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EFFECT OF BIVALENT CATIONS ON PROPERTIES OF NaCl-STIMULATED ATPase ACTIVITY IN RABBIT SMALL INTESTINAL MUCOSA

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UDC 612.332.015.1.014.46-085.23

KEY WORDS: small intestine; NaCl-stimulated, oligomycin-inhibited ATPase activity; bivalent cations; enzyme regulation.

The writers' previous investigation showed that the mucosa of the rabbit's small intestine contains a highly labile enzyme with ATPase activity, the properties of which suggest that the function of this ATPase may be linked with secretion [1]. A particularly interesting fact is that Ca^{++} or Mg^{++} ions, if added to the incubation medium in a concentration of 0.2-0.5 mM, cause sharp changes in the properties of the ATPase reaction, expressed as loss of the stimulating effect of NaCl and $NaHCO_3$ and disappearance of the inhibitory effect of ethacrynic acid on ATPase activity. The results suggest that this ATPase functions in the cell under conditions which exclude the possibility of access of bivalent cations to it.

The aim of this investigation was to continue the study of the effect of bivalent cations on ATPase activity of the mucosa of the small intestine.

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits weighing 1.2-1.8 kg. The secretogenic agent was histamine, which was injected subcutaneously into the animals in a dose of 2.0-2.5 mg/kg body weight. The animals were decapitated 2-2.5 h after injection of histamine. The mucosa of the small intestine was washed with cold physiological saline and wiped with filter paper, after which the epithelium was carefully scraped off and a 10% homogenate prepared in isolation medium containing 0.25 M sucrose, 5 mM EDTA, and 5 mM HEPES-Tris, pH 7.0. After centrifugation at 700g for 10 min a residue of the membranes was obtained. The residue was resuspended in isolation medium in which EDTA was reduced to a concentration of 0.5 mM or was completely absent. ATPase activity in these membrane preparations was determined from the accumulation

Laboratory of Molecular Basis of Pathogenesis of Infectious Diseases, Central Research Institute of Epidemiology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 8, pp. 66-69, August, 1982. Original article submitted March 5, 1982.

TABLE 1. Changes in Properties of ATPase Activity in Mucosa of Rabbit's Small Intestine after Addition of Bivalent Cations (0.25 mM) to Incubation Medium (M ± m)

Experimental conditions	NaCl in incubation medium, mM	ATPase activity, μmoles P_i/mg protein/h				
		without inhibitors	oligomycin (1 μg/ml)	ethacrynic acid (0.1 mM)	2,4-dinitrophenol (50 μM)	p-chloromercuribenzoate (75 μM)
Control	0	2,81±0,39	1,20±0,22	1,97±0,07	1,98±0,16	1,68±0,16
Control	50	4,29±0,19	1,19±0,31	1,94±0,12	1,74±0,22	1,72±0,21
Mg^{2+}	0	4,99±0,38	5,00±0,24	5,10±0,27	4,84±0,32	4,74±0,30
	50	5,29±0,90	4,99±0,36	4,91±0,45	4,75±0,44	4,97±0,57
Ca^{2+}	0	3,66±0,63	3,67±0,45	3,70±0,38	3,82±0,46	3,32±0,58
	50	3,62±0,67	3,65±0,70	3,37±0,44	3,24±0,44	3,41±0,57
Ba^{2+}	0	2,76±0,37	—	—	—	—
	50	2,80±0,04	1,80±0,17	—	—	—
Zn^{2+}	0	4,48±0,24	—	—	—	—
	50	4,36±0,37	2,93±0,20	—	—	—

Legend. Besides reagents mentioned above, incubation medium also contained 40 mM MES-Tris, pH 6.2, 5 mM ATP, and 0.05 mM EDTA. Here and in Table 2, P_i denotes inorganic phosphorus.

TABLE 2. Changes in Properties of ATPase Activity in Control Animals and in Animals Receiving Histamine, after Addition of EDTA to Isolation Medium (M ± m)

Group of animals	Oligomycin	ATPase activity, μmoles P_i/mg protein/h			
		with EDTA in isolation medium		with EDTA in isolation medium	
		without NaCl	NaCl	without NaCl	NaCl
Control	Absent	1,61±0,15	1,75±0,32	1,76±0,43	2,02±0,5
	Present	1,59±0,21	1,62±0,3	0,80±0,22	0,76±0,28
Experimental	Absent	2,25±0,01	2,97±0,09	2,99±0,15	4,02±0,27
	Present	1,42±0,25	1,47±0,3	0,97±0,03	0,98±0,03

Legend. Besides reagents mentioned above, incubation medium also contained 40 mM MES-Tris, pH 6.2, 5 mM ATP, and 2 mM EDTA.

of inorganic phosphorus [6] in incubation medium containing 5 mM ATP, 50 mM NaCl, 40 mM MES-Tris, pH 6.2. The protein concentration was determined by the biuret method. In cases when contamination of the incubation medium with bivalent cations had to be avoided, EDTA was added to it in a concentration of 2 mM. ATPase activity was determined only in freshly isolated membrane preparations in the course of 2-3 h after decapitation of the animals.

