EFFECT OF HEPARIN, SPERMIDINE, AND Be^{2+} IONS ON PHOSPHATASE AND RIBONUCLEASE ACTIVITY OF RAT LIVER CELL NUCLEI

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The effect of heparin, spermidine, and Be^{2+} ions on adenosine triphosphatase (ATPase), β-glycerophosphatase, and ribonuclease (RNase) activity of rat liver cell nuclei was investigated in vitro. Be^{2+} ions were shown to inhibit ATPase and β-glycerophosphate activity, the former more effectively. Heparin and spermidine, in physiological concentrations, also inhibited the activity of both phosphatases. Heparin and spermidine had a similar action on nuclear RNase activity. The inhibitory effect of these agents on endonucleases was revealed.

KEY WORDS: ATPase; β-glycerophosphatase; RNase; heparin; spermidine; ions; nuclei; rat liver.

Investigation of the function of the nuclear membrane is of great importance to the elucidation of the mechanisms of nucleo-cytoplasmic relations, the components of which include maturation of RNA and its transport from nucleus into cytoplasm.

Investigations conducted in the writers' laboratory for several years have shown that the nuclear membrane possesses a wide spectrum of enzymes, including adenosine triphosphatase (ATPase) [1-3] and certain other phosphatases [3], and also ribonuclease (RNase) [5]. The presence of a nonspecific phosphatase in the nuclear pores has recently been demonstrated [7, 8].

On the other hand, there is evidence in the literature that certain effectors of phosphatases and ribonucleases can influence the outflow of RNA from isolated cell nuclei. It has been shown, for instance, that Be^{2+} ions, inhibitors of alkaline phosphatase [14], inhibit the outflow of RNA from nuclei in vitro [6]. It has also been demonstrated that spermidine, which can protect endoribonucleases against inhibition by polynucleotides [12], also inhibits the outflow of RNA [18].

These findings indicate that enzymes of the nuclear membrane and nuclear pores are involved in nucleocytoplasmic RNA transport. However, information on the effect of agents modifying RNA transport on phosphatase and RNase activity of the nucleus and nuclear membrane in the literature, if present at all, is only fragmentary in character.

Accordingly, in the investigation described below, the effect of heparin, spermidine, and Be^{2+} was studied on ATPase, β-glycerophosphatase, and RNase activity of isolated rat liver cell nuclei.

EXPERIMENTAL METHOD

The liver of noninbred male albino rats weighing 150-200 g was used in the experiments. Isolation and purification of the nuclei were carried out as in [15] in medium containing $MgCl_2$ instead of $CaCl_2$. The purity of the sample of nuclei and their integrity were verified under the light-optical and electron microscopes. High-polymer cytoplasmic RNA was isolated from the rat liver by the phenol method [9]. RNase inhibitor was isolated from rat liver cytosol [17]. Nuclear RNase activity was determined by the method described by Skridonenko et al. [5]. High-polymer cytoplasmic RNA was used as the substrate. ATPase activity was determined

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TABLE 1. Effect of Be^{2+}, Heparin, and Spermidine on ATPase and β-Glycerophosphatase Activity of Rat Liver Nuclei (mean results of 3 or 4 experiments)

Agent	Concentration	ATPase activity			β-glycerophosphatase activity		
		P_i^*/μg protein·min		degree of inhibition, %	P_i^*/μg protein·min		degree of inhibition, %
		control	experiment		control	experiment	
Be^{+2}, mM	0,1	14,25	12,59	12	1,056	—	—
	1,0		6,7	53		0,600	42
	3,0		0,0	100		0,575	46
	5,0		0,0	100		0,040	97
Heparin, μg/ml	150	15,4	10,76	30	0,810	0,646	20
	500		10,17	34		0,510	37
Spermidine, mM	0,5	13,84	11,92	14	0,810	—	—
	1,0		11,76	15		0,538	34
	2,0		11,12	20		—	—
	3,0		11,90	14		0,505	38
	5,0		10,11	23		0,544	33

* P_i: inorganic phosphorus.

TABLE 2. Action of Natural RNase Inhibitor, Heparin, and Spermidine on RNase Activity of Rat Liver Nuclei (mean results of 3 or 4 experiments)

Agent	Concentration	Activity, optical density, units/mg protein·h		
		control	experiment	degree of inhibition, %
Natural RNase inhibitor, ml	0,1*	2,10	1,39	34
	0,2†		0,99	63
Heparin, μg/ml	20	2,12	1,96	8
	50		1,70	19
	150		1,68	20
	500		1,77	17
Spermidine, mM	0,05	2,07	2,00	0
	0,5		0,50	76
	5		0,36	81

* Equivalent to 6 mg cytosol.

† Equivalent to 12 mg cytosol.

as in [10] and β-glycerophosphatase activity at pH 6.4 as in [14]. Protein was determined by Lowry's method [13].

EXPERIMENTAL RESULTS

The results of investigation of the effect of Be^{2+}, heparin, and spermidine on ATPase and β-glycerophosphatase activity of the isolated nuclei are given in Table 1. It will be clear from Table 1 that Be^{2+} ions inhibit the activity of both enzymes. In a concentration of 1 mM, Be^{2+} gave 50% inhibition of ATPase, and in a concentration of 3 mM it caused total inhibition of activity. By contrast, total inhibition of β-glycerophosphatase activity required a concentration of 5 mM of Be^{2+}. The sensitivity of the nuclear ATPase and β-glycerophosphatase to the inhibitory effect of Be^{2+} thus differed.

