ROLE OF GROWTH HORMONE IN REGULATION OF POLYNUCLEOTIDE-PHOSPHORYLASE ACTIVITY IN RAT LIVER

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Hypophysectomy in rats is followed by a significant increase in polynucleotide-phosphorylase (PNPase) activity in ribosomal fractions of the liver. Injection of growth hormone into hypophysectomized animals leads to inhibition of PNPase activity. Within the dose range from 5 to 100 μg the dose-effect curve is linear. The action of growth hormone is most marked 18 h after a single injection.

KEY WORDS: growth hormone; hypophysectomy; polynucleotide phosphorylase; liver ribosomes.

One of the most active hormonal stimulators of protein synthesis in vivo is growth hormone [4, 5]. Among the possible ways whereby this hormone achieves its action its effect on enzyme activity has been examined [6, 7]. There are as yet no data from which general conclusions can be drawn on the mechanism of the effect of growth hormone on the corresponding systems of RNA and protein metabolism.

Meanwhile, some interesting work has been carried out in this direction. In particular, attention is drawn to studies of the effect of growth hormone on RNA-depolymerase activity. For instance, in an investigation of total alkaline ribonuclease (RNase) of the postmitochondrial fraction of rat liver, a twofold increase in enzyme activity was observed 18 h after hypophysectomy. Intraperitoneal injection of growth hormone depressed total RNase activity to the normal level [8].

Another enzyme of interest in this connection is polynucleotide phosphorylase (PNPase), which is located in liver cells in the microsomal fraction [1] and, for that reason, plays a role in the regulation of the quantity of protein formed in them.

The object of this investigation was to study the character of the effect of growth hormone on PNPase activity.

EXPERIMENTAL METHOD

Experiments were carried out on intact and hypophysectomized female Wistar rats. Hypophysectomy was performed on rats aged 4 weeks and weighing 65-70 g by the method described previously, using an apparatus for transauricular removal of the pituitary [2]. The animals were used in the experiments 1, 2, and 6 weeks after the operation.

PNPase activity in the polysomal fractions of the liver was determined by phosphorolysis of polyadenylic acid in the presence of labeled orthophosphate-^{32}P, with determination of ^{32}P-labeled nucleoside diphosphates adsorbed on Norite as the reaction product.

The fractions of total, free, and bound ribosomes were isolated by the method of Blobel and Potter [3].

Human growth hormone (Kaunas Endocrine Preparations Factory) was used. The hormone was injected subcutaneously in 0.2 ml physiological saline.

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TABLE 1. Effect of Hypophysectomy on PNPase Activity (in specific activity units) of Total Polysomal Fractions of Rat Liver (M ± m; n = 5)

Experiment No.	Hypophysectomized rats	Intact rats (control)	P
	1 week		
1	8,5±0,3	0,96±0,09	≤0,001
2	17,3±0,1	11,3±0,4	≤0,001
3	4,1±0,3	3,1±0,2	≤0,05
4	17,1±0,6	9,9±0,3	≤0,01
	2 weeks		
1	7,5±0,2	1,14±0,04	≤0,01
2	6,2±0,9	0,80±0,01	≤0,01
3	12,60±0,05	6,6±0,2	≤0,001
4	11,5±0,3	5,0±0,5	≤0,001
5	9,7±0,5	4,8±0,4	≤0,01
6	18,0±0,6	7,9±0,7	≤0,001
7	8,20±0,07	4,3±0,1	≤0,001
	6 weeks		
1	3,8±0,2	1,17±0,13	≤0,01
2	2,0±0,6	0,94±0,17	≤0,01

Legend: 1, 2, and 6 weeks – times after hypophysectomy.

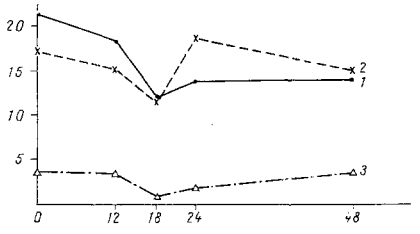

Fig. 1

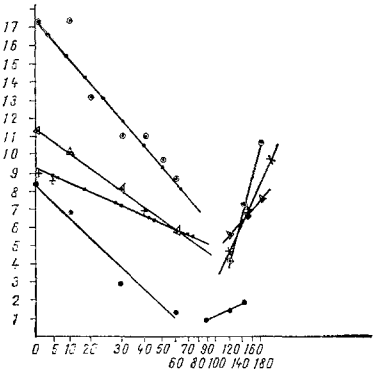

Fig. 2

Fig. 1. Changes in liver PNPase activity of hypophysectomized rats depending on duration of action of growth hormone (60 μg). Abscissa, time (in h); ordinate, specific activity (in units/mg protein). 1) Total ribosomes; 2) membrane-bound ribosomes; 3) free ribosomes.

Fig. 2. Dose-dependence of effect of growth hormone on liver PNPase activity of hypophysectomized rats. Results of four experiments illustrated. Enzyme activity determined in samples of liver polysomes 18 h after injection of hormone. Abscissa, content of growth hormone (in μg); ordinate, specific activity of PNPase (in units/mg protein).

EXPERIMENTAL RESULTS

The results showing the effect of hypophysectomy on rat liver PNPase activity are given in Table 1. Clearly the stimulating effect of hypophysectomy on enzyme activity was absolutely reproducible and statistically significant and it was observed for a long time after the operation. It can tentatively be suggested that after hypophysectomy, which is known to be followed by a sharp decrease in the rate of protein synthesis and growth of animals, certain hormonal factors constantly inhibiting PNPase activity are eliminated. Growth hormone may play the leading role among these factors.

The effect of exogenous growth hormone on liver PNPase activity in hypophysectomized rats in different ribosomal fractions is demonstrated in Fig. 1. Most activity of the total polysomal fraction clearly was accounted for by PNPase located in the fraction of membrane-bound ribosomes. Free ribosomes accounted for less than 20% of total PNPase activity. A single injection of growth hormone led to inhibition of PNPase activity, which was most marked after 18 h. Characteristically inhibition of enzyme activity was distinctly observed in all ribosomal fractions and coincided in time.

The dose dependence of inhibition of PNPase activity by growth hormone after a single injection is shown in Fig. 2. Within the dose range from 5 to 100 μg this dependence is linear in character. With a further increase in dose the PNPase activity gradually increased, and with a dose of 200 μg per animal in some experiments it returned to the initial level of activity. This region of the dose-dependence curve also is linear in character. It is difficult at present to explain the opposite action of "superphysiological" concentration of growth hormone.

These experiments thus demonstrate a direct role of growth hormone in the regulation of liver PNPase activity. The inhibitory action of growth hormone on the activity of this hepatic enzyme is in full agreement with its characteristics as an anabolic hormone. By inhibiting PNPase function it evidently prevents destruction of mRNA by this enzyme, and thus ultimately activates protein synthesis in the hepatocytes.

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