < 0.01$: 1,6; 6,10; $p < 0.005$: 1,10; 2,11; 5,11; 8,11; $p < 0.001$: 1,11; 3,11; 4,11; 6,11. The apparent stoichiometric ratio at $\Delta\psi = 0$ (J^a_{Na0}/J^{sb}_{r0}) is also shown.

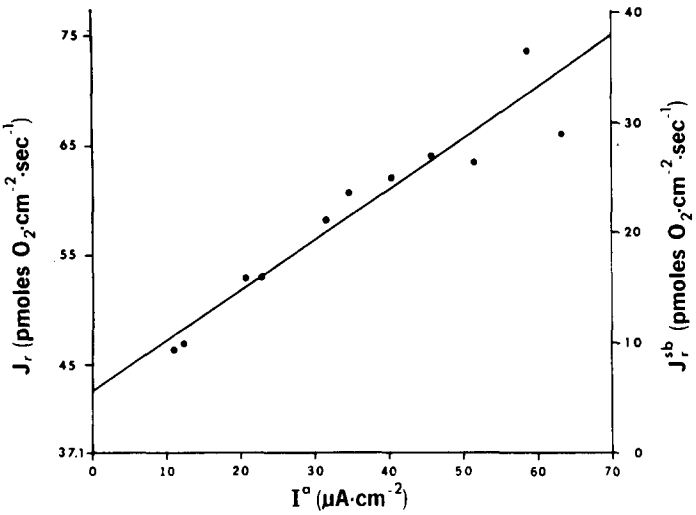

Fig. 4. Example of the relationship between J_r and I^a. J_r is linearly related to I^a, varied by clamping the potential difference across the tissue sequentially at 0, ±40, ±80 mV. Data from tissue 6. Slope equals 0.461 pmol $O_2 \cdot \mu A^{-1} \cdot s^{-1}$. Note that $dJ_r^{sb}/dJ_{Na}^a < J_{r0}^{sb}/J_{Na0}^a$ and that $(J_r^{sb})_{J_{Na}} = 0, \Delta\psi \neq 0 > (J_r^{sb})_{J_{Na}} = 0, \Delta\psi = 0$ (see also Fig. 2).

Relationship between J_{Na}^a and J_r when $\Delta\psi$ is altered

In view of the linearity between J_r and J_{Na}^a under short-circuit conditions it is of interest to examine the relationship between J_r and J_{Na}^a when the flows are altered by variation of $\Delta\psi$. Fig. 4 shows an example in which data were recorded from the same tissue shown in Fig. 2. Again a linear relationship is observed. The mean correlation coefficient r for eleven tissues evaluated individually is 0.953 ± 0.021.

As before, we can estimate by extrapolation the rate of oxygen consumption in the absence of sodium transport, and the inverse slope defines an apparent stoichiometric ratio dNa^+/dO_2. Two points are of special interest. First, at the potential for which active sodium transport is zero the mean rate of suprabasal oxygen consumption in 11 tissues, $(J_r^{sb})_{J_{Na}^a=0}$, evaluated by extrapolation, is equal to 4.4 ± 1.9 pmol $\cdot$ $cm^{-2} \cdot s^{-1}$ and is significantly greater than zero ($p < 0.025$). Second, dJ_{Na}^a/dJ_r^{sb} does not equal J_{Na0}^a/J_{r0}^{sb}. The inverse slopes measured with varying $\Delta\psi$ are significantly greater than those measured under short-circuit conditions (Table I) when compared by rank order analysis ($p < 0.02$).

Discussion

The results of our studies show that the rate of active sodium transport varies linearly with the rate of oxygen consumption in toad urinary bladders, but the relationship depends on the conditions in which the measurements are made. This may be explained by fundamental non-equilibrium thermodynamic considerations, interpreted in terms of a simple model of the transepithelial sodium transport system (Fig. 1).

Transport energetics have been described by a two-flow linear formulation

[6]. According to this formulation, when identical solutions are placed on both sides of the tissue the rate of active sodium transport J^a_{Na} is related to the rate of suprabasal oxygen consumption J^{sb}_r by the following equations:

