

LETTERS TO THE EDITOR

The Na-K Pump as a Current Source

*Affinity-driven Transport Has No
Reversal Potential but
Its Metabolic Cost Does*

Dear Sir:

In an earlier letter on another matter, Chapman and Johnson (1978) identified a paradox facing a widely held stoichiometric model for ATP utilization in active ion transport. Their well-reasoned and carefully documented argument summarized two apparently conflicting points of evidence. First, the net rate of ATP hydrolysis not only varies with imposed brief displacements of the transmembrane electric potential difference, but ceases at, and reverses direction to net synthesis beyond, a reversal potential that may not be far from the cell's resting potential. Second, the active transport fluxes of sodium and potassium remain essentially constant over this same range of transmembrane potentials. According to the stoichiometric concept in question, the net hydrolysis of one ATP molecule drives a stoichiometrically fixed number of ions of each actively transported species in a specific direction through the cell membrane. One then expects the net ATP turnover rate¹ and the active transport rates to remain strictly proportional, or tightly coupled, during displacements of the membrane potential, and that all should go to zero simultaneously at the reversal potential for net ATP hydrolysis. The evidence just cited is therefore paradoxical for this net-turnover stoichiometric (NTS) model.

Chapman and Johnson argued that this difficulty can be resolved in a way that preserves the overall stoichiometry, as well as essentially voltage-independent pump fluxes over much of the physiological range, provided certain kinetic relationships obtain among fluxes, ATP turnover, substrate concentrations, and membrane potential (see also Chapman et al., 1979; Chapman, 1981). Moreover, they suggested new experiments to test for simultaneous reversal of active pump fluxes and net ATP turnover. But they also cautioned that unless simultaneous reversal can be demonstrated experimentally, a major rethinking of the pump's mechanism and stoichiometry may be in order.

The purpose of this letter is to point out that Kedem's (1961) concept of

¹ I use "hydrolysis rate," "turnover," and "turnover rate" interchangeably in this discussion.

affinity-driven transport offers an alternative solution, when one sets aside the assumption of stoichiometry between net ATP turnover and active transport. Taken together with Chapman and Johnson's second point, it then indicates that the Na-K pump ought to behave as an ideal current source whose Na and K pumping rates are independent of the transmembrane potential, but whose metabolic cost is not. It suggests further that the basic problem in active transport may be viewed as follows: the electrochemical potential differences of Na and K, which drive the electrodiffusion of these ions, cannot also directly drive their active transport. But the Onsager reciprocity principle lets these potentials make the ATP to supply the transport power, and the free energy of the ATP then drives the ions. The pump provides the mechanism for this reciprocal power exchange, and as with any fuel-consuming machine, and essential role of the net ATP hydrolysis is to cover the pump's obligatory dissipative losses, i.e., its "second law overhead."

The argument derives from the linear nonequilibrium thermodynamic formulation of Kedem (1961), Katchalsky and Curran (1965), Essig and Caplan (1968), and Katchalsky (1970) for force-driven flows. The fundamental assumption is that active transport fluxes are driven by the thermodynamic driving force for ATP hydrolysis—the DeDonder affinity A —rather than by the net ATP hydrolysis rate J_r . This assumption, and not the thermodynamic formalism used, is the essential difference between the present approach and the one taken in the NTS model. The present model in fact contrasts directly with Rapoport's (1970) linear nonequilibrium thermodynamic analysis of the NTS model of Chapman and Johnson (1978), Tanford (1981), and others.

Accordingly, ion fluxes and the ATP hydrolysis rate are considered to be coupled to a set of thermodynamic driving forces by a set of linear conductance coefficients L . In terms of molar fluxes J and driving forces per mole X , the relationships and attendant assumptions are conveniently expressed in matrix form as $J = LX$, or :

$$\begin{bmatrix} J_{\text{Na}} \\ J_{\text{K}} \\ J_{\text{Cl}} \\ J_r \end{bmatrix} = \begin{bmatrix} L_{\text{Na}} & 0 & 0 & L_{\text{Na}r} \\ 0 & L_{\text{K}} & 0 & L_{\text{K}r} \\ 0 & 0 & L_{\text{Cl}} & 0 \\ L_{r\text{Na}} & L_{r\text{K}} & 0 & L_r \end{bmatrix} \begin{bmatrix} F(V - E_{\text{Na}}) \\ F(V - E_{\text{K}}) \\ -F(V - E_{\text{Cl}}) \\ A \end{bmatrix}. \quad (1)$$