Unlike Be^{2+}, heparin proved to be an equally effective inhibitor of ATPase and β-glycerophosphatase. Heparin, in concentrations of 150-500 μg/ml, depressed the activity of both enzymes by 30-40%. Addition of spermidine to the incubation mixture also resulted in some degree of inhibition of activity of these phosphatases.

Neither heparin nor spermidine caused complete suppression of ATPase and β-glycerophosphatase activity, and the degree of their inhibition was practically independent of the concentration of the inhibitors within wide limits. This may indicate that the action of heparin and spermidine on nuclear phosphatases, unlike the action of Be^{2+} ions on them, is indirect.

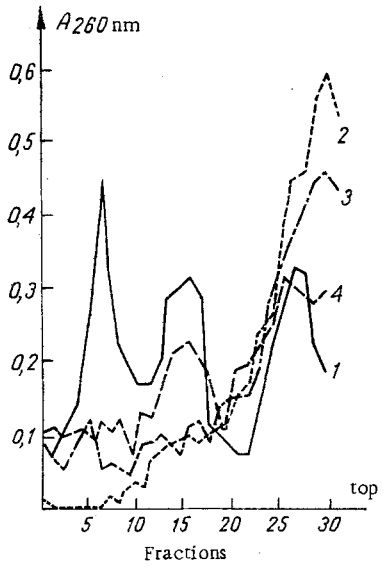

Fig. 1. Sedimentation profile of RNA hydrolysis products in a 5-20% sucrose concentration gradient. Abscissa, fractions; ordinate, optical density at 260 nm. 1) Original RNA; 2) RNA after incubation with nuclei; 3) RNA after incubation with nuclei in the presence of 0.5 mM spermidine; 4) RNA after incubation with nuclei in the presence of 150 μg/ml heparin.

Data showing the effect of heparin, spermidine, and the natural RNase inhibitor isolated from liver cytosol on nuclear RNase activity are given in Table 2. As Table 2 shows, all three agents inhibited RNase activity, and the inhibitory effect increased with an increase in the concentration of the inhibitors. Appreciable inhibition of nuclear RNase activity was observed under the influence of heparin and spermidine in concentrations comparable with physiological.

The sedimentation profile of hydrolysis products of cytoplasmic RNA in a sucrose concentration gradient after incubation with nuclei for 30 min at 37°C is shown in Fig. 1. It follows from the sedimentation profile that heparin and, to a lesser degree, spermidine protect RNA mainly against endonuclease action.

The results thus indicate that the activity of the phosphatases and ribonucleases of liver cell nuclei can be controlled in vitro by a number of physiological agents that are present in the cytoplasm of various tissues and, in particular, by heparin and spermidine. The presence of RNase and phosphatase activity in the nuclear membrane and the possibility of its regulation by cytoplasmic factors may be of great importance in the light of data in the literature [4, 11, 16, 18] showing that cytoplasmic factors have a regulatory influence on the nuclear mechanisms of RNA transport.

It was accordingly decided to investigate the influence of effects of RNase and phosphatase on transport of newly synthesized RNA from liver cell nuclei in vitro. These experiments showed that heparin stimulates, whereas spermidine inhibits the outflow of RNA from the nuclei.

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CHANGES IN ACTIVITY OF KEY ENZYMES OF GLYCONEOGENESIS IN THE LIVER AND KIDNEY OF RATS EXPOSED TO SUBEXTREMAL AND EXTREMAL FACTORS

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Activity of the key enzymes of glyconeogenesis – phosphoenolypyruvate carboxykinase (PEPCK), fructose-1,6-diphosphatase (FDPase), and glucose-6-phosphatase (G6Pase) – in the liver and kidneys of rats was investigated during simultaneous exposure of the animals to subextremal and extremal factors. The low initial PEPCK activity in the liver and its high variability under the influence of extremal stimuli are evidence that this enzyme plays the role of limiting stage of glyconeogenesis. PEPCK activity in the kidneys was comparable with FDPase activity and significantly higher than G6Pase activity, PEPCK activity in the kidneys was 5-6 times higher than in the liver. Under the influence of extremal factors, PEPCK and G6Pase activity in the kidneys rose whereas FDPase activity was practically unchanged. The absence of any distinctly synchronized changes in the activity of the key enzymes of glyconeogenesis in the liver and kidneys indicates that there is no single operon in the cells of these organs for PEPCK, FDPase, and G6Pase with a common mechanism of regulation.

KEY WORDS: glyconeogenesis; phosphoenolpyruvate carboxykinase; fructose-1,6-diphosphatase; glucose-6-phosphatase; subextremal and extremal factors.

Glyconeogenesis plays and extremely important role in the maintenance of carbohydrate homeostasis in the body when in a state of prolonged functional stress. For example, in states such as starvation this process is the only source of glucose. The effectiveness of glyconeogenesis in vivo depends primarily on the combined operation of three key enzymes, which can reverse the processes of glycolysis in the tissues: phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-diphosphatase (FDPase), and glucose-6-phosphatase (G6Pase). Some workers regard these enzymes as a single genetically determined enzyme ensemble with common mechanisms of regulation [1, 11]. Glyconeogenesis is known to take place in only two organs, the liver and kidneys. This is because the activity of enzymes such as PEPCK is absent in the other organs [6, 10]. Investigations to study the coordination of functions of the key enzymes on which the effectiveness of operation of the corresponding "metabolic conveyors" depends have virtually not been undertaken.

In this study an attempt was made to examine coordination of the function of PEPCK, FDPase, and G6Pase simultaneously in the liver and kidneys during exposure of animals to subextremal and extremal factors. Within the framework of this problem the results of a comparative evaluation of activity of the

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