

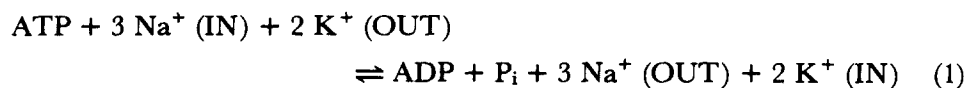
LETTER TO THE EDITOR

Equilibrium State of ATP-driven Ion Pumps in Relation to Physiological Ion Concentration Gradients

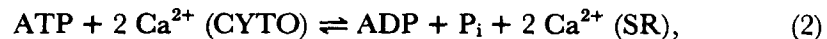
Dear Sir:

The purpose of this communication is to show that the Ca ion concentration gradient across the sarcoplasmic reticulum membrane of a resting skeletal muscle cell is close to what would be predicted for the equilibrium state of the ATP-driven Ca pump. The Na and K gradients across the plasma membranes of erythrocytes or of a resting squid axon, however, fall far short of the predicted equilibrium of the ATP-driven Na,K pump. The basis for the apparent inefficiency of the Na,K pump is not obvious. It may be physiological, arising from competing ion fluxes produced by other ion-translocating systems in the membrane, or it may be biochemical, arising from a factor such as imperfect discrimination by the pump protein between Na and K ions.

The calculated equilibria are based on the assumption that ATP hydrolysis and ion transport are tightly coupled by the pump proteins, but no assumption about the detailed mechanism is required. With the generally accepted stoichiometries (Glynn and Karlsh, 1975; Hasselbach, 1979), the coupled overall reactions are



and



with ATP and its hydrolysis products confined in each case to the cytoplasmic side of the membrane. It is possible that the Ca pump (analogous to the Na,K pump) also carries another cation in the opposite direction, and it has been suggested that Mg and/or K may play that role (Kanazawa et al., 1971). This possibility does not affect the calculation, because significant concentration gradients in Mg or K are not established (Somlyo et al., 1977). Absence of a gradient in K concentration is assured by the existence of channels that make the membrane leaky to Na and K (McKinley and Meissner, 1977), and these channels also dissipate any membrane potential that might otherwise exist.

The fact that the reactants and products of reactions 1 and 2 are not all in the same phase does not affect the calculation of the equilibrium state according to the stoichiometry of the reaction. The standard free energy change (ΔG°) is obtained in the usual way as the sum of standard chemical

potentials ($\mu^{0'}$) for the products less the sum for the reactants, and since $\mu^{0'}$ for an ion in an aqueous medium is the same on either side of the membrane, $\Delta G^{0'}$ has the same value for both reactions, numerically equal to the standard free energy of hydrolysis of ATP. With $\Delta G^{0'} = -RT \ln K'$, this means that each reaction also has the same equilibrium constant, $K' = K_{\text{ATP}} = [\text{ADP}][\text{P}_i]/[\text{ATP}]$ at equilibrium in the absence of competing reactions. The values given by Guynn and Veech (1973) as a function of the Mg concentration have been used, corrected for temperature when necessary by use of an enthalpy change of -5 kcal/mol (Alberty, 1969). Values of intracellular ionized Mg concentrations from the recent literature were used to allow for the small effect of Mg on K_{ATP} . Concentrations are expressed in molar units, per liter or kilogram of cell water for intracellular concentrations. For ATP and its hydrolysis products, concentrations represent each species in all its forms (different ionization states, complexes with Mg, etc.). For the metal ions the concentrations are those of the free ions, exclusive of ions bound to proteins or to the membrane, and the activity coefficients of the unbound ions are assumed to be the same on the two sides of the membrane. For Na and K the fraction of bound ions under any conditions of interest is almost certainly negligible (Shporer and Civan, 1977), so that [Na] and [K] effectively represent total concentrations of these ions.

Equilibria predicted by reactions 1 and 2 with $K' = K_{\text{ATP}}$ depend on the initial conditions and other constraints. To predict the equilibrium gradients under physiological conditions, we make use of the fact that the cytoplasmic concentrations of ATP and its hydrolysis products are maintained at constant values by metabolic processes unrelated to the pump (e.g., Veech et al. [1979]). When the pump is operating far from equilibrium, it may transiently perturb these concentrations, but this is not a factor in a calculation based on the premise that the pump has been allowed to attain thermodynamic equilibrium. Similarly, the membrane potential ψ may in part be determined by the pump reaction, but only measured values are needed for an equilibrium calculation. The equilibrium conditions for the two pumps may thus be written as

$$\left\{ \frac{[\text{Na}^+(\text{OUT})]^3 [\text{K}^+(\text{IN})]^2}{[\text{Na}^+(\text{IN})]^3 [\text{K}^+(\text{OUT})]^2} \right\}_{\text{eq}} = K_{\text{ATP}} e^{\psi F/RT} \left\{ \frac{[\text{ATP}]}{[\text{ADP}][\text{P}_i]} \right\}_{\text{CYTO}} \quad (3)$$

and

$$\left\{ \frac{[\text{Ca}^{2+}(\text{SR})]^2}{[\text{Ca}^{2+}(\text{CYTO})]^2} \right\}_{\text{eq}} = K_{\text{ATP}} e^{4\psi F/RT} \left\{ \frac{[\text{ATP}]}{[\text{ADP}][\text{P}_i]} \right\}_{\text{CYTO}}, \quad (4)$$

all known parameters being combined on the right-hand side.

