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ADP controls the electrogenicity of Na/Na exchange catalyzed by dog kidney Na,K-ATPase proteoliposomes

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Abstract

Sodium pump mediated Na/Na exchange was studied using Na^+-loaded proteoliposomes prepared from dog kidney Na,K-ATPase. Measurements of both $^{22}Na^+$ influx and pump-generated electrical potentials were carried out, the latter using the anionic dye, oxonol VI. In the presence of ATP, the formation of a strophanthidin-sensitive membrane potential confirms that Na/Na exchange associated with ATP hydrolysis can be electrogenic depending on the source of the enzyme. With the addition of varying concentrations of ADP, electrogenic exchange is progressively inhibited and replaced by electroneutral exchange. ADP is equally effective in activating (ATP + ADP)-dependent electroneutral exchange. With sufficient ADP, electrogenic Na/Na exchange is completely replaced by electroneutral exchange.

Keywords: ATPase, Na^+/K^+-; Sodium/potassium ion pump; Sodium–sodium ion exchange

1. Introduction

In the absence of extracellular K^+, the sodium pump can effect two modes of Na/Na exchange. One mode is associated with ATP hydrolysis whereby Na^+ ions at the extracellular surface act as surrogate K^+ ions [1–3]; the other, first described in squid axons [4] and studied in erythrocytes [5], requires ADP as well as ATP and is associated with Na^+-dependent ADP–ATP exchange (for review, see Ref. [6]). Evidence to support the conclusion that the ATP-dependent Na/Na exchange associated with ATP hydrolysis is electrogenic was obtained with the enzyme purified from the shark rectal gland [7] and mammalian kidney [8,9] and presumably reflects a $3Na^+$-for-$2Na^+$ exchange per molecule ATP hydrolyzed based on a Na/ATP ratio of close to 3.0 observed with inside-out membrane vesicles derived from red cells [2] and purified kidney Na,K-ATPase proteoliposomes [8].

Abercrombie and DeWeer [10] showed directly that, in the absence of extracellular K^+, the sodium pump of the squid axon catalyzes an (ATP + ADP)-dependent electroneutral cardiotonic-steroid sensitive Na^+ influx. In studies with the mammalian (pig kidney) enzyme reconstituted into proteoliposomes, Goldschleger et al. [9] showed that ADP accelerated Na^+ influx into vesicles carrying out ATP-dependent electrogenic Na/Na exchange without affecting the magnitude of the electrical potential, consistent with the conclusion that (ATP + ADP)-dependent electroneutral Na influx occurs in addition to electrogenic exchange. In contrast, in studies carried out with the rabbit kidney enzyme, ADP inhibited electrogenic Na/Na exchange noncompetitively with respect to ATP [8]; whether or to what extent this inhibition was associated with electroneutral exchange replacing electrogenic exchange was not determined. The experiments described in this paper were carried out similarly to those described by Apell et al. [8], except that Na^+ influx and pump-generated membrane potential were studied concurrently.

2. Materials and methods

Na,K-ATPase was purified from the red outer medulla of freshly obtained dog kidneys by the procedure described by Jørgensen [11] and stored at −70° C. Specific activities ranged from 12–18 μmol/mg per min at 37° C. The enzyme was solubilized with deoxycholate, and reconsti-

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tuted into proteoliposomes by the cholate dialysis procedure described by Apell et al. [8], using the same buffers and sulfate salts of Na^+, K^+ and Tris described by those authors. Changes in membrane potential were monitored by fluorescence measurements as described by Apell et al. [8] using the potential-sensitive dye oxonol VI at a final concentration of 11 μM. ^{22}Na influx was assayed by rapid ion exchange as described by Karlish and Pick [12].

