INCORPORATION OF GLYCINE-^{14}C INTO PLASMA MEMBRANE PROTEINS OF THE NORMAL AND REGENERATING LIVER

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Incorporation of glycine-^{14}C into plasma membrane proteins of the intact and regenerating liver was studied at the beginning of the G_1-period of the mitotic cycle. Electrophoretic analysis of plasma membrane proteins of the regenerating liver, labeled with glycine-^{14}C and soluble in 0.05 M Na_2CO_3, showed that uptake of label was greatest into proteins with a molecular weight of about 60,000. The difference between incorporation of glycine-^{14}C into plasma membrane proteins of the intact and regenerating liver insoluble in 0.05 M Na_2CO_3 during electrophoretic separation was not significant.

KEY WORDS: protein synthesis; plasma membrane; regeneration of the liver.

The state of the plasma membrane of the cells evidently plays an essential role in the initiation of the cell cycle, and for that reason investigation of the synthesis of proteins and other components of the plasma membranes in the early states of preparation of the cell for division is of great interest.

During the study of synthesis of plasma membrane proteins in regenerating liver cells the writer found the maximal increase in the rate of synthesis of plasma membrane proteins soluble in 0.05 M K_2CO_3 solution 1 h after partial hepatectomy, i.e., at the beginning of the G_1-period of the mitotic cycle [1]. It was suggested that an increase in the rate of synthesis of these proteins is one of the earliest biochemical events in cells of the regenerating liver preparing for division.

The aims of the investigation described below were electrophoretic separation of plasma membrane proteins soluble and insoluble in 0.05 M Na_2CO_3 (for brevity they will be called simply soluble and insoluble proteins), and determination of the rate of incorporation of glycine-^{14}C into these proteins in the normal and regenerating liver.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 170-200 g, which were subjected to partial hepatectomy. A mock operation (laparotomy) was performed on the control animals. The animals were killed 1 h after the operation. The liver from 9-11 partially hepatectomized rats and three or four control animals was used in each experiment. Glycine-^{14}C was injected intraperitoneally in a dose of 0.5 μCi/g body weight into each rat 30 min before sacrifice. The specific activity of the glycine-^{14}C was 50 mCi/mmole.

Plasma membranes were isolated by Neville's method [5]. The purity of the isolated membrane preparations was verified by testing for activity of the enzymes Na,K-ATPase and glucose-6-phosphatase in them. Details of the method of determining activity of the enzymes and obtaining the soluble proteins were described previously [1]. The only difference was that, when obtaining soluble proteins, 0.05 M Na_2CO_3 was used instead of 0.05 M K_2CO_3. The results of determination showed that activity of marker enzyme Na-K-ATPase in the plasma membranes was 3 to 6 times higher than in the homogenate, and activity of glucose-6-phosphatase was only 33-50% as high as in the homogenate. Inorganic phosphorus was determined by the method of Fiske and Subbarow [4] and protein concentration by Lowry's method.

The membrane proteins were fractionated by electrophoresis in polyacrylamide gel (PAG) with sodium dodecylsulfate (DDS-Na). The bottom gel (pH 9.18) had the following composition: 11% acrylamide, 0.1% N,N'-methylene-bis-acrylamide, 0.43 M Tris, 0.03 N HCl, 0.15% TEMED, 0.05% ammonium persulfate; the top gel (pH 6.1) contained 3% acrylamide, 0.2% N,N'-methylene-bis-acrylamide, 0.06 M Tris, 0.03 M H_2SO_4, 0.15%

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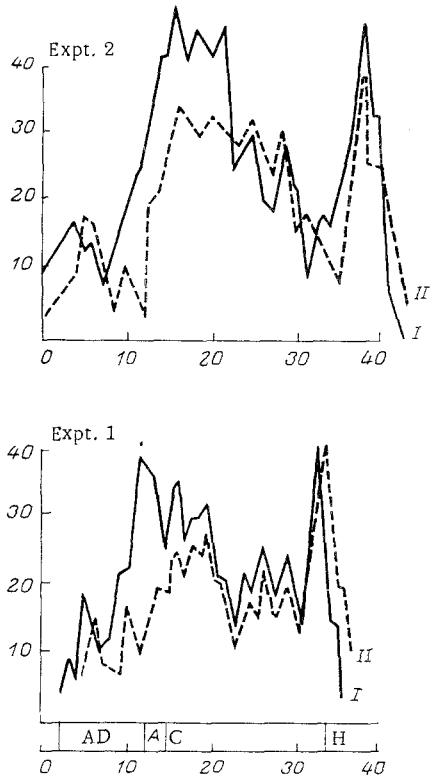

Fig. 1

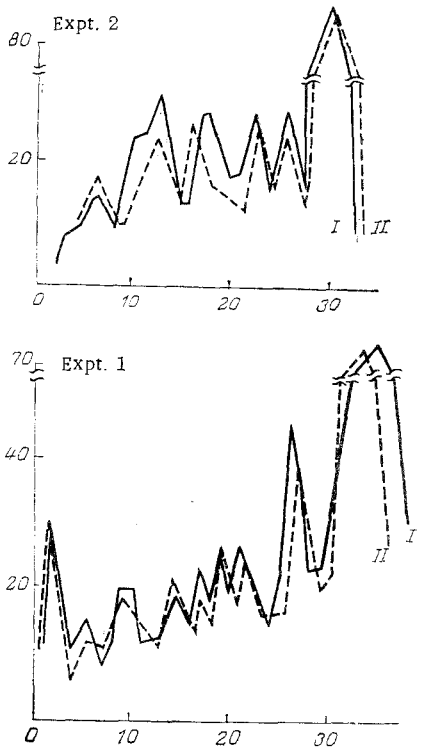

Fig. 2

Fig. 1. Radioactivity of electrophoretic fractions of soluble plasma membrane proteins of intact and regenerating rat liver 1 h after operation and 30 min after injection of glycine-^{14}C. I) Hepatectomized rats; II) controls. Abscissa, serial numbers of fractions and diagram of PAG after electrophoretic fractionation of proteins, using albumin dimer (AD), albumin (A), catalase (C), and hemoglobin (H) as reference substances. Ordinate, radioactivity (in cpm/mg protein).