The most important factor on the right-hand sides of these equations is the cytoplasmic $[\text{ATP}]/[\text{ADP}][\text{P}_i]$ quotient. A series of recent studies by Veech and co-workers, summarized by Veech et al. (1979), has convincingly demonstrated that cytosolic ADP concentrations in mitochondrion-containing cells are lower than earlier analytical data would indicate, and this leads to correspondingly larger values for the concentration quotient $[\text{ATP}]/$

$[ADP][P_i]$, i.e., $\sim 30,000 \text{ M}^{-1}$ in muscle and brain cells. Similar data from Nishiki et al. (1978), for isolated rat hearts at low work levels, substantially agree. An alternative way to express these results is in terms of free energy available from ATP hydrolysis to drive ion transport, $-\Delta G_{ATP} = RT \ln \{K_{ATP}[ATP]/[ADP][P_i]\}$. The data of Veech et al. (1979) yield about 14 kcal/mol ATP hydrolyzed, significantly more than earlier estimates. These results do not apply directly to squid axoplasm, but the difficulty inherent in using direct analytical ADP concentrations for squid axoplasm has been pointed out by Mullins and Brinley (1967), and the $[ATP]/[ADP][P_i]$ quotient of $2,000 \text{ M}^{-1}$ given for the resting squid axon by Hurlbut (1970), and the corresponding value of $-\Delta G_{ATP} = 11.2 \text{ kcal/mol}$, are almost certainly too small. Values of $30,000 \text{ M}^{-1}$ were used for both squid axoplasm and muscle

TABLE I
PHYSIOLOGICAL GRADIENTS ESTABLISHED BY THE Na,K PUMP

	Erythrocyte*	Squid axon†
$[Na(IN)], mM$	11	50
$[Na(OUT)], mM$	138	440
$[K(IN)], mM$	135	400
$[K(OUT)], mM$	4.4	20
ψ, mV	-9	-70
$\frac{[Na(OUT)]^3[K(IN)]^2}{[Na(IN)]^3[K(OUT)]^2}$	2×10^6	3×10^6
Predicted by Eq. 3	1.4×10^9	6×10^8

* Data for human red cells at 38°C. $[Na(IN)]$ from M. Haas and T. McManus (personal communication), ψ from Hoffman and Laris (1974), other data from Funder and Wieth (1966). Use of alternative sources for the experimental data would not significantly alter the result.

† Hodgkin (1964). Use of alternative sources for the experimental data (e.g., Hurlbut, 1970) would not significantly alter the result.

cytoplasm to obtain calculated gradients according to Eqs. 3 and 4. For the erythrocyte, which lacks mitochondria, the lower value of $5,700 \text{ M}^{-1}$ given by Veech et al. (1979) was used.

In comparing predictions based on these equations with physiological concentration gradients, it must be recognized that the latter are not true equilibrium values, but represent a steady state resulting from the combined effects of the ion pump and of passive fluxes. The major passive fluxes (e.g., Na and K channels of the nerve) are turned off in a resting cell, but slower fluxes ("leaks") persist. The forward and reverse rates of the pump approach equality as equilibrium is approached, and the net rate will eventually become slow enough to enable even slow fluxes to compete with it. It is energetically advantageous, however, if passive flux competition does not manifest itself until the pump reaction is very close to equilibrium, so as to minimize the rate at which ATP has to be utilized to maintain the steady state. The data of Tables I and II show that this condition is satisfied for the sarcoplasmic reticulum Ca pump, but not for the Na,K pump. For the latter, both in

erythrocytes and squid axon, the physiological Na and K gradients are far smaller than those calculated by Eq. 3.

Table II includes data derived from in vitro experiments using oxalate-loaded vesicles to control the intravesicular concentration of free Ca (Makinose and Hasselbach, 1966). The maximal ratios $[\text{Ca}(\text{SR})]^2/[\text{Ca}(\text{extravesicular})]^2$ obtained in these experiments are lower than the estimated physiological ratios (though still considerably higher than the corresponding ratios for the Na,K pump in Table I). This may reflect a smaller value for the extravesicular $[\text{ATP}]/[\text{ADP}][\text{P}_i]$ quotient than under physiological conditions (measurements were not made), but it is not likely that this can account for the total difference: probably the pump flux rate becomes sufficiently small at extravesicular Ca concentrations in the nanomolar range to allow the rate of

