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EVIDENCE FOR GLUCOKINASE TRANSLOCATION BY GLUCOSE IN RAT HEPATOCYTES

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Summary: By an immunohistochemical technique, we examined changes in the subcellular distribution of rat liver glucokinase in response to external stimuli. Glucokinase immunoreactivity was found predominantly in the nucleus of hepatocytes. *In situ* perfusion of the liver with 20 mM glucose for 10 min caused a marked decrease in nuclear immunoreactivity and an increase in cytoplasmic immunoreactivity. Insulin (10 nmol/l) potentiated this glucose effect. However, no change took place when 5 mM glucose was perfused. These results indicate that glucokinase is translocated from the nucleus to the cytoplasm in response to a high concentration of glucose.

The primary function of the liver is to maintain the blood glucose levels through glycolysis, glycogen metabolism, and gluconeogenesis (1). Glucokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1) is one of the hexokinase isozymes and is often called hexokinase IV or high-Km hexokinase (2). This enzyme has been identified only in glucose-sensing cells such as hepatocytes, pancreatic islet cells, and some neuroendocrine cells (3). Glucokinase is considered to play a major regulatory role in hepatic glucose metabolism (2, 4-6).

Earlier we examined the subcellular distribution of glucokinase in rat liver and found that glucokinase is present in both nucleus and cytoplasm, being predominantly in the nucleus (7). This finding led us to hypothesize that the localization of glucokinase in the nuclei may minimize the futile substrate cycle between glucose and glucose 6-phosphate (G/G-6-P cycle) and that translocation of glucokinase between the nucleus and the cytoplasm may take place to regulate glucose metabolism. If our hypothesis is true, it is highly possible that the subcellular distribution of glucokinase is changed in response to external stimuli such as glucose infusion. We examined this possibility in the present study.

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MATERIALS AND METHODS

Affinity purification of anti-glucokinase antibody

Antiserum against homogeneous rat liver glucokinase was raised in rabbits as described previously (7). This serum was adsorbed onto protein-A Sepharose by the batch-wise method, anti-glucokinase IgG was eluted with 100 mM glycine-HCl (pH 2.8), and the eluate was immediately neutralized with 2 M Tris-HCl (pH 8.0). Anti-glucokinase IgG was further purified by sequential adsorption and elution from rat liver glucokinase immobilized onto poly(vinylidene difluoride) membranes as follows: The membrane (1 cm^2) was kept at 4°C for 24 h in 1 ml of 200 μg/ml pure glucokinase, blocked in 5 % non-fat dry milk in phosphate-buffered saline (PBS), and incubated at 37°C in 1 ml of anti-glucokinase IgG (2 mg/ml) obtained from the protein-A Sepharose chromatography. After several washes in PBS containing 5 % non-fat dry milk and 0.05 % Tween-20, the glucokinase-specific antibody was eluted with 1 ml of 100 mM glycine-HCl buffer (pH 2.8) containing 5 % bovine serum albumin, neutralized with 32 μl of 2 M Tris-HCl (pH 8.0), and stored at 4°C until used. Non-immune IgG and pre-immune IgG were purified by batch adsorption of the respective non-immune and pre-immune sera onto protein-A Sepharose.

Liver perfusion and fixation

Male Wistar rats weighing 180-220g were given free access to water and food (MM-3; Funabashi Farm, Japan). The rats were placed under a 24-h-fast prior to the liver perfusion. The animals were anesthetized with pentobarbital (60 mg/kg body wt.), and the liver was perfused *in situ* without recirculation at 37°C via the portal vein. Krebs-Henseleit buffer equilibrated with O_2/CO_2 (19:1) was used as perfusion medium. The perfusion flow rate was 3 ml/min per g of liver. After a 10-min perfusion, the liver was excised, sliced at a 2-3 mm thickness, and immersed overnight in Zamboni's solution.