J_{Na} , J_{K} , and J_{Cl} are the respective net ion fluxes ($\text{mol s}^{-1} \text{m}^{-2}$) (SI units are used throughout) through the membrane, taken as positive when from inside the cell to outside. The expressions of the form $F(V - E_{\text{Na}})$ are the conjugate driving forces for the respective ion fluxes. Each is the electrochemical potential difference per mole of ion from inside to outside (J mol^{-1}); F is the Faraday constant, V is the transmembrane electric potential difference in volts from inside to outside, E_{Na} , etc. is the Nernst ionic equilibrium potential of the respective ion, and the sign of the term is as the ion's valence.

J_r is the net ATP hydrolysis rate ($\text{mol s}^{-1} \text{m}^{-2}$) occurring throughout the thickness of the membrane, per unit membrane area, J_r^{tot} of Katchalsky (1970), and as in Chapman and Johnson (1978), is positive for the forward hydrolysis reaction. Its conjugate driving force is the DeDonder affinity A (J

mol⁻¹) for ATP hydrolysis (the negative of the Gibbs free energy per mole; $-\Delta G_{\text{ATP}}$ and $-A$ of Chapman and Johnson, 1978, Eq. 6); it is positive for the energy-yielding reaction.

The L 's are the conductance coefficients. "Straight" conductances L_{Na} , L_{K} , and L_{Cl} are proportionality coefficients between the voltage-dependent, electrodiffusional component of each ion flux and its conjugate force. They are the operationally defined passive ionic conductances of the membrane in molar units; multiplied by F^2 they become the passive electrical conductances g_{Na} , g_{K} , and g_{Cl} (S m⁻²). They are positive for both cations and anions. For this linear model the second law of thermodynamics requires that L_{Na} and L_{K} comprise "pump-leak" components $L_{\text{Na}p}$ and $L_{\text{K}p}$, respectively, in parallel with pump-independent channel conductances $L_{\text{Na}c}$ and $L_{\text{K}c}$, such that:^{2,3}

$$L_{\text{Na}} = L_{\text{Na}c} + L_{\text{Na}p}, \quad (2a) \quad \text{and} \quad L_{\text{K}} = L_{\text{K}c} + L_{\text{K}p}. \quad (2b)$$

Straight conductance L_r is the proportionality coefficient between ATP affinity and the "level flow" hydrolysis rate (Essig and Caplan, 1968) that would obtain if the electrochemical potential differences for the pumped ions were zero (Eq. 5); it is also positive.

The zeros in the first three rows and columns are "cross" conductances between ion fluxes and nonconjugate ionic driving forces, representing the assumption that the conjugate force for one ion species does not drive flow of another.³

Cross conductances $L_{\text{Na}r}$ and $L_{\text{K}r}$ couple the ion fluxes to ATP. They are proportionality coefficients between voltage-independent flux components and the ATP affinity A . The net sodium flux is therefore:

$$J_{\text{Na}} = L_{\text{Na}c}F(V - E_{\text{Na}}) + L_{\text{Na}p}F(V - E_{\text{Na}}) + L_{\text{Na}r}A. \quad (3)$$

The expression for potassium is similar. According to Kedem's (1961) postulate, the affinity-driven fluxes $L_{\text{Na}r}A$ and $L_{\text{K}r}A$ oppose diffusion, so that $L_{\text{Na}r}$ must be positive and $L_{\text{K}r}$ negative. This discussion assumes chloride is not pumped, so the corresponding cross-conductance $L_{\text{Cl}r}$ is zero (row 3, column 4) and chloride flux J_{Cl} includes only the passive term.

