ROLE OF Na,K-ATPase IN ABSORPTION OF ISOTONIC FLUID BY FROG GALL BLADDER EPITHELIUM

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Experiments on isolated frog gall bladders showed that the addition of ouabain ($1 \cdot 10^{-4}$ M) or noradrenalin ($3 \cdot 10^{-6}$ M) to the incubation Ringer's solution from the side of the serous surface of the organ or replacement of the K^+ in the solution by the equivalent Na^+ concentration causes a decrease in the rate of absorption of fluid by the epithelium and a decrease in the Na,K-ATPase activity in its cells. Noradrenalin reduces Mg-ATPase activity also. Significant positive correlation was found between the rate of transport of isotonic fluid by the epithelium and the Na,K-ATPase activity in its cells.

KEY WORDS: *frog gall bladder; Na,K-ATPase; absorption of fluid; noradenalin.*

The epithelium of the gall bladder reabsorbs inorganic ions (Na^+, Cl^-, HCO_3^-) and water from the liver bile and transports it into the blood as an isotonic fluid. The study of the mechanism of transport of the isotonic fluid by the gall bladder epithelium is important, for this type of transcellular ion transport lies at the basis of the secretory and absorptive function of the glandular and epithelial cells of various organs. Several hypotheses have been put forward to explain the nature of the mechanism of transport of isotonic fluid by the gall bladder epithelium [7, 9, 10, 13, 15]. According to one of them, an essential role in this process is played by the Na,K-ATPase of cells of the epithelial layer of the gall bladder wall [3, 5, 10, 15]. It has been shown histochemically that this enzyme system is located in the region of the lateral-basal surface of the epithelial cell membrane [8]. According to one suggestion, Na,K-ATPase transports Na^+ from the cells outwardly in the direction of the serous surface of the gall bladder and so participates in the transcellular transport of these ions [7, 10].

To continue the study of this mechanism, changes in the absorptive function of the organ and in the Na,K-ATPase activity of the epithelial cells were compared in experiments on isolated frog gall bladders exposed to the action of ouabain, noradrenalin (NA), and other factors.

EXPERIMENTAL METHODS

Isolated gall bladders were tied on to thin polyethylene cannulas, the lumen of the organ was filled with Ringer–Bentley solution, and the gall bladder was then immersed with its serous surface in a vessel containing similar solution, through which oxygen was bubbled. The gall bladders were incubated for 90-120 min at 25±0.5°C. Every 15 min the gall bladders were weighed on torsion scales and the intensity of fluid transport from the lumen of the organ to the outside was judged from the decrease in their weight. The velocity of the absorption of fluid was expressed in μl/h/100 mg wet weight of gall bladder wall. After the end of the experiments the gall bladders were incised and the mucosa curetted in the cold, and activity of Mg- and Na,K-ATPase in a homogenate prepared from the scrapings was determined as described previously [4]. In individual series of experiments ouabain ($1 \cdot 10^{-4}$ M) or Na ($3 \cdot 10^{-6}$ M) was added to the incubation medium in contact with the serous surface of the gall bladder, or its K^+ ions were replaced by Na^+ ions.

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TABLE 1. Effect of Transport Inhibitors and NA on Rate of Absorption of Ringer–Bentley Solution from Lumen of Isolated Frog Gall Bladder and on ATPase Activity (in μmoles P_i/mg protein/h) in Epithelial Cells (M±m)

Experimental conditions	V	Mg-ATPase	Na, K-ATPase
Control (n = 10)	74,8±7,5	3,6±0,1	3,4±0,8
Ouabain, 30 min (n = 3)	30,0±8,8*	3,3±0,2	0,9±0,3*
Ouabain, 60 min (n = 3)	10,5±5,1*	1,8±0,6*	0,8±0,5*
Ringer's solution without K^+, 30 min (n = 9)	30,5±4,1*	3,1±0,5	0,9±0,1*
Ringer's solution without K^+, 60 min (n = 9)	14,0±3,6*	4,2±0,5	0,9±0,1*
NA, 30 min (n = 8)	6,2±3,0*	1,5±0,2*	1,4±0,3*

Notes. 1. Ouabain and NA added to solution on side of serous surface of gall bladder; K^+ removed from solution on side of serous surface of gall bladder.
2. n — Denotes number of determinations, V velocity of absorption of Ringer–Bentley solution from lumen of gall bladder (in μl/h/100 g wet weight of gall bladder wall).
3. Values for which P < 0.05 compared with control marked by asterisk.

TABLE 2. Effect of NA on Velocity of Absorption of Ringer–Bentley Solution and on ATPase Activity (in μmoles P_i/mg protein/h) in Epithelial Cells of Frog Gall Bladder during Its Incubation in Potassium-Free Solution (M±m)

Index studied	Ringer's solution without K^+		Ringer's solution without K^+ and with NA			
	incubation for 45 min (I)	incubation for 60 min (II)	incubation for 15 min (III)	P_{I-III}	incubation for 30 min (IV)	P_{II-IV}
V	3,3±0,9 (n=18)	2,8±0,7 (n=19)	7,4±1,9 (n=22)	0,05	1,7±0,8 (n=11)	0,5
Na, K-ATPase	0,9±0,1 (n=10)	0,9±0,1 (n=9)	3,2±0,8 (n=6)	0,001	1,1±0,2 (n=4)	0,5
Mg-ATPase	3,1±0,5 (n=10)	4,2±0,5 (n=9)	3,1±0,4 (n=6)	0,5	4,4±0,6 n=4	0,5

Note. V) Velocity of absorption of Ringer–Bentley solution from lumen of isolated gall bladder (in μl/15 min/100 mg wet weight of gall bladder wall). Remainder of note as in Table 1.

