DIRECT EVIDENCE OF SODIUM RELEASE IN THE COURSE OF Na^+,K^+- DEPENDENT ATPase REACTION

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Received December 1, 1969

Na^+,K^+-dependent ATPase connected with microsomal material of a number of tissues is considered to be a part of the sodium transporting system (Skou,1965; Albers,1967; Lisovskaja,1967). The discovery of the ability of this material to bind sodium ions in presence of ATP and Mg^{++} much strengthened this point of view (Järnefelt,1961; Järnefelt and Stedingk,1963; Charnock and Post,1963). A decrease in ^{22}Na binding capacity found when K^+ ions are present in the medium may be considered an important but still indirect evidence for a proposed Na^+ for K^+ exchange reaction in the course of ATP hydrolysis. The labeling methods used previously to establish this fact showed a difference of Na^+ binding dependent on the presence of K^+ but not the potassium stimulated release of Na^+ ions itself.

Since the mechanism of Na^+-active transport is still obscure it is of importance to establish the Na^+ for K^+ exchange as a real stage of Na^+,K^+-dependent ATPase hydrolysis. For this purpose potentiometric Na^+-activity measurements of microsomal suspension in the course of ATPase reaction performed with the aid of Na^+-sensitive glass

electrodes was found to be a suitable method. Microsomal ATPase material was prepared by Post and Sen (1967) method from guinea pig kidney cortex. A volume of 5 ml of the suspension in 10 mM imidazole-buffer containing 12-15 mg of microsomal protein was used in each experiment. Stable Na^+--sensitive electrodes made of $NABS_{25-05-09}$ glass (Nicolsky et al.,1967; Beljustin and Lev,1967; Beljustin et al.,1968) were immersed in test samples after a calibration in NaCl solutions. Changes of Na^+-activity were automatically recorded in the course of sequential additions of 3 mM $MgCl_2$, 2.5 mM Na salt of ATP and 2.0 mM KCl. 30 experiments of this kind were carried out and in each case a control measurement on enzyme-free media were performed to take into account all sorts of non-specific effects including some influence of K^+ ions on Na^+-selective glass electrode. In several special series, the action of 0.1-3.0 mM of ouabain added to the mixture together with ATP, the addition of 2.0 mM $CaCl_2$ and the omission of ATP or substitution of ATP for ADP were studied.

ATP hydrolysis measured for the same samples as well as in a parallel experiments with longer timing showed that at the conditions used, ATPase activity was about 60% when compared with the optimal activity for the same suspension. This decrease seems to be natural if one takes into account a 5-fold reduced concentration of Na^+ and K^+ (Na/K ratio kept unchanged) as compared with optimal ionic conditions for Na^+,K^+-dependent ATPase. This alteration of optimal ionic concentrations was found necessary in all cases to obtain detectable Na^+-activity changes in the course of reaction.

As is seen from table 1, a release of Na^+ after the addition of K^+ into the test mixture is statistically

Table 1. Increase of Na^+-activity caused by the addition of 2.0 mM/l KCl to microsomal suspension

Experimental variants	Omission (-) or addition (+)*	No of exper.	Increase of Na^+ activity M±m (mM)	P_{diff} (in respect to Complete)
1 Complete	None	30	1.11 ±0.11	-
2 Control	- ATPase	24	0.68 ±0.10**	< 0.01
3	- ATP	9	0.67 ±0.14	< 0.01
4	- ATP; + ADP	4	0.65 ±0.08	< 0.01
5	+ $CaCl_2$	8	0.67 ±0.15	< 0.02
6	+ Ouabain	14	0.92 ±0.12	>0.2

* Omissions and additions are given in respect to complete reaction mixture.

** Non-specific effect including the influence of K^+ ions on Na^+-sensitive electrode.

significant. This Na^+ release is completely inhibited by $CaCl_2$, omission of ATP, or substitution of ATP by ADP, but not decreased significantly with ouabain. On the other hand ATP hydrolysis was reduced in all the above mentioned cases. Described changes of Na^+ release are in a perfect agreement with data on ^{22}Na binding obtained by Charnock and Post (1963) and Järnefelt and Stedingk (1963). This

coincidence shows that Na^+ release is completely determined by the enzyme binding capacity. The fact that ouabain did not inhibit Na^+ release caused by the addition of K^+ is evidence that this inhibitor cannot alter Na^+-for-K^+ exchange.

Considering a turnover number for Na^+,K^+-dependent ATPase system at conditions used of about 3,000 per minute (Post et al.,1965) and the rate of inorganic phosphate formation of 10 mM per minute per liter, the ratio of about 100 Na^+ ions released for 1 inorganic phosphate formed was found. This value for the ratio is of the same order as that calculated by Järnefelt and Stedingk (1963) from experiments where ^{22}Na binding and ^{32}P incorporation had been studied. Stoichiometry of 3 or 2 Na^+ ions transported per 1 inorganic phosphate, as established from transport experiments on intact cells where Na^+ ions were pumped up against a high electrochemical gradient, should not be directly compared with the idle-transport conditions of the above experiments performed on membrane fragments.

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