EFFECT OF GROWTH HORMONE ON POLYNUCLEOTIDE PHOSPHORYLASE ACTIVITY IN A PRIMARY MONOLAYER CULTURE OF RAT HEPATOCYTES

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Experiments in vivo showed previously that hypophysectomy in rats is accompanied by a significant increase in polynucleotide phosphorylase (PNPase) activity in the ribosomal fractions of liver [1]. Injection of growth hormone into hypophysectomized animals inhibits the activity of this enzyme, restoring it to its initial level, whereas in intact rats, rat growth hormone did not reduce PNPase activity.

To study the problem of whether this effect of growth hormone is direct or takes place through certain other humoral factors, experiments were carried out in vitro on a culture of rat hepatocytes. In the opinion of many workers, investigations of hormone-dependent changes in liver enzyme systems are best carried out on a primary monolayer cell culture and not on a cell suspension. The reason is that during culture the cells recover their specific functions and also restore the receptor apparatus of their membranes, which is injured during treatment of the liver tissue with enzymes [2, 4, 5].

By the use of a monolayer culture the conditions are thus created for direct contact between hormone and target cells.

EXPERIMENTAL METHOD

Hepatocytes were cultured by a method developed in the Laboratory of Biological Standardization of Hormones. The liver of Wistar rats of both sexes, aged 8 and 50 days, was removed under sterile conditions, treated with 0.02% EDTA solution with antibiotics, and pieces of the large lobe (500 mg) were carefully minced. The pieces of liver, washed in 0.02% EDTA to remove blood, were treated with 7 ml of 0.25% trypsin solution (from Serva, West Germany) and incubated for 8-10 min with periodic shaking. The trypsin solution was then removed, medium No. 199 with 10% embryonic calf serum in a volume of 7 ml was added, and the tissue was dispersed by pipeting 10-15 times. The primary suspension thus obtained was allowed to stand for 15-20 min at 20°C. Hepatocytes are known to be much larger than other types of liver cells [3], and they therefore sediment more rapidly. The supernatant was next removed and the floccular residue of hepatocytes was diluted in 50 ml of medium with 10% embryonic calf serum. The final suspension, containing 5×10^6-6×10^6 cells/ml medium, was poured in doses of 2 ml into Carrell flasks and allowed to stand at a constant temperature of 37°C in an atmosphere of 95% air and 5% CO_2. The cells were cultured for 1-3 days and the medium was changed every 24 h. After the last change the medium contained no serum. Growth hormone was added to the test cultures in a dose of 1 μg/ml (human somatotropin, obtained from Kaunas Endocrine Preparations Factory) in a volume of 0.1 ml and an equal volume of medium No. 199 was added to the control cultures. After incubation for 3 h the medium was removed and the cells were separated from the glass to obtain a cell suspension. The cells were subjected to osmotic shock for 1 h, then homogenized in a glass homogenizer containing 1 mM Tris-HCl, pH 8.0, 2 mM $MgCl_2$, and 2 mM 2-mercaptoethanol.

PNPase activity was revealed by studying phosphorolysis of poly(A) in the presence of ^{32}P-orthophosphate. ^{32}P-nucleoside diphosphates, adsorbed on Norite, were determined as the reaction products. PNPase activity

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TABLE 1. Effect of Growth Hormone on PNPase Activity in Cultures of Hepatocytes Obtained from Intact and Hypophysectomized Rats (M ± m)

Age of animals, days	Duration of culture, days	Experimental conditions	PNPase activity, units/mg protein
8	1	Intact	5,50±0,12
8	1	Intact + growth hormone	2,45±0,07*
8	2	Intact	4,60±0,05
8	2	Intact + growth hormone	2,80±0,05*
8	3	Intact	7,67±0,15
8	3	Intact + growth hormone	4,26±0,10*
50	1	Intact	8,40±0,19
50	1	Intact + growth hormone	4,20±0,10*
50	1	Intact	10,05±0,04
50	1	Intact + growth hormone	5,50±0,11*
50	1	Hypophysectomized	83,30±0,09
50	1	Hypophysectomized + growth hormone	7,85±0,04*

*P < 0.001 compared with control.

was expressed in units/mg protein. The unit of activity of the enzyme was taken to be that amount of it which catalyzed incorporation of 1 nanomole ^{32}P-orthophosphate into material adsorbed on charcoal during incubation for 60 min at 37°C. The protein content was determined by Lowry's method.

Liver from hypophysectomized rats (1 week after hypophysectomy) also was used in the experiments. The pituitary was removed through a transauricular approach on an instrument designed in the Laboratory of Biological Standardization of Hormones [6].

EXPERIMENTAL RESULTS

The results given in Table 1 reflect both the original state of PNPase activity in a culture of hepatocytes from intact and hypophysectomized rats, and also the activity of this enzyme in the cells during the action of growth hormone. It will be clear from Table 1 that PNPase activity of hepatocytes from 50-day-old rats was somewhat higher than from 8-day-old newborn animals. The sensitivity of the cells to growth hormone was the same in rats of both groups – in both cultures enzyme activity was reduced by half. PNPase activity in hepatocytes of intact animals was found to be 4 to 8 times lower than in hepatocytes from hypophysectomized rats, confirming the results of experiments in vivo [1]. This could indicate that slow protein synthesis still continues in the liver cells of hypophysectomized animals when transferred to culture conditions. Unlike results obtained in vivo, addition of growth hormone to a culture of hepatocytes from intact animals revealed a marked decrease (by half) in PNPase activity in the polyribosomal fraction of the liver cells. Meanwhile growth hormone, if added to hepatocytes of hypophysectomized rats, caused the same sharp decline (by four-fifths) in PNPase activity in the experiments in vivo. The absence of effect of growth hormone in vivo and the discovery of its effect in vitro can evidently be explained on the grounds that under conditions of isolation the hepatocytes lose the extracellular factors which regulate the activity of their enzyme systems. In particular, cells grown for a long time in vitro are deprived of the influence of pituitary hormones, including growth hormone, as a result of which they become more sensitive to exogenous growth hormone. This view is confirmed by the results of experiments on hypophysectomized animals. As the writers showed previously, removal of endogenous growth hormone by hypophysectomy facilitates manifestation of the high sensitivity of the liver PNPase to exogenous growth hormone [1].

The results of this investigation show that hepatocytes are target cells for growth hormone and that PNPase activity can be regulated through direct contact with hepatocytes.

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