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Thermodynamic Analysis of Active Sodium Transport and Oxidative Metabolism in Toad Urinary Bladder

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Summary. Measurements of electrical current and oxygen consumption were carried out concurrently under voltage clamp conditions in 11 toad hemibladders. Inhibition of active transport with amiloride then permitted evaluation of the passive conductance and the rate of basal oxygen consumption J_r^b, allowing the simultaneous determination of the rates of active sodium transport J_{Na}^a and suprabasal oxygen consumption J_r^{sb}. J_{Na}^a and J_r^{sb} were linear functions of the electrical potential difference over a range of ± 80 mV. This allowed the comprehensive application of a linear nonequilibrium thermodynamic formalism, leading to the evaluation of the affinity A (negative free energy) of the metabolic reaction driving transport, all phenomenological coefficients, and the degree of coupling q relating transport to metabolism. Values of A determined by two techniques were $A_1 = 56.0 \pm 5.8$ and $A_2 = 58.2 \pm 6.5$ kcal per mole. Values of q determined by two techniques agreed well and were less than 1, indicating incompleteness of coupling, and hence lack of fixed stoichiometry between Na transport and O_2 consumption. The affinity and the electromotive force of sodium transport E_{Na} are not closely correlated, reflecting the fact that E_{Na} comprises both kinetic and energetic factors.

Symbols

A	affinity of metabolic reaction (kcal · mole^{-1})
E_{Na}	electromotive force of sodium transport (mV) $(=(\Delta\psi)_{J_{Na}^a=0})$
F	Faraday's constant (kcal · mV^{-1} · Equiv^{-1})
I	electrical current (μA · cm^{-2})
J_{Na}^a	rate of active sodium transport (pmoles · cm^{-2} · s^{-1})
J_r	rate of oxygen consumption (pmoles · cm^{-2} · s^{-1})
J_r^{sb}	rate of suprabasal oxygen consumption (pmoles · cm^{-2} · s^{-1})
J_r^b	rate of basal oxygen consumption (pmoles · cm^{-2} · s^{-1})
L_{Na}, $L_{Na,r}$, L_r	phenomenological coefficients (μmoles^2 · cm^{-2} · s^{-1} · kcal^{-1})
q	degree of coupling $L_{Na,r}/(L_{Na}L_r)^{1/2}$ relating active transport and metabolism
κ	total conductance (mmho · cm^{-2})
κ^a	active conductance (mmho · cm^{-2})
κ^p	passive conductance (mmho · cm^{-2})
$\Delta\psi$	potential difference $(\psi_{serosal} - \psi_{mucosal})$ (mV).

The subscript zero indicates measurements made at $\Delta\psi = 0$.

In a previous publication [4] a formulation was presented for the analysis of epithelial active sodium transport as a coupled two-flow linear process. In this view the rates of both active sodium transport and the associated oxidative metabolism are linear functions of the electrochemical potential difference of sodium across the epithelium and the affinity (negative free energy) of the metabolic driving reaction. Various aspects of this approach have been separately tested in frog skin [19], toad skin [3] and toad bladder [15]; we present here a more detailed analysis in the toad bladder. The formulation allows the evaluation of phenomenological (kinetic) coefficients and energetic parameters under a wide variety of operating conditions. In particular, the data permit a calculation of the degree of coupling between transport and metabolism, and a comparison of the energetic parameters of the nonequilibrium thermodynamic representation and the classical equivalent circuit model.

Materials and Methods

The following is a brief summary of techniques which have been reported elsewhere [8]. Isolated urinary bladders of *Bufo marinus* (Dominican Republic) were mounted in Lucite chambers of 7.1 cm^2 cross-sectional area, and bathed by glucose-Na Ringer's solution containing gentamicin sulfate. The electrical potential difference $\Delta\psi$ across the bladders was regulated automatically, and the current I was recorded continuously. Oxygen consumption was measured with Clark electrodes [8, 18, 19]. After equilibration for 2.5 hr, $\Delta\psi$ was clamped sequentially at 0, ± 40, ± 80 mV for 6-min periods. Measurements were made during the final 2 min, when 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, 1×10^{-5} M mucosal amiloride (Merck, Sharp & Dohme, N.J.) was used to depress the short-circuit current $I_0 (I_{\Delta\psi=0})$ to near zero and oxygen consumption was again measured.

