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KINETICS OF BIDIRECTIONAL ACTIVE SODIUM FLUXES IN THE TOAD BLADDER

DANIEL WOLFF and ALVIN ESSIG

Department of Physiology, Boston University School of Medicine, Boston, Mass. 02118 (U.S.A.)

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Summary

The kinetics of isotopic Na^+ flows was studied in urinary bladders of toads from the Dominican Republic. Initial studies of the potential dependence of passive serosal to mucosal $^{22}Na^+$ efflux demonstrated the absence of isotope interaction and/or other coupling with passive Na^+ flow. The electrical current I and mucosal to serosal $^{22}Na^+$ influx were then measured with transmembrane potential clamped at $\Delta\psi$ = 0, 25, 50, 75 or 100 mV. Subsequent elimination of active Na^+ transport with mucosal amiloride permitted calculation of the rates of active Na^+ transport J^a_{Na} and active and passive influx $\vec{J}^a_{Na}$ and $\vec{J}^p_{Na}$. The results indicate that for Dominican toad bladders mounted in chambers only Na^+ contributes significantly to transepithelial active ion transport; hence $J^a_{Na} = J^a$. J^a was abolished at $\Delta\psi = E$ = 96.3 ± 1.9 (S.E.) mV. As $\Delta\psi$ approached E, active efflux $\overleftarrow{J}^a$ became demonstrable. At $\Delta\psi$ = 100 mV, $\overleftarrow{J}^a$ exceeded $\vec{J}^a$, so that J^a was negative. Experimental values of $\vec{J}^a$ agreed well with theoretical values predicted by a thermodynamic formulation: $\vec{J}^a_{exp} = 0.985\ \vec{J}^a_{theor}$ (r = 0.993). The dependence of $\vec{J}^a$ on $\Delta\psi$ is curvilinear.

Introduction

Tracers are used extensively to evaluate membrane permeability and the forces promoting transport. Often, however, the interpretation of tracer data is difficult, since in some cases the tracer permeability differs appreciably from the permeability to net flow and the flux ratio is "abnormal" [1—3].

These problems have been analyzed in terms of a variety of models [1—3]. Kedem and Essig [4] have provided a more general thermodynamic analysis, according to which both of the above anomalies are attributable to coupling between the flows of the abundant and tracer species. Experimental studies in synthetic ion-exchange membranes have demonstrated the existence of such "isotope interaction" in passive systems, and have supported the validity of the

above thermodynamic formulation [5,6], as have studies of passive ion fluxes in the toad bladder [7].

Chen and Walser [8] have recently further examined the kinetics of sodium isotope flows in the toad bladder, demonstrating bidirectionality of sodium movements through the active pathway [8]. The compatibility of their results with the general thermodynamic formulation indicates also the possibility of evaluating the extent of isotope interaction [9].

The aim of this work is to test further the validity of the thermodynamic formulation of isotope flows in the case of an active transport system.

Materials and Methods

(A) General methods

Urinary bladders of female toads (*Bufo marinus*, Dominican Republic) (National Reagents, Bridgeport, Conn.), were studied by methods described previously [10,11]. The membranes were mounted in modified Ussing-Zerahn chambers of 7.1 cm^2 cross-sectional area. In order to minimize edge damage membranes were mounted carefully without stretching. Each reservoir was filled with 20 ml Na^+/Ringer solution. The electrical potential difference $\Delta\psi$ ($\psi_{\text{serosal}} - \psi_{\text{mucosal}}$) was regulated with a voltage clamp and the current I, mucosa to serosa, was recorded continuously. A membrane was rejected if the open circuit potential was less than 50 mV 30 min after mounting, or if the fraction of total conductance attributable to the active pathway κ^a/κ (see below) was less than 0.5. In studies of Results, No. 4, initial mean open circuit potential was 64.7 ± 5.0 (S.E.) mV, mean short circuit current was 29.9 ± 6.1 (S.E.) $\mu A/cm^{-2}$, and mean κ^a/κ was 0.64 ± 0.03 (S.E.). ($n = 9$).

(B) Isotope fluxes

(1) Characteristics of passive pathway. The kinetic properties of the passive pathway were evaluated by the determination of serosal to mucosal tracer sodium flux. Bladders were mounted for 30 min at open circuit and $\Delta\psi$ was then clamped at 0 mV. 30 min later 25 μCi of ^{22}Na were added to the serosal bath. Following an equilibration period of 30 min, 500-μl samples were taken from the mucosal bath at 30-min intervals for three periods, the original volume always being restored with non-radioactive Na^+/Ringer solution. Additional samples were then taken at $\Delta\psi$ = 50 and 0 mV for three sequential periods of 30 min at each potential as above. Samples of the serosal medium were taken at the beginning and end of the experiment. The mucosal samples and 1 : 20 dilutions of the serosal samples were counted in a Nuclear Chicago gamma counter.

The serosal to mucosal unidirectional flux $\overleftarrow{J}$ was computed from the quotient of the isotope flux J^* and the specific activity, ρ, of the serosal medium. (The contribution of the specific activity of the mucosal medium to the gradient $\Delta\rho$ was trivial.)