$$J^a_{Na} = L_{Na}(-F\Delta\psi) + L_{Na\,r}\,A \tag{5}$$

$$J^{sb}_r = L_{Na\,r}(-F\Delta\psi) + L_r A \tag{6}$$

F is the Faraday constant, $\Delta\psi$ is the electrical potential difference at which the bladder is clamped, A is the affinity, essentially the negative free energy change $-\Delta G$ of the metabolic reaction driving transport, and the L's are phenomenological coefficients which represent kinetic factors. In terms of the model of the sodium transport system of Fig. 1, J^a_{Na} can be determined from measurements carried out in the presence and absence of amiloride (Eqn. 3). The demonstration that J^a_{Na} and J_r are linear functions of $\Delta\psi$ in both frog skin and toad bladder supports the validity of Eqns. 5 and 6 and indicates that the affinity and the phenomenological coefficients are unaffected by brief perturbations of $\Delta\psi$ (refs. 7 and 13, and Lang, M.A., Caplan, S.R. and Essig, A., manuscript in preparation). The applicability of the thermodynamic formulation is not restricted to the simple model of Fig. 1 but extends to any linear system. However, it should be noted that the choice of experimental protocol may affect the state of the system. The studies reported here were conducted using tissues first equilibrated at short circuit ($\Delta\psi = 0$) after which the potential was varied symmetrically around short-circuit for periods of 6 min. The resulting linearity extended over the range of ±80 mV. If the tissues had first been equilibrated at open-circuit, or some other predetermined fixed value, and the potential then varied, and/or the duration of the potential perturbation altered, we might still observe linear relationships, but the tissue properties might be altered, yielding different values of the thermodynamic parameters. Nevertheless, these parameters would characterize the system in the state under investigation. This issue requires further study.

Nellans and Finn [2] concluded that J^a_{Na}/J^{sb}_r varies with J^a_{Na}, the transport rate. However, as can be seen from the example shown in Fig. 5, their conclusion is valid only in so far as J^a_{Na} varies as a consequence of manipulation of $\Delta\psi$. At $\Delta\psi = 0$, J^a_{Na}/J^{sb}_r is constant when the transport rate varies spontaneously or is depressed with amiloride. From Eqns. 5 and 6, when $\Delta\psi = 0$:

$$dJ^a_{Na}/dJ^{sb}_r \equiv (J^a_{Na}/J^{sb}_r)_{\Delta\psi=0} = L_{Nar}/L_r \tag{7}$$

whereas when $\Delta\psi$ is varied:

$$(dJ^a_{Na}/dJ^{sb}_r)_A = L_{Na}/L_{Na\,r} \tag{8}$$

Therefore, on fundamental theoretical grounds we see that the slope dNa^+/dO_2 need not have the same value under all conditions of transport.

Similar considerations apply to the rate of metabolism in the absence of transport. The thermodynamic conditions associated with zero active sodium transport in Figs. 2 and 4 are quite different. In the former case (Fig. 2) the tissue is not working against an electrochemical potential difference. Active transport varies as a result of changes attributable to undefined metabolic and/or permeability factors, and when flow is zero there may be no need for

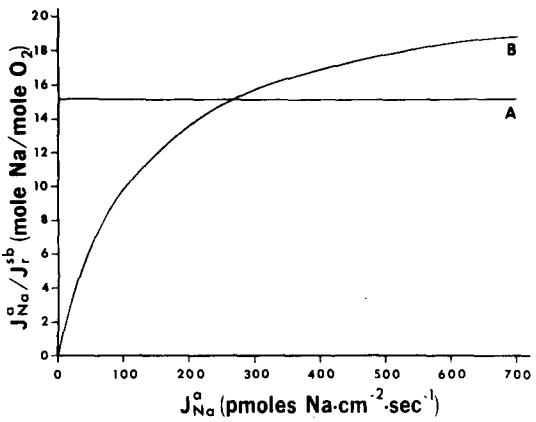

Fig. 5. Example of the relationship between Na^+/O_2 ratio and active sodium transport. The Na^+/O_2 ratio (J^a_{Na}/J^{sb}_r) at different transport rates (J^a_{Na}) is shown using data from tissue 6. When $\Delta\psi = 0$, Na^+/O_2 is constant (line A). When $\Delta\psi$ is varied, Na^+/O_2 increases with the rate of active transport, indicating incomplete coupling between transport and metabolism (line B). In line A the ordinate represents the quotients of corresponding values of $I_0/F \equiv J^a_{Na0}$ and J^{sb}_{r0} obtained from the least-squares line of Fig. 2 ($\Delta\psi = 0$). In line B the ordinate represents the quotients of corresponding values of $I^a/F \equiv J^a_{Na}$ and J^{sb}_r obtained from the least-squares line of Fig. 4 ($\Delta\psi$ varying).

the expenditure of metabolic energy. Hence the rate of metabolism under these conditions may be considered to be basal.

