Importance of Cell Integrity for $Li^+ - Na^+$ Isomorphism in the Activation of $Na^+ + K^+$)-ATPase

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Abstract. Potency of Li^+ as a substitute for Na^+ in activating the ouabain-sensitive ATPase of the rat kidney outer medulla was compared simultaneously on three forms of enzyme preparation differing in the extent of structural intactness: a) tissue slices, b) crude membrane fraction, c) purified enzyme, washed off endogeneous Na^+ and K^+. The ATPase activity of all the preparations was determined under similar conditions. Additionally, the ATPase activity of tissue slices was measured cytospectrophotometrically. In slices the equal rate of ouabain-sensitive ATP hydrolysis was stimulated by $(Li^+ + K^+)$ and $(Na^+ + K^+)$. Considerable ouabain-sensitive activity was also observed in the presence of Li^+ alone. On the contrary, in a highly purified ouabain-sensitive ATPase, the activity of the $(Li^+ + K^+)$ or the $(Li^+ + Li^+)$-ATPase did not exceed 5—10% of that induced by $(Na^+ + K^+)$. In a crude membrane fraction with a high background of Mg^{2+}-ATPase such a low increment of the Li^+-stimulated ATPase activity was not detectable. A suggestion is made that the $Li^+ - Na^+$ isomorphism is strongly dependent on the cell integrity. It appears that the Li^+/Na^+ selectivity of Na^+-binding sites is sensitive to the procedure of tissue homogenization and cell destruction.

Key words: Lithium — Sodium — ATPase — Ion selectivity

Introduction

Considerable disagreement exists regarding the extent of $Li^+ - Na^+$-isomorphism in catalizing Na-dependent reactions of the ouabain-sensitive ATPase. Close similarity between Li^+ and Na^+ was revealed in the ion transport across the frog skin epithelium. By contrast, the ouabain-sensitive activity of the $(Li^+ + K^+)$-ATPase studied in the same epithelia proved to be unexpectedly low as compared to that of the $(Na^+ + K^+)$-ATPase (Reinach et al. 1975; Siegel et al.

1975). Much higher potency of Li^+ as a substitute for Na^+ was found in studies performed on renal slices (Willis and Fang 1975) and red blood cell ghosts (Whittam and Ager 1964; Dunham and Senyk 1977; Hoffman 1978) although in these preparations a possible contribution of endogeneous Na^+ could not be neglected. Convincing evidence that the Na^+-dependent sites of the ouabain-sensitive ATPase can be activated by Li^+ was obtained on a highly purified enzyme (Beaugé 1978) but the level of the Li^+-stimulated activity was one order of magnitude lower than the activity of $(Na^+ + K^+)$-ATPase.

We suggested that various procedures employed in the enzyme preparations might perturb the structural integrity of the ATPase environment affecting thereby the Li^+/Na^+-selectivity of the Na^+-binding sites. To check this supposition, the ability of Li^+ to cause a Na^+-like effect in activating the ouabain-sensitive ATPase was compared for three kinds of renal preparations differing in the extent of their structural intactness: a) tissue slices, b) crude membrane fraction, and c) purified ouabain-sensitive ATPase. The amounts of endogeneous Na^+ and K^+ were far below their activating concentrations. The main finding of the present work is that the disintegration of the enzyme containing tissue is accompanied by a drastic decrease of $Li^+ - Na^+$-isomorphism in stimulating the ouabain-sensitive ATPase.

Material and Methods

Male rats of Wistar strain were used throughout the experiments. Rats were sacrificed by decapitation and kidneys were immediately removed. The kidney medulla was cut out, frozen in liquid nitrogen and kept in cryostate at $-20°C$. Three modifications of the ATPase preparation obtained from the same zone of the outer medulla were used: tissue slices, a crude membrane fraction and a highly purified ouabain-sensitive ATPase. Frozen slices were prepared in cryostate at $-20°C$ and mounted on coverslips. In this form they were found to be fully accessible to all the cofactors used in both biochemical and cytochemical determinations of the ATPase activity. Thickness of slices, about 10 μm, permitted to estimate the enzyme activity in a monolayer of relatively preserved cells. For the preparation of the crude membrane fraction a portion of thawed tissue was homogenized in a cold solution containing (mmol.l^{-1}): sucrose 250, KCl 20, Na_2EDTA 3, $MgCl_2$ 2 and imidazole 10 (pH 7.2). The homogenate was centrifuged at 1500 g for 10 min. The supernatant fraction was removed and sedimented at 22000 g for 60 min. The sediment was used for further investigations. Purified preparation was obtained according to Post and Sen (1967) followed by a treatment with sodium dodecyl sulphate according to Jørgensen (1974). Replacement of the tissue sodium and potassium for lithium was achieved by a threefold washing of the preparations with a lithium containing Tris-maleate buffer, pH 7.4 at 0°C. Residual content of Na^+ and K^+ in the slices ranged from 3 to 7 μmol/g dry wt., while that in other two preparations did not exceed 1 μmol.l^{-1}. Biochemical assay of the ATPase activity of slices, crude membrane fraction and purified preparation was performed identically in a basic medium ($MgCl_2$ 3 mmol.l^{-1}, Tris-maleate buffer 35 mmol.l^{-1}, pH 7.4, Tris-ATP 3 mmol.l^{-1}) containing chlorides of monovalent cations (mmol.l^{-1}): Na^+ 150, K^+ 10 (Medium I), Li^+ 150, K^+ 10 (Medium II), Na^+150, Li^+ 50* (Medium III), Li^+200 (Medium IV). Contamination of the Na^+- or K^+-free media by Na^+ or K^+, respectively, did not exceed 30 μmol.l^{-1}. The ATPase activity was