EXPERIMENTAL RESULTS

As Table 1 shows, the isolated membrane preparations could catalyze the ATPase reaction in the absence of bivalent cations in the incubation medium. ATPase activity observable under these conditions was stimulated by NaCl and inhibited by ethacrynic acid, oligomycin, p-chloromercuribenzoate, and dinitrophenol. Addition of Ca^{++} or Mg^{++} ions to the incubation medium in a final concentration of 0.25 mM caused sharp changes in the properties of ATPase activity, namely, disappearance of the stimulating effect of NaCl and a sharp decrease in the action of inhibitors on the velocity of ATP hydrolysis. It was also shown that close correlation exists between sensitivity to oligomycin of NaCl-stimulated ATPase activity and the Ca^{++} and Mg^{++} concentrations in the incubation medium. The graphs illustrated in Fig. 1 show that with an increase in the concentration of bivalent cations in the incubation medium, the inhibitory action of oligomycin on the rate of ATP hydrolysis was gradually reduced. Parallel with these changes the sensitivity of the ATPase reaction to NaCl decreased, and in the presence of 0.2 mM Mg^{++} or Ca^{++} the effect of oligomycin and NaCl on enzyme activity virtually ceased to be detectable.

Since several enzymes participating in ATP hydrolysis could be present in the available membrane preparations, it might be supposed that the ATPase activity observed in the presence of bivalent cations was functionally connected with activity of what is known as basal ATPase, on which the inhibitors mentioned above, and also Na^+ and Cl^- ions, have no effect. Meanwhile, Ca^{++} and Mg^{++} ions may have an inhibitory effect on activity of NaCl-stimulated oligomycin-

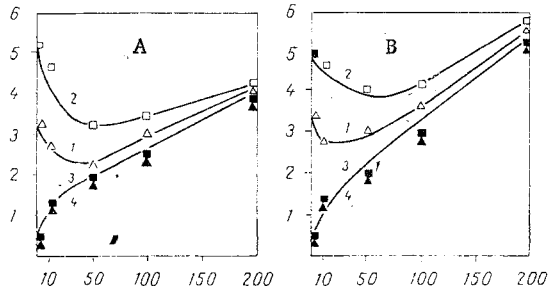

Fig. 1

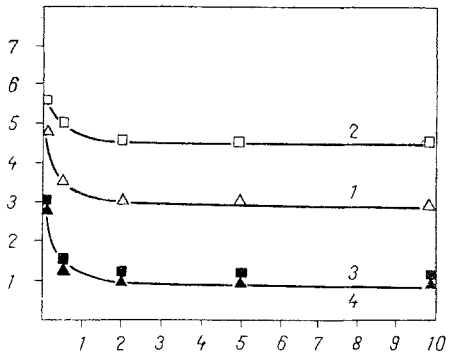

Fig. 2

Fig. 1. Effect of increasing concentration of Ca^{++} and Mg^{++} ions on ATPase activity in mucosa of rabbit small intestine. 1) ATPase activity in absence of NaCl; 2) the same, in presence of 50 mM NaCl; 3) effect of oligomycin on ATPase activity in absence of NaCl; 4) the same, in presence of 50 mM NaCl. Abscissa, concentration (in μM): A) Ca^{++}; B) Mg^{++}; abscissa, concentration (in μmoles P_i/mg protein/h).

Fig. 2. Effect of increasing concentrations of EDTA on ATPase activity in mucosa of rabbit small intestine. Abscissa, EDTA concentration (in mM). Remainder of legend as to Fig. 1.

inhibited ATPase. The suggestion that NaCl-stimulated ATPase does not need bivalent cations is confirmed by the results of experiments in which the effect of different concentrations of EDTA was studied on ATPase activity. The graphs in Fig. 2 show that an increase in the EDTA concentration in the incubation medium to 0.5 mM caused some decrease in ATPase activity in the absence of NaCl, but potentiated the stimulating action of NaCl on the velocity of ATP hydrolysis. However, a further increase in the EDTA concentration to 5-10 mM had no significant effect on oligomycin-sensitive NaCl-stimulated ATPase activity. It will be evident that addition of EDTA to the incubation medium led to binding of contaminating bivalent cations, and this evidently enabled the better detection of NaCl-stimulated ATPase and, at the same time, it allowed activity of ATPases exhibiting absolute dependence on Mg^{++} or Ca^{++} ions to be reduced. It will be noted that it is impossible by means of EDTA to get rid of all the ATPase activity observed in the absence of NaCl, and this may perhaps indicate that this enzyme activity is not connected with activity of Mg- or Ca-dependent ATPases.