Immunohistochemistry

After the fixation, liver pieces were dehydrated, embedded in paraffin, and sectioned at a thickness of 2-3 μm. The sections were placed on slides precoated with 0.1 % poly-L-lysine solution (Sigma, U.S.A.), deparaffinized with Histo-Clear (National Diagnostics, U.S.A.), and treated with a solution (0.1 mg/ml in 50 mM Tris-HCl buffer, pH 8.0) of Pronase E (Sigma, U.S.A.) for 10 min. Next, the sections were placed in PBS containing 0.3 % (w/v) H_2O_2 for 30 min to block endogenous peroxidase activity, washed with PBS, and incubated with normal goat serum for 20 min. Then, they were blotted with wet filter paper and incubated consecutively with affinity-purified anti-glucokinase IgG (diluted 1:20 in 5 % normal goat serum) overnight at 4°C, with biotin-labeled goat serum against rabbit IgG (Seikagaku Kogyo, Japan) for 1 h at 37°C, and with streptavidin-peroxidase (Seikagaku Kogyo, Japan) for 1 h at 37°C. Each incubation was followed by 3-4 washings with PBS. The sections were then placed in 0.05 % 3,3'-diaminobenzidine·4HCl containing 0.05 % (w/v) H_2O_2 for 15 min for staining, washed with distilled water, dehydrated, mounted on Entellan New (Merck, Germany), and examined by light microscopy.

In control experiments, semi-thin sections were incubated with anti-glucokinase rabbit IgG adsorbed by rat liver glucokinase, with non-immune rabbit IgG or with pre-immune rabbit IgG, followed by consecutive incubation with biotin-labeled goat serum against rabbit IgG and streptavidin-peroxidase. Some sections were directly incubated with the second antibody without any preceding incubation with anti-glucokinase IgG.

Assays of glucokinase and protein

The enzyme activity was measured according to the method of Hara et al. (8). Protein was assayed by the Biuret method with bovine serum albumin as standard.

RESULTS

Glucokinase immunoreactivity was found predominantly in the nucleus of hepatocytes in sections prepared from untreated rat liver (Fig. 1A). The perfusion of the liver with a