Measurement of the Rate of Suprabasal Oxygen Consumption, J_r^{sb}

J_r^{sb} is 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 being related to all other activities [8]. Since there is a linear relationship between $J_{r0} (J_r$ at $\Delta\psi = 0)$ and I_0, whether these parameters vary spontaneously or following amiloride, the intercept at $I_0 = 0$ is taken as J_r^b, and

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

Measurement of Passive Conductance, κ^p

κ^p was evaluated by the use of amiloride, which depresses I_0 by selectively blocking the active conductance, so that

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

The conductance κ was evaluated by setting $\Delta\psi$ alternately at ± 10 mV for 5 sec and applying Ohm's law [15].

Measurement of the Rate of Active Sodium Transport, J^a_{Na}

For Dominican toad bladders mounted in chambers the only significant transepithelial active ionic transport is that of sodium [9, 10, 11], and the current-voltage relationship is linear, both in the absence and presence of 10^{-5} M amiloride [8]. Accordingly, $I = FJ^a_{Na} - \kappa^p \Delta\psi$, and so

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

In carefully mounted toad bladders κ^p remains constant for some 2–8 hr [14], so that κ^p is conveniently determined at the conclusion of the experiment.

Interpretation of Data

With identical solutions bathing both sides of the tissue the nonequilibrium thermodynamic representation for active sodium transport in toad bladder takes the form of two linear equations:

$$J^a_{Na} = L_{Na}(-F\Delta\psi) + L_{Na,r}\,A, \tag{4}$$

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

Here A is the thermodynamic affinity of the driving process, and the L's are phenomenological coefficients representing kinetic factors.

Standard statistical procedure were used [16], and results are presented as mean values $\pm$ standard errors of the mean (SE).

Results

Dependence of J^a_{Na} on $\Delta\psi$

Linearity of the rate of active sodium transport J^a_{Na} in the electrical potential difference $\Delta\psi$ was inferred in a previous study from linear current-voltage relationships in tissues in which a high fraction of total conductance was attributable to the active pathway [15]. In the present study J^a_{Na} was evaluated according to Eq. (3), employing the amiloride technique described in Methods to measure κ^p. An example of the relationship between J^a_{Na} and $\Delta\psi$ is shown in Fig. 1*a*. A linear relationship over a range of ± 80 mV was observed consistently. Values of $dJ^a_{Na}/d(\Delta\psi)$ and the linear correlation coefficient r are given in Table 1.

Dependence of J_r on $\Delta\psi$

Measurements of the rate of oxygen consumption J_r were carried out simultaneously with the above measurements of J^a_{Na}. Again a linear