EXPERIMENTAL RESULTS

The results of control experiments showed that the intensity of the absorption of fluid by the gall bladder correlated significantly and positively with Na,K-ATPase activity in the epithelial cells; the coefficient of correlation (r) was 0.62 at P < 0.001.

Addition of NA [2] or of ouabain [6] to the incubation medium on the side of the serous surface of the isolated gall bladder is known to inhibit its absorptive function. It has also been shown that the presence of these substances in the incubation medium of a homogenate of the epithelial cells of the gall bladder with ATP inhibits Na,K-ATPase activity [1, 14].

The present experiments showed that the addition of ouabain or NA to the external incubation solution, or the replacement of its K^+ by Na^+ ions, caused a regular decrease in the rate of absorption of isotonic fluid from the lumen of the gall bladder and also a decrease in the Na,K-ATPase activity in the epithelial cells, which could already be detected after homogenization of the mucous membrane and its incubation with ATP. The effect of NA also was accompanied by a decrease in Mg-ATPase activity in the epithelial cells (Table 1). The degree of inhibition of the absorptive function of the gall bladder and of Na,K-ATPase activity in its cells depended on the duration of contact of the organ with the inhibitory factors. After incubation of the gall bladder with ouabain or in external solution not containing K^+ for 30 min the rate of absorption of fluid by the gall bladder was reduced by 60 and 62%, respectively, and after incubation for 60 min by 83 and 81%. Activity of Na,K-ATPase was reduced mainly during the first 30 min of action of the inhibitors.

Comparison of the results of these two series of experiments thus shows that ouabain and absence of K^+ in the solution of the serous side have the same effect on the transport func-

tion of the gall bladder epithelium. Unlike these two factors, NA also inhibited Mg-ATPase activity.

If the absorptive function of the gall bladder was first inhibited by incubating the organ in potassium-free solution, and NA was then added, the opposite effect was obtained: The velocity of absorption of fluid by the gall bladder and the Na,K-ATPase activity of its cells were increased. This reaction was detected only during the first 15 min of action of the mediator on the gall bladder; during second 15 min the rate of absorption of fluid and the Na,K-ATPase activity were reduced to the initial level (Table 2). The de-inhibiting effect of NA may be due to the outflow of K^+ from the cells and to activation of Na,K-ATPase, for NA is known to increase the permeability of the surface membranes of the liver cells for K^+ [11, 12]. The possibility cannot be ruled out that NA also acts in the same way on the membranes of the gall bladder epithelial cells.

The results given in Fig. 1 show that not only in the control experiments, but also during the action of the various agents on the absorptive function of the gall bladder positive correlation still remained between the rate of fluid transport from the lumen of the gall bladder and Na,K-ATPase activity in the epithelial cells. In the experiments with ouabain and NA, for instance, the coefficient of correlation between these values was 0.76 ($P < 0.05$) and 0.75 ($P < 0.001$), respectively. During the action of NA while the organ was incubated in potassium-free solution, and also during incubation of the gall bladders in potassium-free solution only, the value of r fell to 0.58 ($P < 0.01$) and 0.34 ($P < 0.02$), respectively.

When these data are examined it must be emphasized that changes in Na,K-ATPase activity were discovered after all procedures connected with removal and homogenization of the mucous membrane. Consequently, the action of ouabain and NA on the intact gall bladder wall and also removal of K^+ from the external incubation caused conformational changes in Na,K-ATPase which did not disappear during subsequent incubation of the enzyme under optimal conditions.

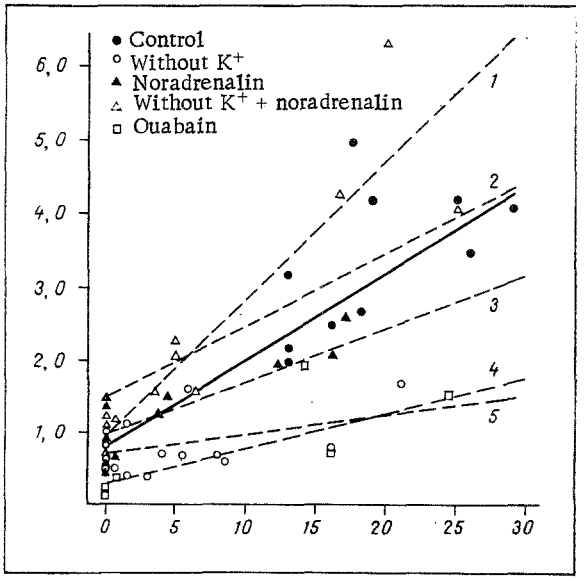

Fig. 1. Relationship between velocity of absorption of isotonic fluid and Na,K-ATPase activity in epithelial cells of frog gall bladder. Broken lines denote regression functions for corresponding indices in control (2) and during exposure of gall bladder to NA and potassium-free (1) and normal (3) solutions, during action of ouabain (4), and replacement of K^+ by Na^+ ions (5); continuous line denotes mean regression function for all series of experiments. Abscissa) rate of absorption of fluid, in μl/15 min/100 mg wet weight of gall bladder wall; ordinate) Na,K-ATPase activity (in μmoles P_i/mg protein/h).

The results of these investigations thus showed that the rate of absorption of isotonic fluid by the frog gall bladder epithelium correlates positively with the change in Na,K-ATPase activity in the epithelial cells. Factors inhibiting the absorptive function of the organ simultaneously cause inhibition of enzyme activity. These results confirm the view that Na,K-ATPase plays a role in the mechanism of absorption of isotonic fluid by the frog gall bladder epithelium.

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