Fig. 2. Radioactivity of electrophoretic fractions of insoluble plasma membrane proteins of intact and regenerating liver 1 h after operation and 30 min after injection of glycine-^{14}C. Legend as to Fig. 1.

TEMED, and 0.05% ammonium persulfate; the top buffer solution (pH 8.64) was made up from 0.04M boric acid, 0.04M Tris, and 0.1% DDS-Na, and the bottom buffer solution (pH 9.18) from 0.42M Tris and 0.03N HCl.

DDS-Na was added at the rate of 8 mg/mg protein to the solution of protein in 0.05M Na_2CO_3 before application to the PAG, after which β-mercaptoethanol was added up to 10% and the gel was incubated for 2 h at 37°C. Proteins were applied to the gel in a dose of 400-500 μg and electrophoresis continued for about 3 h. The columns of gel were then cut into 40 fragments, proteins were eluted from them in 0.25% aqueous solution of DDS-Na, and radioactivity was counted with a Mark-II liquid scintillation counter in Bray's system [2]. The molecular weight of the proteins was determined by electrophoresis in PAG [6].

EXPERIMENTAL RESULTS

The results relating to electrophoretic fractionation of proteins were obtained from five experiments. The pattern of electrophoretic separation of soluble proteins in PAG with DDS-Na is illustrated in Fig. 1. In each experiment plasma membrane proteins of both normal and regenerating liver were represented. It will be clear from Fig. 1 (experiment 1) that the rate of incorporation of glycine-^{14}C into different plasma membrane protein fractions differed in the intact and regenerating liver. Comparison of radioactivity levels of soluble plasma membrane proteins from normal and regenerating liver showed that a clear increase in the incorporation of labeled amino acids into plasma membrane proteins of the regenerating liver corresponded to fractions occupying the middle position on the gel. In experiment 2 (Fig. 1) the region of increased activity was in the same part of the curve.

To determine the molecular weight of these proteins, proteins of known molecular weight (albumin, catalase, hemoglobin) were used as reference substances. A mixture of these proteins was fractionated by electrophoresis in PAG simultaneously and under the same conditions as the soluble plasma membrane proteins (Fig. 1). Since the region of increased activity on the gel corresponded to the mobility of albumin, it can be concluded that the molecular weight of the polypeptides, synthesis of which was stimulated 1 h after hepatectomy, was about 60,000-70,000.

Insoluble proteins also were fractionated by electrophoresis in PAG with DDS-Na (Fig. 2). The results of protein fractionation in two experiments are given in Fig. 2. The radioactivity curve of the insoluble proteins differed from that of the soluble proteins. Uptake of labeled amino acid was maximal into insoluble proteins with higher electrophoretic mobility. However, the rate of incorporation into proteins from intact and regenerating liver did not differ significantly.

The maximal increase in synthesis of soluble plasma membrane proteins of the regenerating liver 1 h after partial hepatectomy, at the beginning of the G_1-period of the mitotic cycle, was thus linked with proteins with a molecular weight of about 60,000.

LITERATURE CITED

1. A. V. Pospelova and N. V. Gorelova, Byull. Éksp. Biol. Med., No. 5, 589 (1978).
2. J. A. Bray, Anal. Biochem., 1, 279 (1960).
3. P. Emmelot, C. J. Bos, et al., Biochim. Biophys. Acta, 90, 126 (1964).
4. C. H. Fiske and J. A. Subbarow, J. Biol. Chem., 66, 375 (1925).
5. D. M. Neville, J. Biophys. Biochem. Cytol., 8, 413 (1960).
6. D. M. Neville, J. Biol. Chem., 246, 6328 (1971).
7. M. R. Stetten, J. Biol. Chem., 239, 3576 (1964).

EFFECT OF HYPOBARIC HYPOXIA ON THE RATE OF INCORPORATION OF ACETATE-1-^{14}C INTO HYDROPHILIC AND HYDROPHOBIC COMPONENTS OF BRAIN PHOSPHOLIPIDS

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Acetate-1-^{14}C was injected intraperitoneally into rats which were kept for 2 h in a pressure chamber under a pressure of 240 mm Hg. Hypobaric hypoxia reduced the incorporation of labeled acetate practically equally in both components of phospholipids (PL), but dependence of the degree of decrease in turnover rate on the depth of hypothermia accompanying the hypoxia was much more marked for the carbon skeleton of the fatty acids than for the hydrophilic components of the total PL fraction. The similarity in the degree of decrease of incorporation of carbon- and phosphorus-labeled precursors during hypoxia suggests that the carbon-containing parts of the hydrophilic components of PL (glycerol and bases) and orthophosphoric acid residues react to hypoxia as a single entity.

KEY WORDS: phospholipid metabolism; hypoxia.

In the study of the functional biochemistry of nerve tissue phospholipids (PL) various procedures which modify the level of functional activity of the nervous system are widely used [1, 8-11]. In the writers' laboratory, acute hypoxia is used for this purpose, during which oxidative metabolism in general and the rate of metabolism in nerve tissue are depressed [6]. In earlier investigations to study PL metabolism, phosphorus-labeled inorganic phosphate was used as precursor, but this enabled changes in metabolism only of the hydrophilic moiety

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