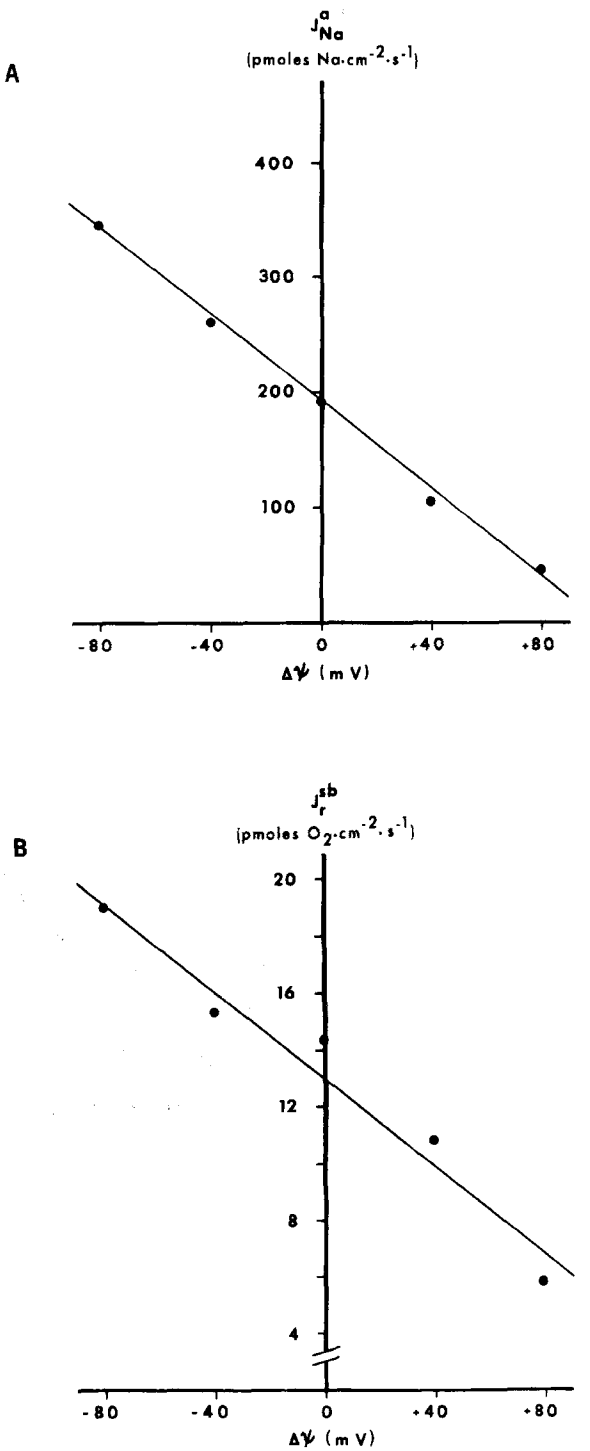

Fig. 1. Relationships between (*a*) J^a_{Na} and $\Delta\psi$, and (*b*) J^{sb}_r and $\Delta\psi$ (tissue #5)

Table 1. Relationships between J^a_{Na}, J_r, and $\Delta\psi$ for 11 hemibladders[a]

tissue #	$-dJ^a_{Na}/d(\Delta\psi)$		$-dJ_r/d(\Delta\psi)$	
	pmoles · cm^{-2} · s^{-1} · mV^{-1}	$-r$	pmoles · cm^{-2} · s^{-1} · mV^{-1}	$-r$
1	2.50	0.994	0.068	0.945
2	1.39	0.995	0.062	0.999
3	3.12	0.954	0.142	0.967
4	2.09	0.999	0.100	0.980
5	1.88	0.999	0.077	0.978
6	1.68	0.945	0.081	0.913
7	1.42	0.981	0.070	0.858
8	4.22	0.999	0.231	0.997
9	1.65	0.999	0.129	0.998
10	2.63	0.996	0.189	0.999
11	3.77	0.999	0.221	0.999
$\bar{x}\pm$SE	2.40±0.29		0.125±0.019	

[a] r is the correlation coefficient.

relationship with $\Delta\psi$ was observed over a range of ±80 mV. The behavior of the hemibladder of Fig. 1*a* is shown in Fig. 1*b*. This dependence was previously shown to hold for frog skin [12, 19]. Values of $dJ_r/d(\Delta\Psi)$ and r are shown in Table 1.

