The Effect of Insulin and Glucagon on the Insulin Receptor of Cultured Hepatoma Cells

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Summary

To elucidate the mechanism of glucagon-insulin (G-I) therapy, the effect of insulin and/or glucagon on the insulin receptor was studied in an experiment utilizing cultured cells (JTC-16) of rat ascites hepatoma. Insulin specific receptors were present on JTC-16 cells and were similar in nature to the receptors of primary culture rat hepatocytes. There were two kinds of insulin receptors. One had a high insulin affinity and the other had low insulin affinity. In the experiment involving addition of insulin, the number of insulin receptors decreased after 24 hrs incubation in proportion to the increase in added insulin concentration. On the other hand, the number of insulin receptors increased with glucagon addition and the increase in proportion to the concentration of glucagon added. In the experiment involving simultaneous addition of insulin and glucagon, a 20% decrease in the number of receptors induced with $10^{-9}$ M insulin was restored to the control level with simultaneous glucagon addition of the same concentration. The number of insulin receptors increased as the concentration of additive glucagon increased. These results show that simultaneous addition of insulin and glucagon inhibits the decrease in number of insulin receptors with insulin alone. These facts may obtain more potent action of insulin in G-I therapy via insulin receptor.

Key Words: Down regulation, Glucagon, Insulin receptor, Up regulation.

Introduction

The principle of current treatment for fulminating hepatic failure consists of protection of liver cell necrosis and temporary support for hepatic function till the hepatic function is restored to minimum essential level. As a result many studies on factors of the promotion of liver regeneration have been done. Among many such factors, hepatotrophic factor and regeneration promoting factor were considered to exist in portal blood. As insulin itself has mitogenic potency and exists in portal blood at high concentration, it has been considered to be one of these factors. In experiments with partially hepatectomized rats, Bucher et al. reported that insulin and glucagon had synergistic action on residual liver cell regeneration.
Though glucagon-insulin therapy has been tried for fulminant hepatic failure in clinical practice since this study was reported, the mechanism of the effect of this therapy is still undetermined.

It is essential that these hormones must bind to each hormone receptor for hepatic regeneration effect. In this study we analyzed the change of insulin receptors of cells cultured with insulin and/or glucagon in the medium.

**Materials and Methods**

**I. Experimental material**

(1) Cultured cell strain

JTC-16 cell of rat ascites hepatoma (AH-7974) which was established by Takaoka et al. was selected for this experiment.

(2) Culture medium

The standard culture medium was DM-160 (Kyokuto Pharmacial Industrial Co. Ltd) supplied with 5% of fetal calf serum (GIBCO, Grand Island Biological Company, Grand Island, NY). JTC-16 cells were maintained in glass culture flasks (TD-40) with flat surfaces in 10 ml of the culture fluid (DM-160-5% FCS). For subsequent experiments, cells were plated in plastic dishes of 35 mm diameter and cultured with hormones in an atmosphere of 5% CO and 95% air at 37°C.

(3) Labeled insulin

$^{125}$I-insulin was labeled with the methods of Cuatrecasas et al. The specific radioactivity of labeled insulin obtained was 150-180 uCi/ug, and 99.5% of the product was precipitated with 10% trichloracetic acid.

**II. Method**

(1) Selection of cultured cell strain suitable for analysis of insulin receptor

In JTC-1 (AH-130), JTC-16 and RLC-10, which were cell strains from hepatocytes, the specific binding of insulin was higher than that of P3HR-1 and Raji which were delivered from lymphoma B cell (Table 1). Particularly receptors were rich in JTC-16 cells. Thus JTC-16 cells were used for the analysis of the nature of insulin receptors for reasons of both the richness of insulin receptors and the characteristic of good cell attachment to plastic dishes.

(2) Culture of JTC-16 cell strain and preparation of culture medium supplied with insulin and/or glucagon

JTC-16 cells were cultured with the medium DM-160-5% FCS. The experiment was started after these cell sheet grew to a confluent state. Concentration of insulin or glucagon was adjusted to $10^{-9}$ - $10^{-7}$ M and each hormone was added to culture medium. The viability of the cells used was over 95% by the methylene blue staining method.

(3) Measurement of $^{125}$I-insulin binding to JTC-16 cell

After removal of culture medium, the cells were washed twice with 2.0 ml phosphate buffered saline and the cells were incubated with 1 ml of DM-160-0.2% bovine serum albumin containing labeled insulin under various experimental conditions. After the cells were incubated with $^{125}$I-insulin, they were washed twice with 2 ml of ice-cold phosphate buffered saline, and finally solubilized with 1 N NaOH for radioactivity determination. Specific $^{125}$I-insulin binding to the cells was obtained by subtracting the nonspecific binding expressed in the presence of excess unlabeled insulin (20...