* *Higher concentrations of Li^+ are needed to induce a K^+-like effect (Skou 1975).*

expressed as μmol P_i/h mg protein released in a 20 min incubation period at 26°C. Inorganic phosphate was determined according to Järnefelt (1972). Protein was assayed by the modified method of Lowry (Sutherland et al. 1949). Cytochemical determination of the ATPase activity was described in detail elsewhere (Krestinskaya and Manusova 1969). In this procedure, frozen nonfixed slices were incubated for 20 min at 26°C in media I—IV containing 0.45 mmol.l^{-1} $Pb(NO_3)_2$. The reaction was stopped by removing the slices from the incubation medium and by washing them with cold distilled water. Then the slices were treated with a diluted (1:200) $(NH_4)_2S$ solution, fixed in 10% formaldehyde for 10—12 hours and mounted in glycerine jelly. As a result of these procedures the inorganic phosphate, liberated due to ATP hydrolysis, precipitated as PbS, the distribution of which corresponds to the enzyme localization.

Optical density of the stained cells was evaluated cytophotometrically. The absorption spectrum of PbS in slices had a maximum at 470—490 nm. The optical density was linearly dependent on the thickness of slices and the incubation time. The two-wavelength method of Mendelson (1958) was used. In each slice the optical density of 30 cells in an identical renal tubule was measured at 497 and 615 nm. The activity of the enzyme was expressed in arbitrary units. In all enzyme preparations the activity of the ouabain-sensitive ATPase (OS-ATPase) was calculated as a difference between the total ATPase activity and that determined in the presence of ouabain (1.10^{-3} mol) or in the basic medium free of monovalent cations. The results are presented as mean values ± SE. The significance of the observed difference was tested with a Student-criterion.

Results

Tissue slices. ATPase activity of slices was determined both by biochemical (Table 1) and cytochemical methods (Fig. 1) and the results obtained were similar. The activity of the $(Na^+ - K^+)$-ATPase (Medium I) was taken as 100%. The activity of the ouabain-sensitive $(Li^+ + K^+)$-ATPase (Medium II) was nearly as

Table 1. Ouabain-sensitive ATPase activity in various forms of enzyme preparations from the rat kidney medulla

Media	Additions (mmol)	Tissue slices (10)*		Crude membrane fraction (7)		Purified OS--ATPase (5)	
		μmol Pi/prot/h	%	μmol Pi/prot/h	%	μmol Pi/prot/h	%
I	Na-150 K-10	30.1 ± 7.2	100	73.3 ± 12.6	100	801 ± 88	100
II	Li-150 K-10**	25.9 ± 4.4	86	0	0	62.3 ± 3.9	8
III	Na-150 Li-50	16.3 ± 2.4	54	17.4 ± 3.0	25	—	—
IV	Li-200	17.8 ± 3.9	60	0	0	54.1 ± 2.6	7

*) Number of experiments

**) In case of purified enzyme the K^+ concentration was reduced to 0.1 mmol.l^{-1} in order to obtain a maximal $(Li^+ - K^+)$ ATPase activity (Beaugé 1978)

high as that of the $(Na^+ + K^+)$-ATPase. At the same time, the activity of the ouabain-sensitive ATPase in K^+ free media (III, IV) made up only 60%. Thus

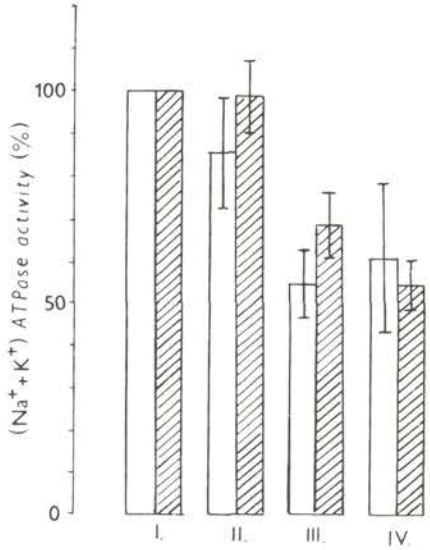

Fig. 1. Activity of the ouabain-sensitive ATPase in rat kidney outer medulla slices. Ordinate — rate of the ouabain-sensitive ATP hydrolysis measured in various media both cytochemically (white columns) and biochemically (dashed columns). Abscissa — additions to the basic incubation media (see Methods). I — 150 mmol.l^{-1} Na^+, 10 mmol.l^{-1} K^+; II — 150 mmol.l^{-1} Li^+, 10 mmol.l^{-1} K^+; III — 150 mmol.l^{-1} Na^+, 50 mmol.l^{-1} Li^+; IV — 200 mmol.l^{-1} Li^+. Vertical bars indicate the standard error of the mean values obtained in 5—7 similar experiments.