Determination of A

Values of $dJ_r/d(\Delta\psi)$ combined with values of the short-circuit current I_0 permit the evaluation of the affinity A [4, 19], here designated A_1:

$$A_1 = -I_0/(dJ_r/d(\Delta\psi)). \tag{6}$$

The measurement of $I_0 = FJ^a_{NaO}$ and $dJ^a_{Na}/d(\Delta\psi)$ permits also the calculation of $(\Delta\psi)_{J^a_{Na}=0}$ and, with dJ^a_{Na}/dJ_r, another set of values of the affinity, designated A_2:

$$A_2 = (dJ^a_{Na}/dJ_r)_A\, F(\Delta\psi)_{J^a_{Na}=0}. \tag{7}$$

The good agreement corresponding values of A_1 and A_2 (Table 2) indicates the consistency of the techniques employed.

Calculation of q

The use of amiloride to depress active transport to near-zero allows not only the evaluation of the passive conductance κ^p, but also the estimation

Table 2. Calculation of the affinity A in 11 hemibladders, evaluated by two different methods[a]

tissue #	I_0	$(\Delta\psi)_{J^a_{Na}=0}$	dJ^a_{Na}/dJ_r	A_1	A_2
	$\mu A \cdot cm^{-2}$	mV	mole Na · mole O_2^{-1}	kcal · mole^{-1}	
1	23.6	98	37.8	83.4	85.1
2	18.1	135	22.5	69.3	69.8
3	34.5	115	23.0	57.8	60.7
4	22.3	110	21.4	52.9	54.1
5	18.2	100	24.6	56.1	56.6
6	26.7	165	22.3	77.7	84.5
7	22.9	167	23.1	78.4	88.9
8	38.2	94	18.2	39.3	39.3
9	17.8	111	12.8	32.8	32.8
10	23.9	94	14.1	30.1	30.3
11	35.8	99	17.1	38.7	38.8
$\bar{x}\pm$SE	25.6±2.2	117±8	21.5±2.0	56.0±5.8	58.2±6.5

[a] $I_0(=FJ^a_{Na0})$ for Eq. (6) and $(\Delta\psi)_{J^a_{Na}=0}$ (equivalent to E_{Na}) for Eq. (7) were evaluated from the line relating J^a_{Na} and $\Delta\psi$ at $\Delta\psi=0$ and $J^a_{Na}=0$, respectively (*see* Fig. 1 *a*).

of the rate of basal oxygen consumption J^b_r, and thus the rate of suprabasal oxygen consumption J^{sb}_r associated with trans-epithelial active sodium transport (Table 3). This in turn permits the evaluation of the degree of coupling by either of two methods [4]:

$$q_1 = \left[1 - \frac{(J^{sb}_r)_{J^a_{Na}=0}}{J^{sb}_{r0}}\right]^{1/2} \tag{8}$$

$$q_2 = \left[\frac{J^a_{Na0}/J^{sb}_{r0}}{dJ^a_{Na}/dJ^{sb}_r}\right]^{1/2}. \tag{9}$$

The good agreement between corresponding values of q_1 and q_2 again indicates the consistency of the techniques employed (Table 3).

Calculation of L_{Na}, $L_{Na,r}$, L_r

Two of the phenomenological coefficients are given directly by data presented above:

$$L_{Na} = (-1/F)\,(dJ^a_{Na}/d(\Delta\psi))_A. \tag{10}$$

$$L_{Na,r} = (-1/F)\,(dJ^{sb}_r/d(\Delta\psi))_A. \tag{11}$$

(The linear relationships between J^a_{Na}, J^{sb}_r, and $\Delta\psi$ indicate that A is invariant on brief perturbation of $\Delta\psi$.) The availability of a means for the