(2) Equivalence of active current and active Na^+ flux. Bladders were mounted for 30 min at open circuit and then $\Delta\psi$ was clamped at 0 mV. 30 min later, 25 μCi of ^{22}Na were added to the mucosal bath. Following an equilibration period of 15—20 min, 500-μl samples were taken from the serosal bath

every 15 min for five periods, the original volume being restored with non-radioactive Ringer solution. Initial and final mucosal samples were diluted appropriately for counting as above. (A few additional studies were carried out at $\Delta\psi$ = 25 and 50 mV; see section B3 below.)

(3) Test of general flux ratio relationship. The bladders were mounted at open circuit for 30 min and then short circuited. 30 min later, 25 μCi of ^{22}Na were added to the mucosal solution. Following 30 min of exposure to the tracer, $\Delta\psi$ was clamped randomly at 0, 25, 50, 75, or 100 mV for 20-min periods, sampling the serosal fluid as above for radioactivity at 10-min intervals. A total of nine membranes was employed. The number of observations made at each potential is given in Table I. At each setting of $\Delta\psi$ the first 10 min was used for equilibration and the second 10 min was used for calculation of $\vec{J}^{*}$ and $\vec{J}$. Mucosal fluid was analyzed as above.

(C) Conductance determinations

The total conductance κ was determined from the change in current caused by 10-s pulses, changing the potential from 0 to ±20 mV automatically at desired intervals: $\kappa = -\Delta I/\Delta(\Delta\psi)$ [10,11].

Passive conductance κ^{p} was determined at the end of the experiment by the addition of amiloride to the mucosal medium to a concentration of 10^{-5} or 10^{-4} M. This brought the short-circuit current I_0 close to zero (2.6 ± 0.5 S.E. % of the initial value). By extrapolation of the plot of I_0 vs. κ, κ^{p} was determined as the intercept in the ordinate at I_0 = 0 [11]. Control studies demonstrated that κ^{p} remained near-constant throughout the experiment: κ^{p} was determined shortly after mounting, and 2 h later in seven hemibladders. The ratio of the final to the initial value was 1.21 ± 0.06 (S.E.), an increase compatible with the effect of the washing procedure used to remove the amiloride, as found earlier [11]. Previous studies had demonstrated that amiloride does not change the passive conductance (as evaluated from serosal to mucosal sodium tracer fluxes [11,12]) and that κ^{p} is constant between −80 and 80 mV [13].

The partial passive conductance κ^{p}_{Na} attributable to sodium was taken as 0.468 κ^{p}. This has been shown to be consistent with the evaluation of κ^{p}_{Na} by a radioactive sodium tracer technique [10,11].

Active conductance κ^{a} was evaluated as the difference of the total and passive conductance.

(D) Measurement of net active sodium flux

I^{a} was obtained from the difference between the total current and the passive current: $I^{a} = I - I^{p} = I + \kappa^{p}\,\Delta\psi$, where I was the mean value obtained by integration of the current over the period of measurement of tracer flux.

The net active sodium flux J^{a}_{Na} was taken as I^{a}/F, where F is Faraday's constant (see Results, No. 2).

(E) Determination of E

The electromotive force of sodium transport was determined from the intercept of the I^{a} vs. $\Delta\psi$ plot with the $\Delta\psi$ axis.

(F) Materials

The composition of the sodium/Ringer solution was 115.9 Na^{+}, 2.5 K^{+},

1.8 Ca^{2+}, 117.8 Cl^-, and 2.4 HCO_3^- expressed in mequiv./l (pH 7.6, 233 mosM/kg H_2O).

^{22}Na was obtained from New England Nuclear Co., Boston, and amiloride was obtained from Merck, Sharp and Dohme Co.

(G) Analysis of data

Standard statistical analyses were used throughout [14]. Results are expressed as mean value ± standard error of the mean (S.E.). Student's *t*-test was used to test significance in the studies of passive conductance and active efflux.

Background

(A) Theoretical

(1) Test of the general flux ratio relationship. In order to analyze the kinetics of isotope flows in the pathway for active sodium transport we shall attempt to apply a thermodynamic formulation relating the rate of net transport J^a and the ratio of unidirectional fluxes [4]:

$$J^a R^{*a} = RT \ln f^a \equiv RT \ln(\vec{J}^a / \overleftarrow{J}^a) \tag{1a}$$

(Since, as discussed below, the only significant transepithelial active transport in our preparation is that of sodium, we shall omit the subscript Na throughout, except when its omission would be confusing). R and T are the gas constant and absolute temperature, and f^a, $\vec{J}^a$, and $\overleftarrow{J}^a$ are the flux ratio, influx, and efflux of the active pathway, respectively. R^{*a}, the phenomenological exchange resistance of the active pathway, is given by the quotient of the specific activity difference $\Delta\rho$ and the tracer flow J^{*a} in the absence of net flow:

$$R^{*a} = -(RT\Delta\rho/J^{*a})_{J^a=0} = RT/\vec{J}_E^a \equiv RT/\overleftarrow{J}_E^a \tag{1b}$$

where E represents the "electromotive force of sodium transport" E_{Na}, the value of the transepithelial electrical potential difference $\Delta\psi$ at which $J^a = 0$ and $\vec{J}^a = \overleftarrow{J}^a$ [3].