The situation differs when tissues are studied in the presence of an electrochemical potential difference. If this is sufficiently large, transport will come to a halt ("static head" [15]). In this case, however, there would be a tendency for the system to relax, with eventual dissipation of the electrochemical potential difference, if there were not a countervailing metabolic force. In general, the prevention of such relaxation requires the expenditure of metabolic energy over and above the basal level. Only for a completely coupled system, in which there is a fixed stoichiometric ratio between rates of transport and metabolism, irrespective of the forces, can an electrochemical potential difference be maintained without the expenditure of metabolic energy. Since $(J^{sb}_r)_{J^a_{Na}=0}$ appears not to be equal to zero, it would be expected that $dJ^a_{Na}/dJ^{sb}_r \neq J^a_{Na}/J^{sb}_r$, and thus whereas dJ^a_{Na}/dJ^{sb}_r is constant, J^a_{Na}/J^{sb}_r will vary with $\Delta\psi$ *.

For completely coupled processes, e.g., chemical reactions, it is useful to consider the stoichiometry which governs the process under all conditions. By analogy, since $(J^a_{Na}/J^{sb}_r)_{\Delta\psi=0}$ is constant, it is attractive to speak of an apparent stoichiometric ratio for active sodium transport. However, it is important to

* Since amiloride has been claimed to influence the electrical potential difference acorss the serosal membrane of the toad bladder [20], presumably the site of the sodium pump, it might be expected that J^a_{Na0}/J^{sb}_{r0} would vary with differing doses of amiloride, rather than being constant as observed.

A possible explanation follows from the linearity of the system and the fact that J_{r0} at $I_0 = 0$ is taken as the rate of basal oxygen consumption J^b_r. Thus, linearity of the overall system in the transepithelial potential difference $\Delta\psi$ indicates linearity in the potential difference at the serosal surface $\Delta\psi_s$. Consider now that, with $\Delta\psi$ clamped at zero, $\Delta\psi_s$ is varied by the application of amiloride. Then, designating by $\Delta\psi^0_s$ the value of $\Delta\psi_s$ for $I_0 = FJ^a_{Na} = 0$, we have $J^a_{Na0}[\Delta\psi_s] = \alpha(\Delta\psi_s - \Delta\psi^0_s)$ and $J_{r0}[\Delta\psi_s] - J_{r0}[\Delta\psi^0_s] \equiv J_{r0}[\Delta\psi_s] - J^b_r \equiv J^{sb}_{r0} = \beta(\Delta\psi_s - \Delta\psi^0_s)$ where α and β are constants. Hence $J^a_{Na0}/J^{sb}_{r0} \equiv \alpha/\beta$ is constant. (This presumes that the conductance of the serosal surface is not affected significantly by mucosal amiloride, an issue currently in dispute [19,20].)

emphasize that the stoichiometry here is only apparent, since it varies with the conditions of operation of the system. If in fact active sodium transport and suprabasal metabolism were completely coupled in the toad bladder it would be found that $(J^a_{Na}/J^{sb}_r)_{\Delta\psi=0} = dJ^a_{Na}/dJ^{sb}_r$ = constant (irrespective of whether $\Delta\psi = 0$), and thus also $(J^{sb}_r)_{J^a_{Na}=0} = 0$. The finding that these conditions are not obeyed suggests incompleteness of coupling, or in terms of Kedem and Caplan's thermodynamic analysis, $|q| < 1$ [15] *. A systematic analysis in these terms will be presented elsewhere.

Possible causes of non-stoichiometric relationships between transport and metabolism have been discussed elsewhere [4,16]. One possibility, indicated in Fig. 1, is recirculation of transported sodium. To the extent that a passive serosal leak permits re-entry of transported sodium into the active transport pool, setting $\Delta\psi$ at a value such that $J^a_{Na} = 0$ would not be associated with inactivity of the pump, but rather with active transport exactly compensated by leak. Hence the rate of suprabasal oxygen consumption $(J^{sb}_r)_{J^a_{Na}=0}$ would not be zero. Under these circumstances, if $\Delta\psi$ were varied experimentally, J^a_{Na}/J^{sb}_r would vary, ranging from some finite value at, say, $\Delta\psi = 0$, to zero at $(\Delta\psi)_{J^a_{Na}=0}$. Furthermore, to the extent that serosal leak differed in various tissues, it would be expected that the apparent stoichiometric ratios would differ. However, in studies in toad bladder Canessa et al. [17] have recently found that the removal of sodium from the serosal bathing solution had no effect on the rate of CO_2 production J_{CO_2} in the presence of amiloride [17]. Hence they concluded that recycling of serosal sodium must be minimal and cannot account for variations in stoichiometry. This demonstration points to other possible causes of variable stoichiometry and uncoupling in the toad bladder. For example, metabolic side reactions might interfere with the coupling of oxidative metabolic processes to ATP production, or there might be partial uncoupling of the mechanism linking ATP utilization and sodium translocation. Thus it would appear that the mechanisms under study cannot be appropriately interpreted in terms of a mechanism such as that of Rapoport which postulates a fixed stoichiometric relationship between the rates of metabolism and transport via the pump [18].

