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Insulin inhibits glucagon-induced glycogenolysis normally in perivenous hepatocytes of Wistar fatty rats

Yoshihiro Ikezawa, Keiichi Yamatani *, Hiroshi Ohnuma, Makoto Daimon [1], Hideo Manaka, Hideo Sasaki

Third Department of Internal Medicine, Yamagata University School of Medicine, 2-2-2 IidaNishi, Yamagata 990-9585, Japan

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Abstract

Wistar fatty (WF) rats are obese, hyperinsulinemic and hyperglycemic, and thus a model of type 2 diabetes mellitus. Since we have found that insulin specifically inhibits glucagon-induced glycogenolysis in perivenous hepatocytes (PVH) from normal rats, we examined the inhibitory effect of insulin on glucagon-induced glycogenolysis in PVH of hyperinsulinemic WF rats.

Basal glucose release was 64.0 ± 4.1 nmol/mg protein/30 min from PVH of lean littermates (WL rats) and 137.0 ± 19.3 nmol/mg protein/30 min from that of WF rats ($p < 0.01$). These were proportional to the glycogen content in PVH of WL and WF rats (56.7 ± 7.2 and 131.0 ± 20.3 μg/mgprotein, $p < 0.01$), and increased to 109.0 ± 8.8 and 225.8 ± 17.9 nmol/mg protein/30 min, respectively, with 0.1 nmol/l glucagon.

When 10 nmol/l insulin was coincubated, 0.1 nmol/l glucagon-induced increase in glucose release decreased to 93.3 ± 10.9 nmol/mg protein/30 min in PVH of WL rats ($p < 0.01$) and to 181 ± 20.7 nmol/mg protein/30 min in PVH of WF rats ($p < 0.01$). Thus, insulin antagonized glucagon-induced glycogenolysis in PVH similarly between WL and WF rats, to $56.7 \pm 13.3\%$ and to $46.1 \pm 7.5\%$, respectively.

Thus, the antagonizing effect of insulin on glucagon-induced increase in glycogenolysis was preserved in PVH of hyperinsulinemic and hyperglycemic WF rats.

Keywords: Insulin; Glucagon; Glycogenolysis; Perivenous hepatocytes

1. Introduction

The liver seems to be the most important target organ for pancreatic hormones such as insulin and glucagon, since these hormones reach the liver first. Following the blood stream, hepatocytes forming hepatic acini are classified into two categories: periportal and perivenous hepatocytes (PPH and

* Corresponding author. Present address: Department of Internal Medicine, Niigata Teishin Hospital, 2-2-8 Yachiyo, Niigata 950-8798, Japan. Tel.: +81 25 244 4700; fax: +81 25 247 6216.
E-mail address: kyamatan@lily.ocn.ne.jp (K. Yamatani).
[1] Tel.: +81 23 628 5316; fax: +81 23 628 5318.

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PVH, respectively) [1]. PPH and PVH are functionally different, PPH being predominantly gluconeogenic, and PVH glycolytic [1]. Glucagon induces glycogenolysis, and the effect is greater in PVH than in PPH [2]. Insulin inhibits glucagon-induced glycogenolysis in PVH more than in PPH in normal Wistar rats [2,3].

Wistar fatty (WF) rats are obese, hyperinsulinemic and hyperglycemic, and often cited as a model of type 2 diabetes mellitus. The decreased insulin sensitivity in WF rats has been confirmed by the steady-state blood glucose method [4] and by an euglycemic-hyperinsulinemic glucose clamp study [5]. Therefore, we examined here whether the inhibitory effect of insulin on glucagon-induced glycogenolysis in PVH was responsible for the insulin-resistance of WF rats.

2. Materials and methods

2.1. Animals and selective hepatocyte isolation

WF rats were provided by the Research and Development Division of Takeda Chemical Industries (Osaka, Japan) [4] and maintained at the Laboratory Animal Center of Yamagata University School of Medicine (Yamagata, Japan). They were maintained on a 12-h day/12-h night cycle with a standard laboratory diet and tap water ad libitum. At 12 weeks of age, WF rats were 450–500 g and their lean littermates (WL rats) were 300–330 g. Fasting plasma glucose concentrations were 8.7–10.3 mmol/l in WF rats and 4.1–4.9 mmol/l in WL rats. The rats were fed ad libitum and anesthetized before surgery by intraperitoneal injection of 100 mg/kg pentobarbital.

PVH were isolated by the digitonin–collagenase method with minor modifications [2,3]. Briefly, after a loose tie was placed around the inferior vena cava above the right kidney, the portal vein was cannulated, and perfusion (15 ml/min) commenced with Ca^{2+}-free Hanks' buffer containing 0.5 mmol/l ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid. The chest cavity was then opened for cannulation of the superior vena cava, and the ligature around the inferior vena cava was fastened.

For preparation of PVH, a 1 mmol/l digitonin solution (Sigma Chemical Co., St. Louis, USA) was prepared by boiling in Hanks' solution, then infused at 37 °C through the cannula into the portal vein at a rate of 10 ml/min for 10 s, followed by Ca^{2+}-free Hanks' buffer for 3 min. Collagenase (95 U/ml, Wako, Osaka, Japan) was then infused for 7 min at a rate of 15 ml/min through the cannula from the inferior vena cava. The liver cells were then dispersed in Eagle's minimum essential medium (Nissui, Tokyo, Japan), filtered through gauze mesh, and washed four times by centrifugation at $50 \times g$ for 1 min. Viability was determined by the exclusion of 0.05% trypan blue, and the cell yield was determined by the hepatocrit method. Cell viability was usually >95%.

2.2. Experimental protocols

Cells were diluted to 10^6 cells/ml in Williams' medium E (Dainippon, Tokyo, Japan) containing 10% (v/v) fetal bovine serum (FBS; BioWhittaker, Walkersville, USA), 1 nmol/l pork insulin (Novo-Nordisk, Copenhagen, Denmark) and 1 nmol/l dexamethasone (Upjohn, Tokyo, Japan). Two milliliters of inocula of hepatocytes was plated in 35-mm collagen-coated dishes and placed in a CO_2 incubator at 37 °C for 60 min. After cell attachment, these monolayers were washed with Krebs–Henseleit buffer twice at 10-min intervals.

For the analysis of glycogenolysis, glucose released from PVH of fed rats was determined during a subsequent 30-min incubation period in Krebs–Henseleit buffer with 0.1 nmol/l pork glucagon (Novo-Nordisk). Insulin (0.1–10 nmol/l) was added.

The medium was collected and stored at −20 °C. Glucose was assayed by a glucose oxidase method (Boehringer-Yamanouchi, Tokyo, Japan). The cells were treated with 1% Triton X-100 (Sigma), scraped, homogenized and stored at −20 °C. The homogenate was stored at −20 °C until the protein assay using the Pyrogallol method (Boehringer-Yamanouchi) or analysis of glycogen using the amyloglucosidase method [2].

2.3. Statistics

Values were shown as the mean ± S.E.M. of seven experiments. The antagonizing effect of insulin on glucagon-induced glycogenolysis was shown as a percent, basal as 0% and glucagon alone as 100%, respectively. One- or two-way analysis of variance was used for the statistical analysis. $p < 0.05$ was considered significant.