Mg^{++} and Ca^{++} ions may perhaps inhibit activity of NaCl-stimulated ATPase and that in the cell this enzyme may function under conditions which prevent access of bivalent cations to it. In this connection, investigations which showed that ATPases whose activity likewise is not absolutely dependent on bivalent cations are present in certain biological objects [2, 4], must be noted.

The inhibitory effect of bivalent cations on activity of NaCl-stimulated ATPase can also be explained on the grounds that Ca^{++} and Mg^{++} ions, when binding with membranes, thereby lose their permeability, as a result of which access of the substrates to this enzyme is lost. In this case, it can be postulated that an increase in membrane permeability, in the presence of detergents for instance, must again lead to the appearance of NaCl-stimulated, oligomycin-inhibited ATPase activity. In fact, according to data obtained in the writers' laboratory [1] and also by other workers [3, 5], Mg-dependent anion-stimulated ATPase, whose activity is considerably increased after treatment of the membranes with detergents, are present in the mucosa of the small intestine. As was stated previously [1], Mg-dependent, HCO_3^--stimulated, SCN^--inhibited ATPase activity, observed in membranes treated with Triton X-100, differs sharply in its properties from NaCl-stimulated ATPase. However, it cannot be definitely concluded from these results that NaCl-stimulated and HCO_3^--stimulated ATPase activities reflect the function of different enzymes.

It must also be noted that Ba^{++} and Zn^{++} ions differ appreciably in their action on activity of NaCl-stimulated ATPase from Ca^{++} and Mg^{++} ions (Table 1). For instance, in a concentration of 0.25 mM these cations abolish only the activating effect of NaCl on ATPase activity, whereas oligomycin, in the presence of these cations, continues to inhibit ATP hydrolysis, al-

though by a lesser degree. Complete cessation of the inhibitory effect of oligomycin on ATPase activity was observed when the Ba^{++} and Zn^{++} concentrations in the incubation medium were increased to 0.5 mM.

Such marked selective sensitivity of NaCl-stimulated ATPase activity to Ca^{++} and Mg^{++} ions could indicate that these cations are regulators of the activity of this enzyme. In fact, the data given in Table 2 shows that in control animals, in six of nine cases in membranes isolated in medium without EDTA, ATPase activity sensitive to NaCl and to oligomycin cannot be detected at all, even when EDTA is present in the incubation medium, whereas isolation of membranes in medium with EDTA facilitates the appearance of ATPase activity sensitive to oligomycin. It should, however, be pointed out that in this case also NaCl had hardly any effect on the velocity of ATP hydrolysis. Meanwhile, in animals receiving histamine, in all cases NaCl-stimulated, oligomycin-inhibited ATPase activity appeared both in membranes isolated in medium without EDTA and in those isolated in the presence of this substance.

The results, however, do not give an answer to the question of what order of events leads to activation of NaCl-stimulated ATPase by histamine. It may be that histamine lowers the affinity of this enzyme for bivalent cations, and this is accompanied by conformational changes in the protein and the transition of NaCl-stimulated ATPase into the active state as a result of the outflow of cations from their binding sites. The possibility likewise cannot be ruled out that histamine changes membrane permeability and thereby regulates interaction of this enzyme with bivalent cations.

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EFFECT OF PROTEIN INTAKE ON PROTEIN TURNOVER IN SUBCELLULAR FRACTION OF RAT LIVER

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UDC 612.352.3-06:612.398

KEY WORDS: protein turnover; subcellular fractions; low- and high-protein diets.

Protein turnover in intracellular structures is a process which controls the steady-state content of the various proteins which provide for a definite level of functional activity. The important role of turnover in adaptive changes in response to the action of an external factor will thus be evident. The dietary factor is particularly important, for besides the function of participating in metabolism, it also performs the role of supplier of precursors for synthesis of biological macromolecules and, in particular, amino acids.

Theoretical analyses and numerous experiments [4, 7, 8] have broadened opportunities for the study of rates of protein renewal with the use of labeled amino acids. However, they have so far found only limited application in the field of biochemistry of nutrition.

The aim of this investigation was to assess dependence of protein turnover in subcellular fractions on long-term feeding on low- and high-protein diets.

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(Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 8, pp. 69-72, August, 1982. Original article submitted February 12, 1982.

0007-4888/82/9408-1092$07.50