Clearly our ability to evaluate the degree of coupling accurately depends on the reliability of our estimates of J^b_r and hence J^{sb}_r. The validity of our calculation of J^{sb}_r by the use of amiloride to evaluate J^b_r requires that the processes of suprabasal and basal oxygen consumption be discrete and that the rate of basal oxygen consumption be unaffected by changes in the rate of active sodium transport. Al-Awqati et al. and Maffly et al. have presented evidence that, in the toad bladder, metabolism which supports transepithelial sodium transport is indeed functionally separate from metabolism which supprts other processes [21,22]. Furthermore, our calculation of constant J_{Na0}/J^{sb}_{r0} ratios indicates that J^b_r cannot be varying significantly with variation in the rate of active sodium transport.

The use of amiloride to evaluate J^b_r also requires that in the presence of this agent metabolism of the sodium transport system be brought to a halt.

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* The degree of coupling is defined as $q = L_{Nar}/(L_{Na}L_r)^{1/2}$. It can be seen that for $0 < q < 1$, $(L_{Nar}/L_r) < (L_{Na}/L_{Nar})$. Therefore it would be predicted that $(dJ^a_{Na}/dJ^{sb}_r)_{\Delta\psi=0} < (dJ^a_{Na}/dJ^{sb}_r)_A$, as was found experimentally.

On first sight it might seem however, that with an incompletely coupled system pump-associated metabolism would continue in the presence of amiloride, as required for the maintenance of static head, as discussed above. Nevertheless, it must be appreciated that the static head, achieved by establishing an appropriate transepithelial potential difference is quite a different state from that achieved by the administration of amiloride. In the former case, assuming passivity of the outer membrane, intracellular sodium is at equilibrium with that in the outer bathing solution. In the latter case, with a very high resistance of the outer membrane in the presence of amiloride, intracellular sodium will be at a substantially lower electrochemical potential than in the outer solution, particularly in view of the recent evidence for minimal recycling of serosal sodium [17]. It would therefore be anticipated that the intracellular sodium concentration would be at the lowest possible level. Under these circumstances kinetic considerations suggest that (Na^+ + K^+)-ATPase activity would be minimal, so that J_r must closely approximate J_r^b.

If despite these considerations amiloride gives an overestimate of J_r^b, q will be overestimated, and vice versa. In either case the present analysis demonstrates that only with complete coupling ($q = 1$) will J_{Na}^a/J_r^{sb} be constant, irrespective of the value of the electrochemical potential difference of sodium across the epithelial membrane.

Glossary

F Faraday's constant ($C \cdot equiv.^{-1}$)
I electrical current ($\mu A \cdot cm^{-2}$) *
I_0 short-circuit current $(I)_{\Delta\psi=0}$ ($\mu A \cdot cm^{-2}$)
J_{Na}^a rate of active sodium transport ($pmol \cdot cm^{-2} \cdot s^{-1}$)
J_{Na0}^a rate of active sodium transport at $\Delta\psi = 0$ ($pmol \cdot cm^{-2} \cdot s^{-1}$)
J_r rate of oxygen consumption ($pmol \cdot cm^{-2} \cdot s^{-1}$)
J_r^{sb} rate of suprabasal oxygen consumption ($pmol \cdot cm^{-2} \cdot s^{-1}$)
J_{r0}^{sb} rate of suprabasal oxygen consumption at $\Delta\psi = 0$ ($pmol \cdot cm^{-2} \cdot s^{-1}$)
J_r^b rate of basal oxygen consumption ($pmol \cdot cm^{-2} \cdot s^{-1}$)
L_{Na}, L_{Nar}, L_r phenomenological coefficients ($\mu mol^2 \cdot cm^{-2} \cdot s^{-1} \cdot kcal^{-1}$)
$Na^+/O_2 \equiv J_{Na}^a/J_r^{sb}$
κ total conductance ($m\Omega^{-1} \cdot cm^{-2}$)
κ^a conductance of the active pathway ($m\Omega^{-1} \cdot cm^{-2}$)
κ^p conductance of the passive pathway ($m\Omega^{-1} \cdot cm^{-2}$)
$\Delta\Psi$ potential difference ($\Psi_{serosal} - \Psi_{mucosal}$) (mV)

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* In general $I_i = z_i F J_i$, where z_i is the valence of species i. Since for Na^+, $z = 1$ equiv./mol, z_{Na} will be omitted throughout.

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