Table 3. Calculation of the degree of coupling q in 11 hemibladders, evaluated by two methods[a]

tissue #	J_r^b	J_{r0}^{sb}	$(J_r^{sb})_{J_{Na}=0}$	q_1	q_2
	pmole · cm^{-2} · s^{-1}				
1	20.6	17.1	10.5	0.62	0.59
2	6.3	19.2	10.7	0.66	0.64
3	37.1	21.8	5.4	0.87	0.82
4	24.4	11.4	0.3	0.99	0.91
5	17.2	13.0	5.3	0.77	0.75
6	23.3	15.6	2.1	0.93	0.84
7	20.4	13.9	2.2	0.92	0.80
8	42.5	39.0	17.3	0.75	0.76
9	23.2	13.2	−1.2	1.04	1.09
10	22.2	16.1	−1.7	1.05	1.08
11	30.5	18.9	−2.8	1.07	1.09
$\bar{x}\pm$SE	24.3±2.9	18.1±2.3	4.4±1.9	0.87±0.05	0.85±0.05

[a] $(J_r^{sb})_{J_{Na}=0}$ was calculated by extrapolating the line relating J_r^{sb} and J_{Na}^a to the point where $J_{Na}^a=0$.

Table 4. Calculation of phenomenological coefficients in 11 hemibladders[a]

Tissue #	L_{Na}	$L_{Na,r}$	L_r
	μmole^2 · cm^{-2} · s^{-1} · kcal^{-1}		
1	108.4	2.93	0.203
2	60.3	2.70	0.276
3	135.3	6.17	0.367
4	90.6	4.35	0.213
5	81.5	3.35	0.230
6	72.9	3.55	0.192
7	61.6	3.02	0.166
8	183.0	10.04	0.991
9	71.6	5.60	0.402
10	114.1	8.20	0.533
11	163.5	9.58	0.487
$\bar{x}\pm$SE	103.9±12.5	5.41±0.83	0.369±0.073

[a] In the calculation of L_r with Eq. (12), A was taken as the mean of A_1 and A_2.

measurement of J_r^b and J_r^{sb} allows also the evaluation of L_r:

$$L_r=(J_r^{sb})_{\Delta\psi=0}/A. \tag{12}$$

Values of L_{Na}, $L_{Na,r}$, and L_r, calculated from the application of Eqs. (10), (11), and (12), respectively, are given in Table 4.

Discussion

Various aspects of linearity of the thermodynamic flows in the electrochemical potential difference have been tested previously [3, 15, 19]. It should be noted that in the previous study in toad bladder [15] the delineation of the current-voltage relationship involved perturbations of $\Delta\psi$ for some 15–30 sec. In this study, in order to conform to the protocol for the measurement of rates of oxygen consumption, $\Delta\psi$ was varied for periods of 6 min.[1] The demonstration of linearity of J_r in $\Delta\psi$ in toad bladder is particularly interesting in that the measurements were performed simultaneously with the measurements of J^a_{Na}.

In claiming linearity in the toad bladder over a range of ± 80 mV, we are referring only to the specific conditions of our experiments. Measurements were made well below the E_{Na}, and it is conceivable that in tissues with a lower E_{Na}, the range of linearity might well be compressed. However, this is of no significance for the application of the thermodynamic formulation.

The above demonstrated linearities provide a partial test of the validity of the nonequilibrium thermodynamic formalism. It has not yet been possible to test linearity in the affinity A and the validity of the Onsager reciprocal relations. Presuming that the formalism is valid, the affinity A of the metabolic driving reaction can be determined by either of two modes of calculation [Eqs. (6) and (7)]. The agreement shown between these two determinations speaks for the self-consistency of the techniques employed.

The determination of J^b_r and κ^p by the use of amiloride allows a complete thermodynamic characterization of the system, comprising the affinity and the three phenomenological coefficients.