It was not convenient for us to test Eqn. 1 as it stands, since this would require the simultaneous measurement of opposing unidirectional fluxes at several settings of $\Delta\psi$; at values in the physiological range, efflux through the active pathway is small and cannot be measured accurately. Therefore we introduce the identity $J \equiv \vec{J} - \overleftarrow{J}$ into Eqn. 1a, in order to obtain two useful relationships between the net flux and the influx:

$$J^a R^{*a} = RT \ln\left(\frac{\vec{J}^a}{\vec{J}^a - J^a}\right) \tag{2}$$

and

$$\vec{J}^a = \frac{J^a}{1 - \exp[-J^a R^{*a}/RT]} \tag{3}$$

Like Eqn. 1a, Eqn. 2 does not permit a convenient test of the general flux ratio relationship, since over most of the physiological range $\vec{J}^a - J^a \equiv \overleftarrow{J}^a$ is small, and its experimental evaluation would involve the error of taking the small dif-

ference of comparably large numbers. However, at a suitably large value of $\Delta\psi$, efflux becomes appreciable, permitting the use of Eqn. 2 to evaluate R^{*a}. Presuming that R^{*a} is independent of $\Delta\psi$, evaluation of $\vec{J}^a$ and J^a over a range of values of $\Delta\psi$ then permits a test of the general flux ratio relationship by determining whether Eqn. 3 is satisfied.

(2) Interpretation of phenomenological resistance coefficients. The above formulation should be applicable irrespective of the nature of the exchange resistance coefficient R^{*a}. A priori there is no basis for knowing whether or not R^{*a} will be equal to the phenomenological resistance coefficient for net flow R^a. On theoretical grounds R^{*a} would be expected to equal R^a if and only if there is no interaction between abundant and tracer flows ("isotope interaction") [4]. In order to examine the nature and relationship of the resistance coefficients more precisely it is necessary to consider the contribution of metabolism to the forces promoting transport [9].

Studies in frog skin and toad bladder have demonstrated a linear relationship between the rate of active sodium transport J^a and the associated rate of oxidative metabolism J_r, and a linear dependence of both these flows on $\Delta\psi$ [10, 13,15—17]. Accordingly, with solutions of identical composition at each surface, we write

$$J^aR^a = -F\Delta\Psi - R_{or}J_r \qquad (\Delta c = 0) \tag{4}$$

where F is the Faraday constant, and R_{or} is the phenomenological resistance coefficient relating transport to metabolism. Combining Eqns. 1a and 4 gives

$$RT \ln f^a = -(R^{*a}/R^a)(F\Delta\Psi + R_{or}J_r) \qquad (\Delta c = 0) \tag{5}$$

showing that only in the absence of isotope interaction does the flux ratio quantify the forces promoting transport.

It is useful to apply Eqn. 5 to the case where $\Delta\psi = E$. Under these circumstances J^a and $\ln f^a = 0$, giving

$$R_{or} = -FE/J_{rE} \tag{6}$$

Since J_r is a linear function of $\Delta\psi$ in the frog skin and toad bladder, $dJ_r/d\Delta\psi$ is constant, so that

$$J_r = J_{r0} + \left(\frac{J_{rE} - J_{r0}}{E}\right)\Delta\Psi \tag{7}$$

where J_{r0} and J_{rE} are the values of J_r at $\Delta\psi = 0$ and E, respectively.

Introducing Eqns. 6 and 7 into Eqn. 5,

$$RT \ln f^a = -(R^{*a}/R^a)(J_{r0}/J_{rE})F(\Delta\Psi - E) \tag{8}$$

Alternatively, it may be useful to introduce Kedem and Caplan's parameter q, which quantifies the "degree of coupling" between transport and metabolism ($0 \leqslant q^2 \leqslant 1$) [18]. Since $J_{rE}/J_{r0} = 1 - q^2$,

$$RT \ln f^a = -[(R^{*a}/R^a)/(1 - q^2)]F(\Delta\Psi - E) \tag{9}$$

Introducing now the definition

$$(R^{*a}/R^a)(J_{r0}/J_{rE}) = (R^{*a}/R^a)/(1 - q^2) \equiv Q^a \tag{10}$$

gives Chen and Walser's Eqn. 10 [8]:

$$\ln f^a = -Q^a F(\Delta\Psi - E)/RT \tag{11}$$

(We have ignored the valence $z_{Na} = 1$.) Q^a is a useful lumped parameter for characterizing the dependence of f^a and the unidirectional fluxes on $\Delta\psi$. Remembering that $J = \vec{J} - \overleftarrow{J}$ and $\vec{J}/\overleftarrow{J} = f$, combining Eqns. 1a, 1b, and 11 gives

$$\frac{\vec{J}^a}{\vec{J}^a_E} = \frac{-Q^a F(\Delta\Psi - E)/RT}{1 - \exp[Q^a F(\Delta\Psi - E)/RT]} \tag{12a}$$

and

$$\frac{\overleftarrow{J}^a}{\overleftarrow{J}^a_E} = \frac{-Q^a F(\Delta\Psi - E)/RT}{\exp[-Q^a F(\Delta\Psi - E)/RT] - 1} \tag{12b}$$