Cross conductances $L_{r\text{Na}}$ and $L_{r\text{K}}$ couple components of the net ATP turnover rate J_r to the electrochemical potentials of the pumped ions. This

² The thermodynamic constraint is that the dissipation function Φ must be positive except at Donnan equilibrium where all driving forces are zero (Katchalsky and Curran, 1965), since the working pump must always dissipate some power entropically. The determinant of the conductance matrix in Eq. 1 must therefore be positive, and must remain so when either $L_{\text{Na}c}$ or $L_{\text{K}c}$ is reduced to zero by channel blocking agents, e.g., tetrodotoxin or amiloride for $L_{\text{Na}c}$ or tetraethylammonium or 4-AP for $L_{\text{K}c}$. The condition is:

$$(L_{\text{Na}p} - L_{\text{Na}r}^2/L_r) (L_{\text{K}p} - L_{\text{K}r}^2/L_r) > (L_{\text{Na}r}L_{\text{K}r}/L_r)^2$$

where each factor on the left is also positive. Certain nonlinear mechanisms could meet this need in other ways.

³ The NTS model requires specific straight pump-leak conductances $L_{\text{Na}r}^2/L_r$ and $L_{\text{K}r}^2/L_r$, and pump-leak cross conductances $L_{\text{Na}r}L_{\text{K}r}/L_r$ between Na and K in Eq. 1, row 1, column 2, and row 2, column 1 (Rapoport, 1970, Eq. 14a, b).

cross coupling is a consequence of the Onsager reciprocity principle, which for the linear case makes reciprocal cross conductances equal:

$$L_{rNa} = L_{Na r}, \quad (4a) \quad L_{rK} = L_{K r}, \quad (4b) \quad \text{and} \quad L_{rCl} = L_{Cl r} = 0. \quad (4c)$$

L_{rNa} is therefore positive, and L_{rK} is negative; these cross conductances will be given as $L_{Na r}$ and $L_{K r}$ in the remaining discussion. The net hydrolysis rate is therefore:

$$J_r = L_{Na r}F(V - E_{Na}) + L_{K r}F(V - E_K) + L_r A. \quad (5)$$

It represents the metabolic cost of the pump. As the potential-driven terms arising from Onsager reciprocity are ordinarily negative, they may be thought of as "reciprocal synthesis." Metabolic cost thus not only varies with transmembrane potential V , but goes to zero at reversal potential V_r :

$$V_r = (L_{Na r}E_{Na} + L_{K r}E_K - L_r A/F)/(L_{Na r} + L_{K r}), \quad (6)$$

which is similar to Chapman and Johnson's $-E_r$ for the NTS case (1978, Eq. 10). The voltage dependence of net hydrolysis in both the NTS model and the present one is therefore consistent with the theoretical and experimental evidence cited in Chapman and Johnson's first point.⁴

As for the voltage independence of active transport cited in their second point, the affinity-driven components $L_{Na r}A$ and $L_{K r}A$ of the net Na and K fluxes (Eq. 3) behave in precisely this way. Moreover, they also satisfy a number of criteria used to define active transport operationally in a variety of experimental preparations.^{5,6} They depend on the integrity of the pump, represented by cross conductances $L_{Na r}$ and $L_{K r}$, they depend on the free energy of ATP, and they are unidirectional fluxes which, at physiological potentials, oppose the electrodiffusion of their respective ions. These affinity-driven fluxes may therefore be taken as identical with active sodium and potassium transport, respectively.⁷

In their electrical behavior, the affinity-driven fluxes are voltage-independent ionic currents. A source of electromotive force that maintains a current

⁴ Supporting data include the reversal of ATP hydrolysis in erythrocyte ghosts (Garrahan and Glynn, 1967) and the marked voltage dependence of O₂ consumption (Lang et al., 1977) and of CO₂ production (Labarca et al., 1977) in toad urinary bladder.

⁵ These include unidirectional tracer fluxes in squid axon (Hodgkin and Keynes, 1954; Brinley and Mullins, 1974) and net pump current inferred from Li- and DNP-sensitive post-tetanic hyperpolarization in crustacean stretch receptor neuron (Nakajima and Takahashi, 1966) and from cold- and ouabain-sensitive electrogenic hyperpolarization in *Anisodoris* G neuron (Marmor, 1971). See also De Weer's (1975) review.

⁶ Lang et al. (1977) and Labarca et al. (1977) reported voltage-dependent "active" transport in toad bladder by an indirect current measurement, but defined it to include an electrodiffusional flux. Chapman and Johnson (1978) criticized related findings in *Helix* neurons on similar grounds.