TABLE II
CONCENTRATION GRADIENTS ESTABLISHED BY THE
SARCOPLASMIC RETICULUM Ca PUMP

	Physiological*	Oxalate-loaded vesicles‡
$[\text{Ca}(\text{CYTO})]$	$<10^{-7}$ M	2–10 nM
$[\text{Ca}(\text{SR})]$	2–5 mM	5–100 μM
ψ	0	0
$[\text{Ca}(\text{SR})]^2/[\text{Ca}(\text{CYTO})]^2$	$>10^9$	6×10^6 to 2×10^7
Predicted by Eq. 4	6×10^9	—§

* Hasselbach (1979), Martonosi (1980). The total concentration of Ca in sarcoplasmic reticulum lumen (in the resting cell) exceeds 10 mM, but much of this is bound to Ca-binding proteins.

‡ Makinose and Hasselbach (1965). The intravesicular concentration replaces $[\text{Ca}(\text{SR})]$ and was adjusted by varying the oxalate concentration. The measured extravesicular concentration at equilibrium replaces $[\text{Ca}(\text{CYTO})]$.

§ Exact prediction not possible because $[\text{ATP}]/[\text{ADP}][\text{P}_i]$ quotient was not determined.

passive Ca leak through the membrane to compete with the pump, and to set up a steady-state gradient below the pump's own equilibrium gradient. The more significant aspect of these results is that the pump is able to reduce the extravesicular Ca concentration to the nanomolar range (1–5 nM; Hasselbach, 1979), which is more than an order of magnitude less than $[\text{Ca}(\text{CYTO})]$ under physiological conditions, and supports the conclusion that the physiological Ca concentration is limited by the thermodynamics of the overall reaction and not by kinetic regulation.

The question of how closely physiological Na and K concentrations approach the equilibrium state of the Na,K pump has not often been posed explicitly. However, Hurlbut (1970), in a review of data for nerve fibers and using $\Delta G_{\text{ATP}} = -11$ kcal/mol, concluded that the ion gradients in the resting state would be close to the pump equilibrium state if the pump stoichiometry were three Na and three K ions transported per ATP hydrolyzed. Using a slightly different approach, Chapman (1973) and Chapman and Johnson (1978) showed that the conditions under which ATP-dependent Na influx and efflux exactly balance each other would correspond to the pump equilib-

rium state with the stoichiometry of reaction 1 if one could set $\Delta G_{\text{ATP}} = -8$ kcal/mol. In light of the analytical data of Veech et al. (1979) and the present evidence against the transport of three K ions per ATP, the possibility that Na and K gradients in nerve fibers or in the red cell even approximately represent the equilibrium state of the Na,K pump is no longer tenable, unless one discards reaction 1 as descriptive of the overall process catalyzed by the pump. The contrastingly close agreement between prediction and experiment for the Ca pump indicates that the failure of the Na,K pump to attain the predicted equilibrium is not trivial. There are three possible explanations for it.

(1) Regulatory processes may affect the *rate* at which the pump operates (without affecting stoichiometry), causing it to become very slow or cease altogether long before equilibrium is attained. This possibility would seem to be excluded, at least for the erythrocyte, by the well-established observation that unidirectional ouabain-sensitive Na and K fluxes remain high under physiological conditions.

(2) The competitive effects of passive fluxes may be much stronger for the Na,K pumps than for the sarcoplasmic reticulum Ca pump. This is perhaps intuitively the most likely explanation, but if it is the true explanation it is not obvious why the results in Table I for erythrocytes and squid axon should be so similar, since the two membranes differ greatly in their passive permeability characteristics.

(3) The failure to attain the equilibrium predicted by Eq. 3 may result from an inherent limitation of the pump itself, perhaps reflecting nature's inability to design binding sites that discriminate perfectly between Na and K ions. This could lead to deviation from the stoichiometry of reaction 1 as $[\text{K(IN)}]$ builds up and $[\text{Na(IN)}]$ decreases. In that event, the physiological state might closely approximate the equilibrium state of the pump, but reaction 1 would no longer be appropriate for calculation of the equilibrium gradients. The demonstration that K can compete for the Na sites of the Na,K pump protein, and vice versa (Robinson and Flashner, 1979; Yamaguchi and Tonomura, 1979), supports this possibility. It has also been shown that the pump protein can promote Na-Na and K-K exchange under some laboratory conditions (Glynn and Karlsh, 1975), but the exchange pathway in these experiments does not involve ATP hydrolysis and could not, therefore, affect pump efficiency.

Explanation 3 would be preferable in terms of free energy conservation. In 2 it is implied that the pump itself is capable of utilizing the free energy of ATP for ion translocation with essentially 100% efficiency, but a continuous high rate of ATP hydrolysis would be required to maintain the steady state. In 3 the pump itself is viewed as <70% efficient under steady-state conditions, but it is implicit that the pump is close to equilibrium under these conditions, so that the rate of ATP utilization would be low.

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