A convenient means of evaluating Q^a is given by combining Eqns. 1a and 11 at $\Delta\psi = 0$ to obtain

$$Q^a = J^a_0 R^{*a}/FE \tag{13}$$

(B) Experimental

The application of the above formulations requires the evaluation of J^a, $\vec{J}^a$, and E. This may be done by the following means.

Given a linear dependence of the rate of active transport on the transmembrane electrical potential, if only sodium is transported actively,

$$J^a = (1/F)(I_0 - \kappa^a \Delta\Psi) \tag{14}$$

where I_0 is the short-circuit current ($I_{\Delta\psi = 0}$) and κ^a is the conductance of the active pathway. The latter quantity is given by

$$\kappa^a = \kappa - \kappa^p \tag{15}$$

where κ and κ^p are the total conductance and passive conductance, measured, respectively, before and after the abolition of active transport with amiloride, as discussed under Materials and Methods.

Taking the passive partial conductance $\kappa^p_{Na} = 0.468\ \kappa^p$ [10], for uncoupled passive sodium flow [19—21],

$$\vec{J}^p_{Na} = \frac{\kappa^p_{Na}\Delta\Psi}{F[\exp(F\Delta\Psi/RT) - 1]} \tag{16}$$

(At $\Delta\psi = 0$, $\vec{J}^p_{Na} = \overleftarrow{J}^p_{Na} = (RT/F^2)\ \kappa^p_{Na}$.) For active sodium flow, if only sodium is transported actively,

$$\vec{J}^a \equiv \vec{J}^a_{Na} = \vec{J}_{Na} - \frac{\kappa^p_{Na}\Delta\Psi}{F[\exp(F\Delta\Psi/RT) - 1]} \tag{17}$$

Finally, the electromotive force of sodium transport is given by

$$E = (\Delta\Psi)_{J^a = 0} \tag{18}$$

Results

Before testing the fundamental relationship (Eqn. 3, and thus Eqn. 1), it was necessary to test various assumptions implicit in the theory.

(1) Characteristics of the passive pathway

At $\Delta\psi \leqslant 50$ mV active efflux is not demonstrable (see below). Accordingly, the kinetic properties of the passive pathway were studied by determining the serosal to mucosal ^{22}Na flux in three membranes in three sequential periods of 30 min each, with $\Delta\psi$ set at 0 and 50 mV, following which κ^p was evaluated by the use of 10^{-5} M amiloride. Assuming that $\vec{J}^p_{Na} = \overleftarrow{J}^p_{Na}$ at 0 mV, and that $\vec{J}^p_{Na}(-50\ mV) = \overleftarrow{J}^p_{Na}(50\ mV)$, then allowed the comparison of values of $\vec{J}^p_{Na}$ (and thus $\overleftarrow{J}^p_{Na}$) predicted theoretically from Eqn. 16 with values of $\overleftarrow{J}^p_{Na}$ determined experimentally. The mean ratio of theoretical and experimental values of $\overleftarrow{J}^p_{Na}$ at 0 and 50 mV were 0.971 ± 0.061 (S.E.) and 0.952 ± 0.036 (S.E.), respectively, which are insignificantly different from 1. This finding supports the validity of Eqn. 16, and therefore indicates the absence of significant isotope interaction and coupling of flows of other ions with passive sodium flow in our preparation, as concluded previously [10].

(2) Equivalence of active current and active Na^+ flux

Since Leaf et al.'s original demonstration, it has been considered that in Dominican toad bladders mounted in chambers, net sodium transport is nearly equivalent to the short-circuit current [22,23]. Recently, however, Walser [8,24,25] has presented evidence that in toad bladders exposed to bicarbonate-containing Ringer's solution and mounted in sacs, net sodium transport accounts for only some 80% of the short-circuit current. For this reason, the equivalence of the short-circuit current and the rate of net active sodium transport was retested under our experimental conditions.