⁷ The NTS model requires that its specific pump-leak fluxes also be considered as active, in order to make net pump-associated flux proportional to net ATP turnover (Rapoport, 1970). However, according to the experimental data, the pump-leak terms do not suppress the unidirectional affinity-driven fluxes and thereby make them inherently voltage dependent, but simply behave as independent, passive counterfluxes that evidently go through some part of the pump. Like the pump-independent passive fluxes, they are not powered by ATP hydrolysis.

that is independent of the potential across it is, by definition, a current source. The affinity-driven Na-K pump is therefore a sodium current source in parallel with one for potassium, with respective pump currents $FL_{Na}A$ and $FL_{K}A$. That makes the present model a current-source model.

The current-source pump is nonstoichiometric with respect to net ATP turnover. But since affinity-driven fluxes and affinity-driven level flow hydrolysis rate L_rA are proportional, the conductance ratios L_{Na}/L_r and $-L_{K}/L_r$ may be thought of as stoichiometric coefficients with respect to level flow. Unlike unidirectional ion fluxes, however, unidirectional forward and reverse components of the net ATP hydrolysis reaction (Eq. 5) cannot be measured explicitly by existing methods. Forward hydrolysis may in fact proceed at the level flow rate, independently of the voltage-dependent "reciprocal synthesis" terms in Eq. 5, in which case affinity-driven transport could occur by a stoichiometric molecular mechanism of the sort usually envisaged for the NTS model. But the effect of these reciprocity terms may be voltage-dependent suppression of forward hydrolysis instead. If so, active fluxes are inherently nonstoichiometric with respect to ATP hydrolysis. This possibility suggests that it may be fruitful to consider inherently nonstoichiometric molecular models for the pump mechanism.

For the current-source model, therefore, there is no fundamental thermodynamic contradiction between voltage-independent active transport and voltage-dependent metabolic cost; what was apparently paradoxical for the NTS model is the expected behavior of the current-source pump.

As for the role of ATP in the energetics of active transport, each model raises a further question: why should the NTS pump always split the same amount of ATP to pump a given number of ions, when the energy required to translocate them varies considerably with their concentrations and the transmembrane potential? Perhaps that is a price paid for some other biological advantage. Where does the current-source pump get its power when the membrane potential is displaced to, and beyond, the reversal potential V_r for net ATP turnover? It gets it mainly from the external device used to displace the membrane potential, acting through a power transfer mediated by the Onsager reciprocity principle. Net turnover is not a principal source of transport power for the current source pump; transport power derives indirectly from the electrochemical potentials of the pumped ions themselves (K. M. Chapman, 1980, 1982, and manuscript in preparation).

The reciprocity mechanism works this way: as potential-driven reciprocal synthesis generates ATP against the affinity A of the ATP pool, it drives power in the amounts $-L_{Na}F(V - E_{Na})A$ and $-L_{K}F(V - E_{K})A$ into the ATP pool. As the affinity-driven currents are pumped against their respective electrochemical potentials, they simultaneously drive an identical power $-(FL_{Na}A)(V - E_{Na})$ and $-(FL_{K}A)(V - E_{K})$ back into the ion pools, in effect consuming the ATP from reciprocal synthesis as fast as it is made. Since no net ATP hydrolysis results from this power transfer, the participating ATP acts, by definition, as a catalyst; it does not appear as metabolic cost.

The power released by net ATP hydrolysis exactly equals the pump's

dissipative losses when the membrane is at steady state. That is, the metabolic cost of the idling pump is not the cost of transport, but of its "second law overhead,"⁸ just as it is in any idling, fuel-consuming machine. During recovery processes, and during imposed displacements of the membrane potential, net turnover may also contribute transport power, or may defer to power drawn from the ion pools and any external power source.

The current-source pump is thus a reciprocity machine that recycles ATP for transport power in maintaining steady active transport rates, but whose metabolic cost varies with the electrochemical load.

Limits of linearity for this thermodynamic model are of some concern. The linear approximation is known to hold near Donnan equilibrium, but many excitable cell membranes operate around two to three times RT from it, and ATP affinity A is probably an order of magnitude greater than that. However, the important question is not whether the physiological system remains linear, but the extent to which it remains reciprocal. I suspect the main conclusions outlined here are not limited to the linear case, but that remains to be shown.

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⁸ Overhead is expressed by the dissipation function $\Phi = J'X$, or the sum of products of flows times conjugate force (Eq. 1). At steady state, all net ionic fluxes are zero, so that Φ equals only the net turnover power $J_r A$.

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