In terms of the phenomenological coefficients the thermodynamic degree of coupling is given by [6]

$$q = L_{Na,r}/(L_{Na}\, L_r)^{1/2}; \tag{13}$$

as a consequence of the second law of thermodynamics, $|q| \leqq 1$. The degree of coupling is a measure of the interdependence of transport and metabolism. In the case of complete coupling ($q^2 = 1$) there is a unique stoichiometric ratio between J^a_{Na} and J^{sb}_r under all conditions of operation, that is J^a_{Na}/J^{sb}_r is independent of $\Delta\psi/A$. When q is zero, on the other hand, J^a_{Na}/J^{sb}_r

1 Because of the square wave response on perturbation of $\Delta\psi$ in the presence of amiloride κ^p was conveniently determined from 5 sec perturbations.

is proportional to $\Delta\psi/A$; the two processes are independent and each flow is proportional only to its conjugate force. As can be seen in Table 4, coupling in the toad bladder is incomplete. This demonstrates that fixed stoichiometry cannot be expected. Although J^a_{Na}/J^{sb}_r measured at "level flow", i.e., in the absence of an electrochemical potential difference for Na, depends only on the kinetic parameter $L_{Na,r}/L_r$, away from level flow the apparent stoichiometric ratio is a function of both kinetic and energetic parameters [8].

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 appropriateness of employing amiloride for this purpose is suggested by the finding elsewhere that J^a_{Na0}/J^{sb}_{r0} appears constant over a wide range of J^a_{Na0} [8]. If, however, amiloride acts directly only on the mucosal surface, so that the pump achieves a static head, J^b_r and q were overestimated. Nevertheless, q is demonstrably less than 1 ($p<0.025$). The same conclusion was reached by Lahav *et al.* in studies of frog skin [7].

Finally, it is worthwhile to consider the relationship between the linear two-flow formulation and the classical equivalent circuit model [17]. While the two treatments are not inconsistent, the latter deals only with output parameters, whereas the former considers input parameters as well.

The quantity A may be considered a fundamental energetic parameter on thermodynamic grounds [4]. This point of view is supported by recent experimental findings [12]. In contradistinction, the electromotive force for Na transport of the equivalent circuit model, E_{Na}, reflects both kinetic and energetic factors. This is seen by considering that at $\Delta c=0$, from Eq. (4):

$$E_{Na}=(\Delta\psi)_{J^a_{Na}=0}=(1/F)\,(L_{Na,r}/L_{Na})\,A. \tag{14}$$

A correlation exists between E_{Na} and A in the eleven tissues studied, but this correlation is loose ($r=0.63$) (Table 2). Also, recent work indicates that E_{Na} and A are influenced differently by different agents [5, 12].

It is pertinent to ask what insight the above thermodynamic analysis can provide into the nature of the active sodium transport process. The significance of the thermodynamic affinity is evident: presuming that the linear thermodynamic formulation is valid, A represents the negative Gibbs free energy of a metabolic reaction driving the transport process, and is thus a fundamental parameter in evaluation of the effectiveness of energy utilization [4]. Furthermore, measurements of A in the presence of agents influencing the rate of sodium transport will help to distinguish between effects on energetic and kinetic parameters.

The utility of the phenomenological coefficients is less evident. According to the currently accepted model of the sodium transport system, sodium enters the cells passively at the mucosal (apical) surface and is extruded by a Na^+-K^+-ATPase at the serosal (basal-lateral) surface. In principle it is possible that there may be some "recycling" of sodium through a serosal leak. In terms of this model, for a system of constant affinity, $L_{Na}=(1/F^2)\,\kappa^a$ [5, 14], and $L_{Na,r}$ reflects the interaction between transport and metabolism [4, 12]. Obviously, however, the precise dependence of the thermodynamic phenomenological coefficients on cell parameters is complex, and requires further investigation. Even pending such investigations, however, the evaluation of the phenomenological coefficients under varying experimental conditions may help to clarify mechanisms. For example, the present results speak against any model of the active transport system which invokes a fixed stoichiometric relationship between transport and metabolism. Since Canessa, Labarca, and Leaf's recent study indicates that variable stoichiometric ratios cannot be attributed to recycling of sodium [2], it is necessary to consider the possible importance of other sources of uncoupling [1, 8, 13]. These would include, for example, partial uncoupling of oxidative phosphorylation, or of the mechanism linking ATP utilization and sodium translocation.

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