3. Results

3.1. Electrogenic pumping in the presence and absence of intravesicular K^+

Using the potential-sensitive fluorescent dye oxonol VI, we have confirmed the earlier finding of Apell et al. [8] that ATP-dependent electrogenic Na/Na exchange can replace electrogenic Na/K exchange when intravesicular (extracellular K^+) is depleted. Thus, as shown in Fig. 1A, when K^+-filled proteoliposomes are suspended in medium containing 75 mM Na_2SO_4 and then equilibrated with oxonol VI, a rapid increase in fluorescence is observed following addition of ATP due to the formation of a membrane potential, inside positive, presumably reflecting the uptake of three Na^+ and efflux of two K^+ ions. The subsequent decline in fluorescence is consistent with depletion of intravesicular (extracellular) K^+ as a result of this exchange. The further slow rise in fluorescence is consistent with the formation of a membrane potential due to ATP-promoted electrogenic Na/Na exchange replacing electrogenic Na/K exchange as observed with the rabbit kidney enzyme reconstituted into proteoliposomes [8]. As explained by Apell et al. [8], the high membrane voltage transiently increases leakage currents until a stable condition of electrogenic Na/Na exchange is attained. The ratio of the initial rate of potential increase observed in the first phase of the reaction ascribed to Na/K exchange to that observed in the second phase ascribed to Na/Na exchange is 22, which is close to the value of 18.5 reported earlier

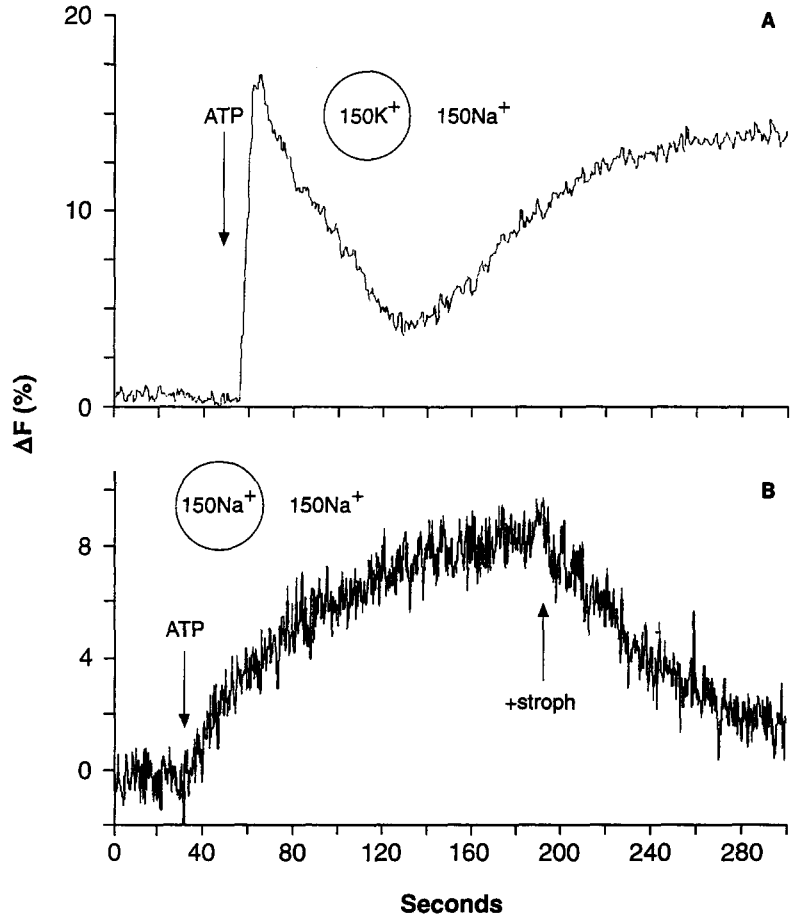

Fig. 1. Electrogenic Na/K and Na/Na exchanges measured by oxonol VI fluorescence. (A) Proteoliposomes (20 μl) containing 150 mequiv./l K^+ (75 mM K_2SO_4) and buffer A comprising 6 mM imidazole, 1 mM EDTA and 5 mM $MgSO_4$, pH 6.2, were added to 2.7 ml medium containing 150 mequiv./l Na^+ (75 mM Na_2SO_4), buffer A and 11 μM oxonol VI (30 μl of a 0.1 mM solution freshly diluted, with water, from a 1 mM stock solution in ethanol). The reaction was initiated by addition of 0.25 mM ATP as indicated. (B) Proteoliposomes containing 150 mequiv./l Na^+ were assayed as described in A. Strophanthidin at a final concentration of 10^{-4} M (6 μl of a 50 mM ethanolic solution) was added as indicated.

