INDUCTION OF KEY GLYCOLYTIC ENZYMES IN MOUSE FETAL LIVER CULTURED IN CIRCUMFUSION SYSTEM BY INSULIN

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SUMMARY

Effect of insulin on key enzymes (Hexokinase, Glucokinase, Pyruvate kinase, and Glucose-6-phosphate dehydrogenase) in carbohydrate metabolism has been studied in mouse fetal liver cultured in a circumfusion system. Specific activities of GK, PK, and G-6-P DH were induced 2-or 3-fold increases in fetal liver after 12 days cultivation by a single dose of insulin. While, HK was hardly influenced by insulin. These findings suggest that mouse fetal liver cultured in a circumfusion system for 2 weeks maintains the same response to insulin as in vivo adult mouse liver.

INTRODUCTION

Rose's circumfusion system culture is highly effective method for maintaining the process of differentiation in mammal embryo tissues over protracted periods, and cultured tissues can be observed with a phase-contrast microscope in this system (1). It has been reported by Rose et al (2) that 13-14-day old fetal mouse liver differentiated after cultivation for 4 weeks, so that it had the same electron microscopic features as that observed in adult liver cells. Therefore, it is important to establish whether the mouse fetal liver cultivated in the system maintains the differentiated functions characteristic of in adult liver.

It is well known that the liver plays an important role in the maintenance of stable levels of glucose in the blood. The control of the blood glucose by the liver is due to the regu-

lation of overall rate and direction in pathways of glycolysis and gluconeogenesis. Weber et al (3) have postulated hepatic role for insulin on the induction of key enzymes involved in glycolysis and the simultaneous suppresion of key gluconeogenic enzymes. It is suggested that the effect of insulin on the synthesis for key enzymes in the liver can be applied as a marker for differentiated functions of the liver.

In the present report we wish to describe the finding that 13-14-day mouse fetal liver cultivated in a circumfusion system for 12 days have the same response to insulin as in vivo adult mouse liver with respect to the induction of key enzymes of carbohydrate metabolism.

MATERIALS AND METHODS

Animals and tissues. Male and female mice of the ICR-strain, weighing 18-20 g, were supplied from Tokyo Jitsuken Dobutsu Co., Tokyo. Animals were fed at 25° on laboratory solid diet (Nipon Clea Co., Tokyo), with unlimited supply of water. Thirteen-or 14-day mouse fetuses were removed under sterile conditions after killing the mother with ether. The ages of the fetuses were determined from mating day. Livers were removed and established in culture as described below.

Rose's circumfusion system culture. Tissue fragments from 13-14-day old mouse fetal liver were placed on a mica coverslip and then covered with a full sheet of unperforated cellulose acetate membrane washed in the culture medium. The chambers were fabricated as reported by Rose (1). The circulation of the culture medium was carried out with the use of a self-contained mechanical system devised by Rose (1). Culture medium was composed of 199 (80 %), calf serum (20 %), penicillin G (1,000 units /mg), and phenol red (0.025 %). It was changed weekly at half volume.

Enzyme extract. Cultured liver (4-6 mg wet weight) in a multi purpose culture chamber was homogenized in a Potter-Elvehjem homogenizer with 0.5 ml of a cold 0.1 M Tris-Hcl buffer (pH 7.5) containing 1 mM EDTA and 10 mM 2-mercaptoethanol. The resulting homogenates were used for the assay of enzyme activity and for the determination of content of protein.

Assay of enzyme activity. The activities of HK* and GK* were assayed by the method of Dipietro and Weinhouse (4). The activities of G-6-PDH* and PK* were assayed according to the method of Glock and McLean (5), and Bücher and Pfleiderer (6), respectively ; the procedures used were the same as that described previously (7,8). One unit of enzymes was defined as the amount of the enzyme that caused a formation of 1 μmole of products per minute. The content of protein was determined by the method of Lowry et al (9) with the use of human serum albumin as the standard.

RESULTS

Typical results are shown in Fig. 1. All the activities in 13-14-day fetal mouse liver reached constant levels of specific activity after 10-12 days cultivation in a multipurpose culture chamber with a cellulose acetate membrane. The constant

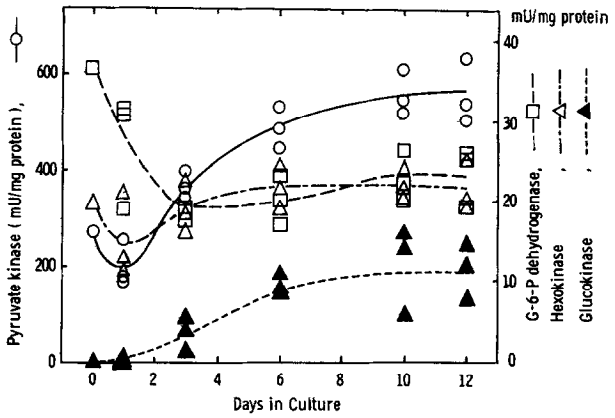

Fig. 1 Developmental patterns of key enzymes for charbohydrate metabolism in mouse liver in a circumfusion system.