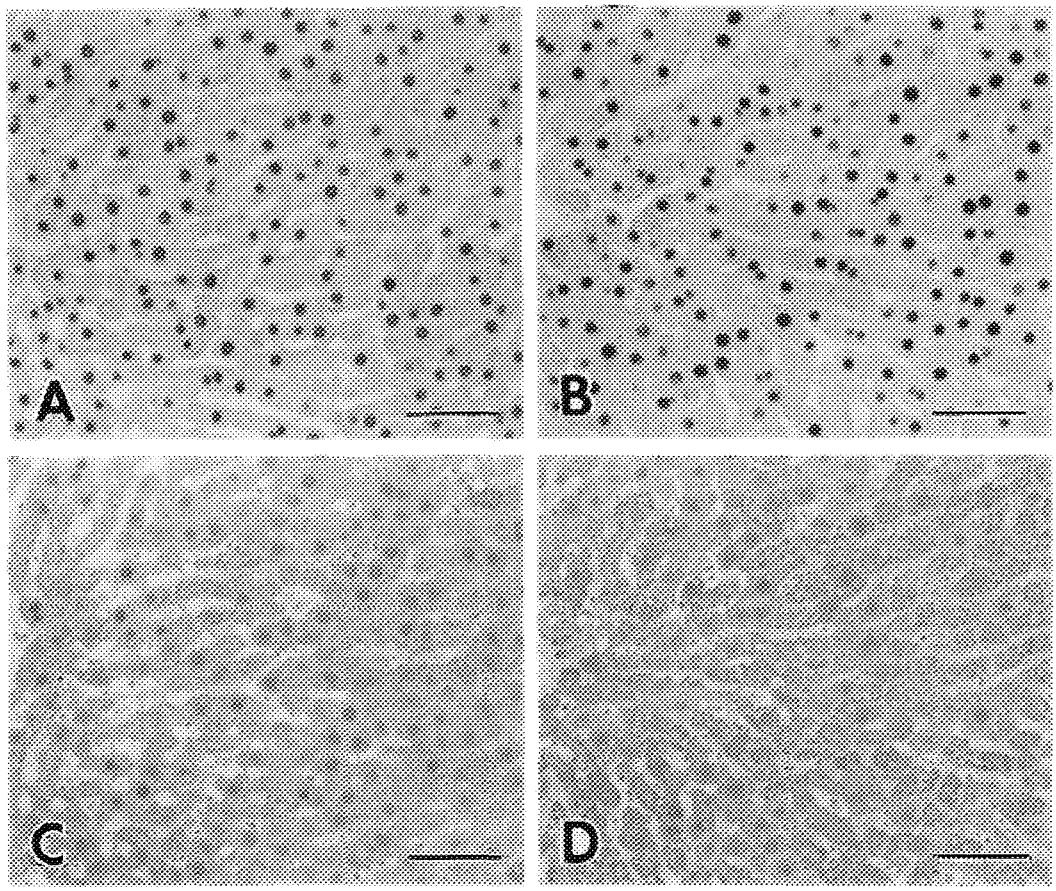

Figure 1. Immunohistochemical distribution of glucokinase in untreated liver (A), liver perfused with 5 mM glucose (B), liver perfused with 20 mM glucose (C), and liver perfused with 20 mM glucose in the presence of 10 nmol/l of insulin (D). Positive staining for glucokinase is revealed by the brown color. Original magnification x 30. Bars = 50 μm.

physiological concentration (5 mM) of glucose for 10 min did not affect the subcellular distribution of glucokinase (Fig. 1B). However, a striking change in the distribution took place in both nuclei and cytoplasm when the organ was perfused with 20 mM glucose; i.e., the nuclear immunoreactivity was decreased, and inversely, the cytoplasmic immunoreactivity was increased (Fig. 1C). Insulin (10 nmol/l) potentiated this effect (Fig. 1D). Liver glucokinase activity was similar (~6 units/mg protein) when measured just after perfusion with 5 mM glucose, 20 mM glucose, or 20 mM glucose plus 10 nmol/l insulin.

DISCUSSION

We previously reported the specificity of anti-glucokinase antibody that was obtained from the same rabbit as used in this study but was not affinity-purified (7). Thus, a single

immunoreactive band migrating identically as glucokinase and with a molecular weight of 52,000 was seen with the antibody after electrophoresis and immunoblotting of extracts from rat liver. The antibody distinguished between glucokinase and hexokinase of rat liver. To increase the specificity of the antibody, we used affinity-purified anti-glucokinase IgG in this study.

We examined the specificity of immunostaining, and found that 1) no staining was observed when non-immune or pre-immune IgG was used instead of anti-glucokinase IgG; 2) immunoabsorption of anti-glucokinase IgG with purified rat liver glucokinase completely prevented immunostaining of glucokinase; and 3) no staining was observed when liver sections were incubated with the second antibody without preceding incubation with anti-glucokinase antibody. Furthermore, no immunostaining was detected in sections of streptozotocin-diabetic rat liver depleted of glucokinase almost completely (data not shown). Also, earlier we confirmed the existence of glucokinase in the rat liver nuclear fraction by the method of isolation of nuclei using a non-aqueous solvent (7). These facts indicate that the immunostaining is specific for glucokinase.

Our present results clearly indicate that glucokinase is translocated from the nucleus to the cytoplasm in response to a high concentration of glucose. Glucokinase immunoreactivity in the nuclei was far stronger than that in the cytoplasm in almost all the hepatocytes of 24-h-fasted rats. We found recently, however, that the nuclear immunoreactivity in some hepatocytes of fed rats is weak or absent, and inversely, the cytoplasmic immunoreactivity in those cells is strong (data not shown). Weak or no nuclear immunoreactivity in those hepatocytes may be caused by translocation of glucokinase from the nucleus to the cytoplasm.

The findings reported here have interesting implications for hepatic glucose metabolism. It is known that the G/G-6-P cycle is involved in the regulation of glucose entry into and exit from the cells (5, 6). In the post-absorptive and fasting states, both glycogenolysis and gluconeogenesis provide a steady supply of G-6-P. Glucose-6-phosphatase converts this metabolite to free glucose to be released into the circulation. In these states, the nuclear localization of glucokinase might prevent the rephosphorylation of glucose that is produced in the cytoplasm. In the absorptive state, the following events are likely to occur: the elevation of glucose concentration in the portal vein causes glucose to enter hepatocytes, glucokinase is translocated from the nucleus to the cytoplasm, glucose in the cytoplasm is phosphorylated to G-6-P by the enzyme, and the G-6-P is converted into glycogen or metabolized via the glycolytic pathway. It is conceivable that translocation of glucokinase from the nucleus to the cytoplasm in response to a high concentration of glucose increases cytoplasmic glucose utilization in hepatocytes.

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