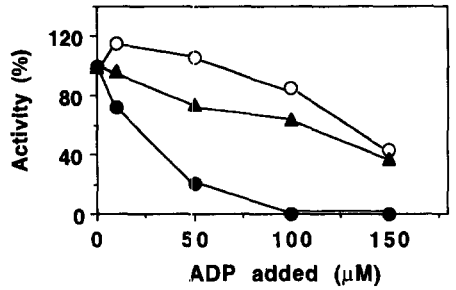

Fig. 2. Effect of varying ADP concentration on ATP-dependent initial rate of oxonol VI fluorescence increase and $^{22}Na^+$ influx. Initial rate of Oxonol VI fluorescence increase, $\partial\Delta F/\partial t$ (●), and $^{22}Na^+$ influx (▲) assayed at room temperature under similar conditions and with 0.25 mM ATP as described in Fig. 1 except that the extravesicular Na^+ concentration was 20 mequiv./l, with the osmolarity maintained with Tris-sulfate. $\partial\Delta F/\partial t$ assayed at 150 mequiv./l. Na^+ (○). For flux assays, the proteoliposome concentration was 20-fold that used for the oxonol VI fluorescence. The flux assay was carried out for 6 min since the ATP-dependent $^{22}Na^+$ uptake increases linearly with time up to at least this period.

[8]. Fig. 1B shows that the established fluorescence formation of a membrane potential in Na^+-filled proteoliposomes suspended in Na^+-containing medium is abolished following addition of the lipophilic cardiotonic steroid, strophanthidin. When the intravesicular Na_2SO_4 was reduced to 10 mM by replacement with Tris-sulfate, an ATP-dependent increase in fluorescence could not be detected (not shown).

3.2. Effect of ADP on Na^+ influx and the generation of a transmembrane potential in Na^+-filled proteoliposomes

When Na^+-filled vesicles are suspended in a medium containing 250 μM ATP and 75 mM Na_2SO_4, the initial rate of increase in oxonol VI fluorescence is not changed following addition of ADP up to about 100 μM (Fig. 2, open symbols). However, as shown in Fig. 2, when extravesicular (cytoplasmic) Na_2SO_4 is reduced to 10 mM, electrogenic pumping, albeit decreased somewhat (≈ 30%), is markedly sensitive to inhibition by ADP.

Under identical conditions and with the same Na^+-filled vesicles as those used for measurements of oxonol fluorescence except for a higher vesicle concentration, the effect of ADP on ATP-dependent $^{22}Na^+$ influx was tested at the lower (20 mequiv./l) Na^+ concentration. In the representative experiment shown in Fig. 2, ADP caused a decrease in both the initial rate of ATP-dependent fluorescence increase ($\partial\Delta F/\partial t$) and Na^+ influx. However, the effect of ADP on $\partial\Delta F/\partial t$ is much greater than its effect on ATP-dependent Na^+ influx. Thus, at 50 μM ADP, ^{22}Na influx is decreased by only ≈ 30% whereas a fluorescence increase is no longer detected. It should be noted that the ADP concentration-dependence of the decrease in $\partial\Delta F/\partial t$ is similar to that reported by Apell et al. [8] using the rabbit enzyme.

4. Discussion

There appear to be differences in the ability of the pump of diverse membrane systems to effect Na/Na exchange coupled to ATP hydrolysis versus Na/Na exchange coupled to ADP-ATP exchange. Thus, in the case of Na^+-filled pig kidney Na,K-ATPase proteoliposomes, Na^+ influx observed with ATP alone is increased by addition of ADP [8]. In contrast, ADP inhibits the influx into proteoliposomes prepared from the enzyme of either rabbit kidney [8] or shark rectal gland [7]. In the present study, we show directly that the mammalian (dog kidney) Na,K-ATPase, like the squid giant axon enzyme studied by Abercrombie and DeWeer [10], can mediate electroneutral Na/Na exchange in the presence of both ADP and ATP. Under the conditions of our experiments, ADP promotes electroneutral exchange at the expense of electrogenic exchange, at least when the cytoplasmic Na^+ concentration is relatively low (20 mequiv./l). This conclusion is based on the assumption that electroneutral exchange is probably a 3 Na^+-for-3Na^+ exchange.

From the results shown in Fig. 2, the proportion of total nucleotide-dependent Na^+ influx due to electrogenic exchange and that due to electroneutral Na/Na exchange can be estimated. Thus, at each concentration of added ADP, the difference in (i) total Na/Na exchange, expressed as a percentage of the exchange observed in the absence of ADP (filled triangles) and (ii) $\partial\Delta F/\partial t$, also expressed as a percentage of $\partial\Delta F/\partial t$ observed in the absence of ADP (filled circles), represents the proportion of total nucleotide-dependent exchange due to electroneutral Na/Na exchange. From the data obtained at 20 mequiv./l extravesicular Na^+, the results of this estimation indicate clearly that as ADP reduces electrogenic exchange, electroneutral exchange is increased in mirror-image fashion.