Mucosal to serosal ^{22}Na flux, $\vec{J}^*_{Na}$, was determined for five periods of 15 min each at short circuit in 12 membranes, allowing the calculation of $\vec{J}_{Na}$. The measurement of conductance prior to and following the near-abolition of I_0 by the application of amiloride permitted the evaluation of κ^p and κ^a (Eqn. 15). In the absence of isotope interaction or other coupled flows for sodium in the passive pathway, the passive influx $\vec{J}^p_{Na}$ is given by Eqn. 16. It was found that at $\Delta\psi = 0$, $\vec{J}^p_{Na}$ and $\vec{J}_{Na}$ were 0.188 (±0.032) × 10^{-10} and 6.02 (±0.96) × 10^{-10} mol · cm^{-2} · s^{-1}, respectively, and $\vec{J}^p_{Na}/\vec{J}_{Na}$ was 0.036 ± 0.011 (S.E.) ($n = 12$), indicating that edge damage was minimal, and permitting the accurate calculation of the active influx $\vec{J}^a_{Na}$ from Eqn. 17. Since at $\Delta\psi = 0$ mV there is no demonstrable active efflux, $\vec{J}^a_{Na}$ equals the rate of net active sodium transport, J^a_{Na}. The mean of I_0/FJ^a_{Na} calculated in this manner was 0.99 ± 0.02 (S.E.) ($n = 12$).

This result suggests that sodium transport accounts completely for active ion transport. However, there remains the slight possibility that under short-circuit conditions active transport of other cationic and anionic species might be overlooked. For example, if K^+ and Cl^- were transported in the same direction at the same rates ther combined flows would not affect the short circuit current. It is possible to test for such possibilities by clamping $\Delta\psi \neq 0$. Under such cir-

cumstances, if active transport of K^+ and Cl^- contribute to active conductance their flows must become unequal, and would no longer compensate for each other electrically. Hence the "active current" I^a, evaluated as $I + \kappa^p \Delta\psi$, would become unequal to $FJ^a_{Na} \equiv FJ_{Na} + \kappa^p_{Na}\Delta\psi$. For settings of $\Delta\psi$ at which there is no active efflux, $J^a_{Na} = \vec{J}^a_{Na}$ and can be evaluated from Eqn. 17. In 10 periods at $\Delta\psi$ = 50 mV, FJ^a_{Na} differed insignificantly from I^a: their mean ratio was 1.06 ± 0.03 (S.E.); in five periods at $\Delta\psi$ = 25 mV their mean ratio was 0.99 ± 0.02 (S.E.). This result demonstrates that in Dominican toad bladders mounted in chambers, under the conditions employed in our experiments, sodium is indeed the only ion which contributes significantly to transepithelial active transport, so that $J^a \equiv J^a_{Na}$ *.

(3) Bidirectional fluxes through the active pathway

Given the above results, measurements of $\vec{J}_{Na}$, I, κ, and κ^p, and the application of Eqns. 14, 15, and 17 permit the calculation of J^a and the influx $\vec{J}^a$. The difference of these two quantities then gives $\overleftarrow{J}^a$, the efflux in the active pathway. The relative magnitudes of efflux and influx at values of $\Delta\psi$ ranging from 0 to 100 mV are shown in Table I. As is seen, significant efflux $\overleftarrow{J}^a$ is demonstrable only at values of $\Delta\psi$ = 75 and 100 mV.

The ability to demonstrate back flux through the active pathway is in qualitative agreement with the findings of Chen and Walser [8]. Our results differ, however, in that they observed active efflux only at $\Delta\psi$ = 150 mV. This difference is explicable in terms of the marked difference in E in the two studies, with their mean value being 158 ± 1 mV, as compared with our mean value of 96.3 ± 1.9 (S.E.) mV. As is consistent with our value of E, it is seen that at $\Delta\psi$ = 100 mV the back flux exceeded the forward flux through the active pathway, so that net active flux was reversed with respect to the usual polarity.

(4) Test of the general formalism

The above results demonstrate the unfeasibility of testing the general flux ratio relationship by use of Eqn. 2. As mentioned, however, the application of Eqn. 2 does permit the evaluation of a quantity of fundamental interest, the tracer exchange resistance of the active pathway, R^{*a}. The above-determined values of J^a and $\vec{J}^a$ at $\Delta\psi$ = 100 mV were used to calculate R^{*a}.

Having determined R^{*a} by measurements at $\Delta\psi$ = 100 mV, it was possible to compare experimental values of $\vec{J}^a$ with those predicted by Eqn. 3, using the determinations of J^a and $\vec{J}^a$ measured at settings of $\Delta\psi$ ranging from 0 to 75 mV. For example, for one experiment, the values of J^a and $\vec{J}^a$ at $\Delta\psi$ = 100 mV were −0.13 and $0.16 \cdot 10^{-10}$ mol · cm^{-2} · s^{-1}, respectively; thus the application of Eqn. 2 gives R^{*a} = 2.54 (in the above units). Additional measurements of $\vec{J}^a$ in the same membrane at $\Delta\psi$ = 0, 25, 50, and 75 mV gave values of $1.35 \cdot 10^{-10}$, $0.95 \cdot 10^{-10}$, $0.54 \cdot 10^{-10}$, and $0.27 \cdot 10^{-10}$ mol · cm^{-2} · s^{-1}, respectively. These are to be compared with the theoretical values of $1.35 \cdot 10^{-10}$, $0.95 \cdot 10^{-10}$, $0.58 \cdot 10^{-10}$, and $0.30 \cdot 10^{-10}$ mol · cm^{-2} · s^{-1}, respectively predicted by

* The above results do not exclude the possibility of an electrically neutral ion exchange process, as described for example Cl^- and HCO_3^- in the colon [26]. However, such a process would not interfere with the use of our techniques for the evaluation of J^a_{Na}.