values of four enzymes in 12 days of the cultivation were 22 ± 3 mU/mg protein for HK, 550 ± 80 mU/mg protein for PK, 23 ± 3 mU/mg protein for G-6-P DH, and 11 ± 4 mU/mg protein for GK, respectively. The GK activity, which is one of the marker

*Abbreviations : HK, Hexokinase (ATP : D-hexose 6-phophotransferase, EC 2.7.1.1) ; GK, Glucokinase (ATP : D-glucose 6-phosphotransferase, EC 2.7.1.2) ; G-6-P DH, Glucose-6-phosphate dehydrogenase (D-Glucose-6-phosphate : NADP Oxidoreductase, EC 1.1.1.49) ; PK, Pyruvate kinase (ATP : Pyruvate phosphotransferase, EC 2.7.1.40).

enzymes in mammalian adult livers, could not detected in liver from fetal mouse. However, the enzyme activity could be detected after 2 or 3 day of cultivation and gradually increased with increasing lengths of the cultivation day to reach the maximum value. These results are also in accordance with the morphological results obtained by the observations of a phase and an electron microscope.

On the 12th-day after the cultivation when all enzyme activities become at the constant levels as shown in Fig. 1, insulin (Insulin Novo, 40 U/ml) was added as a single dose in culture

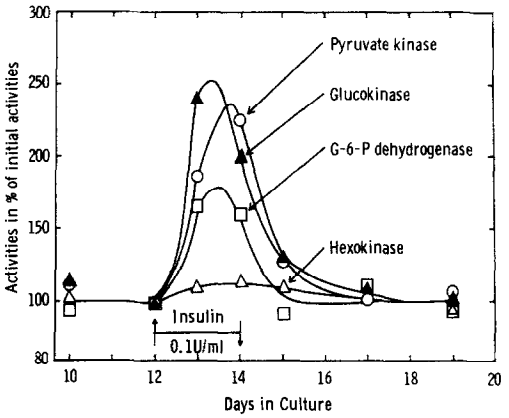

Fig. 2 Effect of insulin on key enzymes for carbohydrate metabolism in mouse liver cultivated in a circumfusion system. Mean values of the results obtained in 3 experiments are given.

medium to a final concentration of 0.1 U/ml medium. As shown in Fig. 2, insulin caused significant induction of GK and PK, which are key glycolytic enzymes, and of G-6-P DH, which occupies a key position in regulating the pentose phosphate pathway. GK activity increased to approximately 2.5-fold of values found in insulin-untreated cultured livers (control livers) after about 30 hrs of the addition of insulin. Increases in the activity of PK and G-6-P DH were, respectively, 2.3-fold and 1.8-fold of

values found in control livers after about 48 hrs of the addition of insulin. When insulin was removed from the culture medium by washing out with Hank's solution after 2 days cultivation, these enzyme activities sharply decreased and, thereafter, returned to the initial levels found in livers before the addition of insulin in 2 or 3 days. Hexokinase activity was hardly influenced by the addition of insulin. These findings were in good aggrement with the results of G. Weber et al (4) which were obtained by in vivo experiments with adult rats.

DISCUSSION

Among the membranes used for culture in the circumfusion system, reconstructed cellulose 11533 and cellulose acetate membranes capable of filtrating 2~3 % of substances of molecular weight 1×10^4, respectively, are most favarable of tissue and organ differentiation (to be published). Therefore, in this experiment cellulose acetate membranes were used.

Although mouse fetal liver cultured in a circumfusion system for 2 weeks maintains, morphologically and functionally, the differentiated characteristics retained in adult liver, several notable differences between adult and the cultured liver are found as follows ; fructose 1, 6-diphosphatase activity, which is key gluconeogenic enzyme, is negligible or undetectable in the cultured liver ; catalase has the same activity as is present in later fetal liver, which is one-seventh of that in the adult liver ; PK and HK are twice as active as in the adult. In addition, GK emerges after the 12-th postnatal day and reaches its adult concentration before the 28-th day, whereas, in the fetal liver cultured in a circumfusion system, GK emerges

in 2-3 days cultivation and reaches the maximum level (value of one-half of the adult concentration) in 10 or 12 days. These observations are unexplained at present.

In order to avoid the complexities of the organism in vivo, in vitro system such as the perfused liver (10), organ culture (11, 12) and cell suspension culture (13) have been employed for investigation about the influence of a hormone on metabolism of the target organ. However, these methods are not satisfactory for the maintenance of functional characteristics over protracted periods. Rose's circumfusion system culture, therefore, is useful for the many types of investigations of in vitro system.

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