50 mmol.l^{-1} Li^+ was not equivalent to 10 mmol.l^{-1} K^+ in the activation of the ouabain-sensitive ATPase.

Crude membrane fraction. Table 1 shows that in the Na^+-free media (II and IV) the ouabain-sensitive ATPase could not be detected. The ability of Li^+ to substitute for Na^+ is drastically decreased. Nevertheless, the K^+-like effect of Li^+ observed in the crude membrane fraction is two times less than that measured in slices. It should be mentioned that in a crude membrane fraction half of the total ATPase activity was represented by the ouabain-insensitive Mg^{2+}-ATPase which evidently decreased the accuracy of determining the presumably low levels of the ouabain-sensitive ATPase stimulated by Li^+ in the absence of Na^+.

Purified preparation of the ouabain-sensitive ATPase. The ouabain-sensitive activity of the purified enzyme preparation measured in Medium I was one order of magnitude higher than that of the crude membrane fraction while the ouabain--insensitive ATPase did not exceed 2% of the total ATPase activity. Under such conditions even a low stimulation of the ouabain-sensitive ATP hydrolysis could be

reliably detected. Table 1 shows that the ouabain-sensitive activity was observed both in Medium II and Medium IV though the absolute level of the $(Li^+ + K^+)$- and $(Li^+ + Li^+)$-ATPase was as low as 7—8% of the maximal rate of hydrolysis observed in Medium I. It is obvious that such a low increment of the ouabain-sensitive activity could not be visualized in a crude membrane fraction.

Discussion

The main finding of this investigation is that the ability of Li^+ to substitute for Na^+ in activating the ouabain-sensitive ATPase drastically decreases in the course of the disintegration of an enzyme containing tissue. Virtually, complete $Li^+ - Na^+$-isomorphism was observed in studying the ouabain-sensitive ATPase of renal slices. Both biochemical and cytochemical determination showed that the $(Li^+ + K^+)$-ATPase activity was close to that of the $(Na + K^+)$-ATPase. A lower level of the ouabain-sensitive ATPase was stimulated by Li^+ alone, which can be accounted for by an incomplete isomorphism of Li^+ and K^+. This $K^+ - Li^+$ similarity was only slightly affected by procedures employed in the preparation of the crude membrane fraction while the $Li^+ - Na^+$ isomorphism totally disappeared. The residual level of both the $(Li^+ + K^+)$- and the $(Li^+ + Li^+)$-ATPase could be revealed only on a purified preparation of the ouabain-sensitive ATPase characterized by a much higher specific activity and a low background of the Mg^{2+}-ATPase. In full agreement with Beaugé (1978) it was shown that in the disintegrated enzyme preparations both the $(Li^+ + K^+)$- and the $(Li^+ + Li^+)$-ATPase activities were one order of magnitude lower when compared to those of the $(Na^+ + K^+)$-ATPase. This difference in the potency of Li^+ to stimulate the ouabain-sensitive ATPase in slices and in preparations with disintegrated structure cannot be attributed to the contamination of incubation media by Na^+ or K^+. They were far below the minimal concentrations capable of activating the ouabain-sensitive ATPase (Fujita et al. 1968). It is tempting to suggest that the extent of the $Li^+ - Na^+$-isomorphism in activating the $(Na^+ + K^+)$-ATPase may be influenced by tissue homogenization. In the course of homogenization and purification cells undergo substantial fragmentation into disoriented particles. On the other hand, the procedure of slice cutting appears to be less damaging. Although, in the process of cutting, freezing and thawing, cell membranes become highly accessible to all enzyme cofactors, the macromolecular structure of cells in slices as well as the orientation of cell membrane surfaces anyhow remain relatively intact. The cell integrity is likely to be of crucial importance for maintaining the $Li^+ - Na^+$-isomorphism at the Na^+-dependent sites of the embedded enzyme. However, the loss of a factor controlling ion selectivity can not be completely ruled out. Thus, the variability in the extent of $Li^+ - Na^+$-isomorphism reported for a variously treated ouabain-sensitive ATPase may be due to the different degree of structural changes affecting the ion selectivity of Na^+-binding sites. In this respect the K^+-like

effect of Li^+ appears to be more resistant to the procedures involved in the enzyme isolation.

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