EFFECT OF ACETYLCHOLINE ON Na,K-ATPase ACTIVITY OF RAT SPLEEN AND LYMPHOSARCOMA HOMOGENATES

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A functional connection exists between the acetylcholine system and the system for active transport of sodium and potassium ions, but its molecular mechanism has not yet been discovered [4]. Low concentrations of acetylcholine are known to increase, whereas high concentrations decrease activity of the key enzyme of transport of these ions, namely Na,K-ATPase [1, 3, 5].

Data in the literature on Na,K-ATPase activity in malignant tumor cells are few in number and contradictory in nature. Activity of this enzyme in Zajdela's hepatoma cells is the same as in normal liver cells [2], but in Morris' hepatoma cells it is low [6]. Low molecular activity of the enzyme is found in Ehrlich's ascites carcinoma cells [7]. Meanwhile during transformation of fibroblasts by oncovirus SV40, Na,K-ATPase activity is increased four to fivefold [11].

The effect of acetylcholine in different concentrations on Na,K-ATPase activity in rat spleen and lymphosarcoma was studied.

EXPERIMENTAL METHOD

Experiments were carried out on seven noninbred albino rats weighing 110-120 g. Eight days before the experiment a Pliss' lymphosarcoma was transplanted into the animals. After decapitation of the experimental rats, the spleen and tumor tissue were studied. Control tests also were done on the spleen of five healthy rats. Before measurements the tissues were homogenized. ATPase activity was estimated from the rate of formation of inorganic phosphate as a result of hydrolysis of ATP. Mg^{++}-ATPase activity was measured in 50 mM Tris-HCl buffer (pH 7.4), containing 150 mM NaCl and 2 mM $MgCl_2$ (medium 1).

Na,K-ATPase activity was measured as the difference between activity in medium 1 and its activity after replacement of 30 mM NaCl by KCl. The samples were incubated at 37°C for 30 min. ATP hydrolysis was stopped by the addition of cold TCA in a final concentration of 4%. Phosphorus was determined by Deniges' method [9] and protein by Lowry's method [12].

Acetylcholine in some experiments was added to the homogenate immediately before the measurements, but in the rest of the experiments pieces of tissue (slices 1 mm thick) were treated with it for 30 min at 4°C.

Statistical analysis of the data was carried out by Wilcoxon's nonparametric method, by means of which the significance of statistical data can be assessed without calculating the standard deviation.

EXPERIMENTAL RESULTS

Keeping pieces of tissue in the cold for 30 min without acetylcholine did not change the Na,K-ATPase activity (Table 1).

Acetylcholine caused activation of Na,K-ATPase in the spleen of healthy rats. The effect was more marked if pieces of tissue were treated with acetylcholine. In that case, as a result of treatment with acetylcholine in a concentration of 10^{-6} M, Na,K-ATPase activity was more than 4 times higher than in the control. In a concentration of 10^{-4} M acetylcholine produced a smaller increase in Na,K-ATPase activity. These data are in agreement with those obtained previously in experiments on frog tissues and on a membrane Na,K-ATPase preparation from bovine brain [3].

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TABLE 1. Na,K-ATPase Activity of Rat Tumor and Spleen Homogenates after Treatment with Acetylcholine in Different Ways (in μmoles P_i/mg protein/h)

Acetylcholine conc., M	Healthy rats		Rats with tumors			
	spleen		homogenate		tumor	
	homogenate	pieces of tissue	homogenate	pieces of tissue	homogenate	pieces of tissue
Control	1,7	1,8	2,2	2,2	2,6	2,7
10^{-6}	3,0	8,5	2,3	5,3	2,1	1,4
10^{-4}	2,3	6,6	2,5	3,8	2,3	1,5

Na,K-ATPase from the spleen of rats with tumors also was stimulated by acetylcholine. The mean increase was 140% with acetylcholine in a concentration of 10^{-6} M and 70% in a concentration of 10^{-4} M. Treatment of splenic homogenates from rats with tumors with acetylcholine caused a very small increase, not statistically significant, in enzyme activity.

The response of tumor Na,K-ATPase to acetylcholine was paradoxical and differed sharply from that of the spleen and other tissues studied previously [3, 5]. Acetylcholine reduced the enzyme activity in tumor cells ($P < 0.05$); with an increase in acetylcholine concentration the effect diminished, by contrast with its inhibitory action on Na,K-ATPase in other objects [1, 5]. The lowest mean value of activity was recorded in pieces of tumor treated with acetylcholine in a concentration of 10^{-6} M (Table 1). The inhibitory action of acetylcholine when added directly to the tumor homogenate was not statistically significant ($P > 0.10$). The response of Na,K-ATPase of tumor cells and of spleen cells from animals with tumors, incidentally, differed qualitatively. This contradicts Greenstein's view [10] that the parallel trend of biochemical changes in the tumor and in the tissues of the tumor-bearing organism is a regular feature, and it supports the alternative view expressed by Shapot [13].

The paradoxical inhibitory effect of low concentrations of acetylcholine on tumor Na,K-ATPase revealed by these experiments requires further study. Is it a property characteristic of malignant cells only? Incidentally, the cholinomimetic carbachol, which causes an increase in the acetylcholine concentration in the liver cells, does not affect its level in a hepatoma [8]. Do the facts described above relate to molecular mechanisms which lie at the basis of changes in sensitivity of tumor cells to regulatory influences?

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