TABLE I

RELATIVE MAGNITUDES (MEAN ± S.E.) OF EFFLUX AND INFLUX IN THE ACTIVE PATHWAY AS A FUNCTION OF TRANSMEMBRANE ELECTRICAL POTENTIAL DIFFERENCE

The ratios differed significantly from zero only at $\Delta\psi$ = 75 mV ($P < 0.05$) and 100 mV ($P < 0.001$).

$\Delta\Psi$ (mV)	n	$\overleftarrow{J}^a$ (10^{-10} mol · cm^{-2} · s^{-1})	$\overrightarrow{J}^a$ (10^{-10} mol · cm^{-2} · s^{-1})	$(\overleftarrow{J}^a/\overrightarrow{J}^a)$
0	9	0.05 ± 0.03	2.19 ± 0.29	0.02 ± 0.02
25	5	0.02 ± 0.02	1.94 ± 0.43	0.01 ± 0.01
50	11	0.05 ± 0.03	1.00 ± 0.19	0.05 ± 0.03
75	6	0.13 ± 0.07	0.70 ± 0.15	0.17 ± 0.06
100	9	0.38 ± 0.11	0.29 ± 0.07	1.27 ± 0.18

Eqn. 3, employing measured values of J^a and the value of R^{*a} = 2.54 determined above.

Fig. 1 shows the relationship of experimental and theoretical values of $\overrightarrow{J}^a$ for all nine membranes studied. As is seen, the plot differs insignificantly from the line of identity.

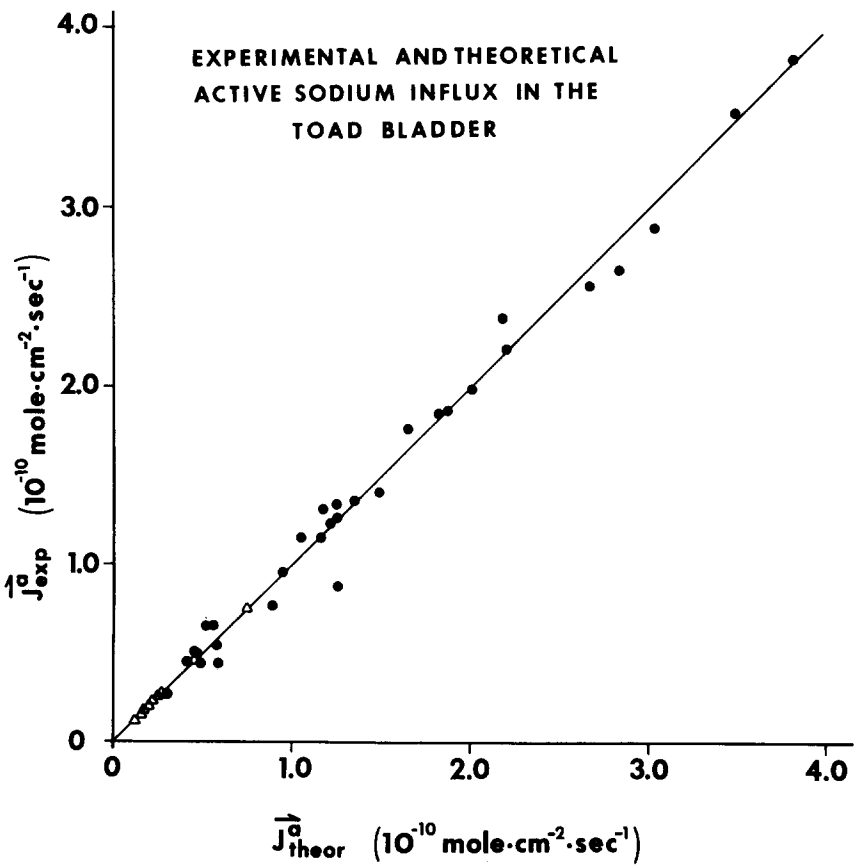

Fig. 1. Experimental and theoretical sodium influx in the active transport pathway. Theoretical values were calculated from Eqn. 3, employing values of R^{*a} determined from the application of Eqn. 2 to measurements of J^a and $\overrightarrow{J}^a$ at $\Delta\psi$ = 100 mV (indicated by the triangles). $\overrightarrow{J}^a_{exp} = 0.985\,\overrightarrow{J}^a_{theor}$ (r = 0.993).