This study confirms the ADP-dependence of inhibition of pump current reported by Apell and co-workers [8] and extends their work to show that non-competitive inhibition of ATP-dependent electrogenic pumping is most likely due to enhanced reversal of the ADP release step ($Na_3 \cdot E_1 \cdot ATP \rightleftharpoons (Na_3)E_1P + ADP$) of the following abbreviated Albers-Post mechanism for the Na,K-ATPase operating under K^+-free solutions, which omits intermediates such as those with less than three Na^+ ions bound to EP (cf. Fig. 22 of Ref. [6]).

$$E_1 \cdot ATP + 3Na_{cyt} \rightleftharpoons Na_3 \cdot E_1 \cdot ATP \rightleftharpoons (Na_3)E_1P \rightleftharpoons$$
$$Na_3 \cdot E_1P \rightleftharpoons Na_3 \cdot E_2P \rightleftharpoons E_2P + 3Na_{ext}$$
$$E_2P \rightleftharpoons E'_2P$$

$$E'_2P + 2Na_{ext} \rightleftharpoons Na_2 \cdot E'_2P \rightleftharpoons (Na_2)E'_2 \rightleftharpoons$$
$$(Na_2)E'_2 \cdot ATP \rightleftharpoons Na_2 \cdot E'_1 \cdot ATP \rightleftharpoons E'_1 \cdot ATP + 2Na_{cyt}$$
$$E'_1 \cdot ATP \rightleftharpoons E_1 \cdot ATP$$

In this reaction scheme, hydrolysis of $Na_2 \cdot E'_2P$ may be rate-limiting in the absence of ADP.

One notable difference in the two studies concerns the inhibitory effect of extravesicular (cytoplasmic) Na^+. Apell et al. [8] consider that the step $(Na_2)E'_2 \cdot ATP \rightleftharpoons Na_2 \cdot E'_1 \cdot ATP$ is shifted towards the left as the cytoplasmic Na^+ concentration is increased. The extent to which the overall reaction is affected by cytoplasmic Na^+ would depend on the poise of the steady-state equilibrium at this step.

Using the shark rectal gland enzyme, Cornelius and Skou [7] failed to observe an ADP-sensitive Na/Na exchange, which they attributed to a poise in the distribution between E_1P and E_2P forms towards E_2P. From the results of their experiments with Na^+-loaded pig kidney enzyme proteoliposomes, Goldschleger et al. [9] suggested that the 'paradoxical' increase in ATP-supported Na/Na exchange by ADP, without diminution of the electrical potential, might be because the rate of the step accelerated by ADP, namely the reversal of the reaction $(Na_3 \cdot E_1 \cdot ATP \rightleftharpoons (Na_3)E_1P + ADP)$ is much slower than other steps, namely the forward rate of phosphorylation of E_1 or the conformational change of E_1P to E_2P. In contrast, in our experiments, the rate-limiting step in the presence of ADP is probably one of the reactions leading to E_2P formation. It is also plausible that there are differences in the poise of the equilibrium between distinct forms of the enzyme which interact with three versus two Na^+ ions at the extracellular surface, i.e., E_2P and E'_2P, respectively, depicted in the foregoing mechanism. It may not be surprising that differences in the relative rates of these steps, possibly secondary to differences in the membrane (lipid) environment, account for differences among the enzymes derived from different tissues. It may be simplistic, however, to assume that in all systems these exchanges are accounted for entirely by the aforementioned 3-for-3 and 3-for-2 exchanges, respectively. Thus, in K^+-free resealed red cell ghosts assayed under conditions supporting Na/Na exchange and in which the ratio of ouabain-sensitive Na^+ efflux to ATP hydrolysis was close to 3.0, two unusual characteristics were observed by Marin and Hoffman [13]: Na^+ efflux was several-fold greater than Na^+ influx and intracellular ADP activated both Na^+ efflux and ATP hydrolysis. Whether this additional complexity of the red cell pump reflects a heretofore unidentified molecular diversification of the sodium pump remains to be determined.

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