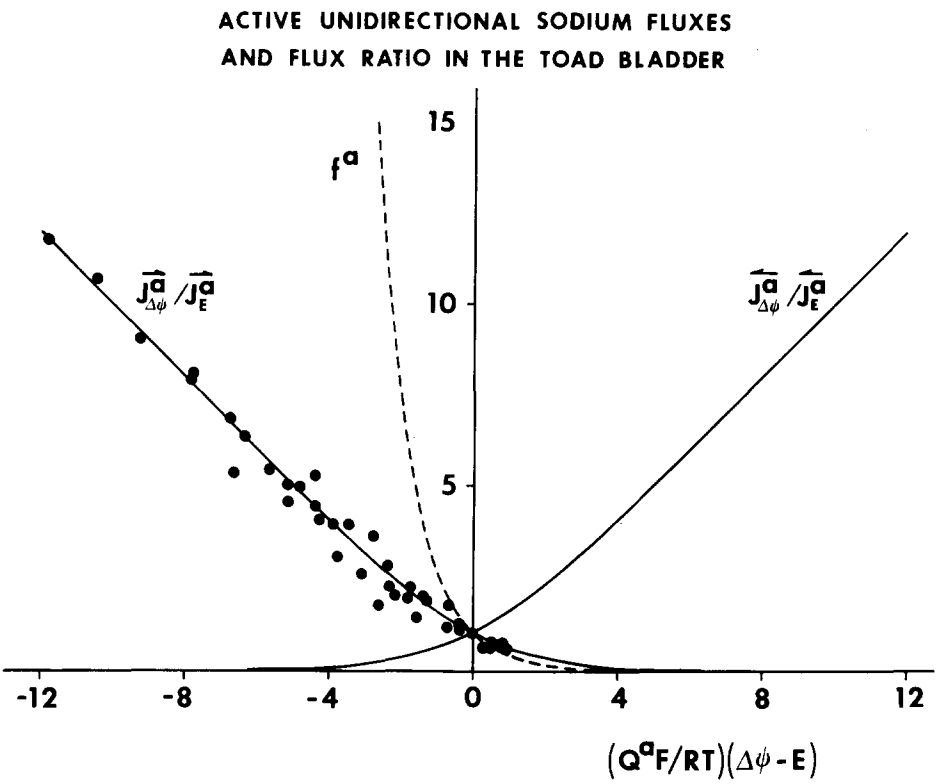

Fig. 2. Influx, efflux, and flux ratio in the active transport pathway. Values of unidirectional flux were normalized relative to the value at $\Delta\psi = E$. The lines represent the theoretical relationships of Eqns. 11, 12a and 12b. The black circles represent experimental values of sodium influx.

(5) Dependence of the bidirectional fluxes on $\Delta\psi$

The validity of the theoretical relations tested above permits us to make meaningful interpretations of the behavior of the two unidirectional fluxes. The values of relevant quantities in the above studies are: R^{*a} (in 10^{10} kcal · cm^2 · s . mol^{-2}) = 2.42 ± 0.35 (S.E.) (range = 0.67—3.66); Q^a = 2.07 ± 0.24 (S.E.) (range = 0.90—3.04); E (in mV) = 96.3 ± 1.9 (S.E.) (range = 85.1—108.1), (n = 9). Given knowledge of R^{*a}, Q^a, and E, it is possible to predict the active influx and efflux at any value of $\Delta\psi$ from Eqns. 12a and 12b. Fig. 2 shows the theoretical relationships for $\vec{J}^a/\vec{J}^a_E$, $\overleftarrow{J}^a/\overleftarrow{J}^a_E$, and f^a, and experimental values for $\vec{J}^a/\vec{J}^a_E$.

Discussion

The thermodynamic formulation of the kinetics of isotope flows has previously been defended on theoretical grounds and supported by studies of passive transport in synthetic membranes and the toad bladder [4—7]. While the incorporation of active transport appeared justified theoretically, experimental substantiation had been lacking. Indeed, until Chen and Walser's recent studies of active sodium transport in the toad bladder there had been no experi-

mental demonstration of bidirectionality of transport in the active pathway. In their study the mean values of active Na^+ influx and efflux at $\Delta\psi = 0$, 100, and 150 mV agreed closely with those predicted theoretically (ref. 8, Table V). Similarly, in the present study the individual values of active Na^+ influx at several settings of $\Delta\Psi$ agree closely with the theoretical predictions of Eqn. 3 (Fig. 1).

A demonstration of the validity of the generalized flux ratio equation in the presence of active transport provides a basis for the meaningful analysis of the kinetics of isotope flows in a wide variety of biological systems. In particular, knowing the values of the pertinent parameters R^{*a}, Q^a, and E, or equivalent information, it is possible to predict and correlate the active influx, efflux, and flux ratio at any value of transmembrane electrical potential. It is of interest that although at usual experimental values of $\Delta\psi$ the dependence of $\vec{J}^a$ on potential appears linear, when $\Delta\psi$ approximates E the dependence of both influx and effux on $\Delta\psi$ is seen to be curvilinear (Fig. 2). This is to be expected even if their difference $J^a = \vec{J}^a - \overleftarrow{J}^a$ remains linear.

In spite of the general agreement between our results and those of Chen and Walser, there are some significant differences: thus, as previously [10], we found no evidence for isotope interaction or other coupling of Na^+ flows in the passive pathway. Secondly, mounting our bladders in chambers (rather than as sacs) and depressing active transport with amiloride (rather than ouabain) we found that only Na^+ is transported actively at a significant rate, simplifying formal analysis. This is the case despite the fact that our Na^+/Ringer's solution contains bicarbonate, which Walser and Chen [25] found it necessary to remove in order to eradicate the discrepancy between the rate of net sodium flux and the short-circuit current. Thirdly, our values for the electromotive force of sodium transport E were smaller, so that it was possible to demonstrate back flux, and even reversal of net flux through the active pathway, at a relatively small value of $\Delta\psi$, 100 mV. Whatever the bases for these differences, it is of interest that the behavior of the unidirectional fluxes and flux ratio in the sodium active transport pathway in the two studies is consistent with the same general formulation.

Of special interest is the nature of Chen and Walser's parameter Q^a, a factor quantifying the extent of deviation of the flux ratio from the prediction of the widely accepted model. In their classic paper Ussing and Zerahn [27] considered that, in the absence of both a concentration difference and an electrical potential difference across the membrane, the electromotive force of sodium transport would be given by $(RT/F) \ln f$ [27]. As is seen from Eqn. 8, this would be unlikely in the frog skin or the toad bladder: even in the absence of isotope interaction (i.e. even if $R^{*a}/R^a = 1$), $(RT/F) \ln f_0^a > E$, since in these tissues $J_{r0} > J_{rE}$ [12,14,15]; hence $Q^a > 1$. The discrepancy may be quite significant: in the present study Q^a averaged 2.07; in Chen and Walser's study Q^a averaged 2.54.

As discussed previously, the above considerations point to the means of determining the extent to which "abnormality" of the flux ratio is attributable to isotope interaction ($R^{*a} \neq R^a$), as against the coupling of transport to metabolism ($J_{r0} \neq J_{rE}$). In order to make a preliminary estimate as to the extent of possible isotope interaction, data obtained in our laboratory from the study of

oxygen consumption were related to data of Chen and Walser obtained from the study of isotope flows. Combining our value of $J_{rE}/J_{r0} = 0.324 \pm 0.075$ ($n = 8$) with Chen and Walser's value of $Q^a = 2.54$ gave a mean value of $R^{*a}/R^a = 0.82$, indicating negative isotope interaction, as in exchange diffusion [9]. It was pointed out, however, that this value of R^*/R cannot be taken too seriously, having been based on studies in different laboratories under different conditions. In the present study isotope flows were analyzed under conditions more closely comparable to those employed in our studies of oxygen consumption. Combining our mean values of $Q^a = 2.07$ and $J_{rE}/J_{r0} = 0.324$ gives a value of $R^{*a}/R^a = 0.67$, but of course a precise determination must await simultaneous measurements of tracer flows and oxygen consumption in the same membrane.

Nevertheless, these results speak for the likelihood of negative isotope interaction, as would be compatible with a system in which ions compete for sites on a carrier, as postulated for active sodium transport in the toad bladder. However, this interpretation cannot be considered definitive, since negative isotope interaction is consistent also with other mechanisms, e.g. circulation of volume flow, and heterogeneity of parallel pathways [4,28,29]. Furthermore, phenomenological coefficients evaluated as above apply to the system as a whole, and may not discriminate the possible influence of unstirred layers and/or other technical factors. It is to be anticipated that with increasingly refined studies of the kinetics of active transport under various conditions, determination of the extent of isotope interaction will provide criteria for the validity of models and elucidate the mode of action of agents that influence transport. Such information would be of fundamental value in the understanding of mechanisms of membrane permeation and the coupling of transport and metabolism.

Symbols

E electromotive force for Na^+ transport (V)
f ratio of unidirectional Na^+ fluxes
F Faraday's constant (C/equiv.)
I total net current (A/cm^{-2})
J net flux of Na^+ (mucosa to serosa) ($mol \cdot cm^{-2} \cdot s^{-1}$)
$\vec{J}, \overleftarrow{J}$ unidirectional Na^+ flux (mucosa to serosa, and serosa to mucosa, respectively)
J_r rate of oxygen consumption ($mol \cdot cm^{-2} \cdot s^{-1}$)
q degree of coupling between transport and metabolism
Q ratio of bulk diffusion coefficient to tracer diffusion coefficient
R gas constant ($cal \cdot K^{-1} \cdot mol^{-1}$)
R phenomenological resistance to net flow ($cal \cdot cm^2 \cdot s \cdot mol^{-2}$)
R^* phenomenological exchange resistance
R_{or} phenomenological resistance coefficient relating transport to metabolism
κ total conductance ($\Omega^{-1} \cdot cm^{-2}$)
κ^p_{Na} partial Na^+ passive conductance
ρ specific activity (Ci/mol)
$\Delta\psi$ potential difference ($\psi_{serosal} - \psi_{mucosal}$) (V)

Superscripts a and p for f, I, J, $\vec{J}$, R, and κ indicate active and passive pathway, respectively; * indicates ^{22}Na tracer isotope. Subscripts 0 and E for I, $\vec{J}$ and J_r indicate $\Delta\psi = 0$ and E, respectively.

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