Impairment of Glucokinase Translocation in Cultured Hepatocytes from OLETF and GK Rats, Animal Models of Type 2 Diabetes*

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Summary. We examined sugar-induced translocation of glucokinase in cultured hepatocytes from Otsuka Long-Evans Tokushima Fatty and Goto-Kakizaki rats, animal models of type 2 diabetes, and compared this with that in Long-Evans Tokushima Otsuka and Wistar rats, respectively, as control strains. When hepatocytes from the four strains were incubated with 5 mM glucose, glucokinase was present predominantly in the nuclei. Higher concentrations of glucose, 5 mM glucose plus 1 mM fructose, and 5 mM glucose plus 1 mM sorbitol all induced the translocation of glucokinase from the nucleus to the cytoplasm in hepatocytes from these rats. The extent of glucokinase translocation under these conditions, however, was less marked in both diabetic rat types than in the control rats. The extent of the phosphorylation of glucose as estimated by the release of H₂O from [2-⁴H] glucose is significantly lower in Goto-Kakizaki rats than in Wistar rats. The results indicate that the translocation of glucokinase is impaired in the hepatocytes of diabetic rats. They also suggest that the impaired translocation of glucokinase is associated with abnormal hepatic glucose metabolism in type 2 diabetes.

Type 2 (non-insulin-dependent) diabetes mellitus is a heterogeneous disorder, caused by an interaction of genetic and environmental factors (DeFRONZO, 1988; HAMMAN, 1992). Hyperglycemia in this form of diabetes results from both a decrease in glucose utilization by the liver and peripheral tissues and an increase in hepatic glucose production (KAWAMORI et al., 1991; MEVORACH et al., 1998).

Glucokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1), often called hexokinase IV, catalyzes the phosphorylation of glucose. It is distinguished from other hexokinase isozymes (types I—III) by its low affinity for glucose and lack of inhibition by glucose 6-phosphate (WEINHOUSE, 1976). This enzyme has been identified only in pancreatic cells, hepatocytes, and some neuroendocrine cells (JETTON et al., 1994). In the liver, glucokinase has been proposed to regulate both glucose uptake and glucose output (MATSCHINSKY, 1996; PRINTZ et al., 1993; O'DOHERTY et al., 1996; TAKUCHI et al., 1996). Recent studies on glucokinase mutations (VELHO et al., 1996; TAPPY et al., 1997), transgenic animals (FERRÉ et al., 1996; HARHARAN et al., 1997), and patients with type 2 diabetes (MÉVORACH et al., 1998) have shown that the lowered functioning of hepatic glucokinase contributes to the pathogenesis of hyperglycemia in diabetes mellitus.

Although glucokinase was believed to be present exclusively in the cytoplasm of hepatocytes, recent studies from our laboratory were the first to show that it is located predominantly in the nucleus under metabolically static conditions, and that metabolic conditions inducing an increase in glucose metabolism cause the translocation of glucokinase from the nucleus to the cytoplasm (MIWA et al., 1990; TOYODA et al., 1994, 1995a, b, 1996, 1997a, b). We also suggested that the translocation of glucokinase between the

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nucleus and the cytoplasm plays a role in the regulation of hepatic glucose metabolism. Agius and Peak (1993, 1997) reported that glucokinase was predominantly in a bound state when hepatocytes incubated with 5 mM glucose were permeabilized with digitonin in the presence of Mg$^{2+}$, and that metabolic changes induced by incubation with high glucose (15–35 mM) or with fructose (50 μM) caused a translocation of glucokinase from its Mg$^{2+}$-dependent binding site to an alternative site.

Otsuka Long-Evans Tokushima Fatty (OLETF) and Goto-Kakizaki (GK) rats, animal models of type 2 diabetes, have been used extensively for investigations on the pathogenesis of type 2 diabetes. The acceleration of hepatic glucose production and suppression of hepatic glucose uptake have been observed in both these diabetic rats (Sugiyma et al., 1989; Suzuki et al., 1993; Ishida et al., 1995; Shibata et al., 1998). In the present study, we examined the sugar-induced translocation of glucokinase in hepatocytes from OLETF and GK rats and compared it with that in Long-Evans Tokushima Otsuka (LETO) and Wistar rats, respectively, as control strains.

**MATERIALS AND METHODS**

**Animals**

Male OLETF and LETO rats were kindly provided by Tokushima Research Institute, Otsuka Pharmaceutical Co., Tokushima, Japan. Male GK and Wistar rats were obtained from Clea Japan, Tokyo, Japan. They were given laboratory chow ad libitum. OLETF and LETO rats and GK and Wistar rats were used for experiments at 40 weeks and 10 weeks of age, respectively. One week ahead of the day of experiments, the rats were deprived of food overnight and subjected to an intraperitoneal glucose tolerance test (1 g/kg body weight). OLETF and GK rats in which plasma glucose at 1 h after glucose injection was more than 11 mM were considered diabetic.

**Antibody**

Antiserum against homogeneous rat liver glucokinase was raised in rabbits as described previously (Toyoda et al., 1994). Monospecific antibodies against glucokinase were affinity-purified as reported earlier (Toyoda et al., 1994).

**Hepatocyte isolation and culture**

Hepatocytes were isolated according to the method by Tanaka et al. (1978) using collagenase perfusion of rat livers. The cells were suspended in Minimum

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**Fig. 1.** Effect of glucose on glucokinase distribution in hepatocytes from LETO (A, C, E) and OLETF (B, D, F) rats. Cells were incubated for 30 min in MEM containing the indicated concentrations of glucose, and then stained for glucokinase by the indirect immunofluorescence method. Bar = 25 μm

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**Fig. 2.** Effect of fructose and sorbitol on glucokinase distribution in hepatocytes from LETO (A, C) and OLETF (B, D) rats. Cells were incubated for 30 min in MEM containing 5 mM glucose plus either 1 mM fructose (A, B) or 1 mM sorbitol (C, D), and then stained for glucokinase by the indirect immunofluorescence method. Bar = 25 μm
Essential Medium (MEM) containing 5% heat-inactivated fetal bovine serum, plated on cover slips in 6-well plates (area of well 15.6 cm²) at a cell density of $6.4 \times 10^4$ cells/cm², and incubated at 37°C in a humidified atmosphere equilibrated with 5% CO₂ in air. After cell attachment (4 h), the medium was replaced with serum-free MEM containing 10 nM dexamethasone, and the cells were cultured further for 16 h. Hepatocyte monolayers were incubated in MEM supplemented with the desired sugar (s) for 30 min before fixation.

**Immunofluorescence staining**

Monolayers were washed once with 2 ml of 150 mM NaCl at room temperature. The fixation medium (4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4) was then added, and the cells were fixed for 40 min at 4°C. After the fixation, the monolayers were washed twice with phosphate-buffered saline (PBS) and permeabilized with 0.5% (v/v) Triton X-100 for 40 min at 4°C. The monolayers were incubated with a solution (serum/milk solution) containing 5% (v/v) normal goat serum and 5% skimmed milk in PBS for 5 h at 4°C to block non-specific staining. They were then incubated consecutively with anti-glucokinase IgG (diluted 1:5 in serum/milk solution) for 6 h at room temperature and with FITC-labeled donkey anti-rabbit IgG antibody (Jackson Immunoresearch, PA, USA; diluted 1:100 in serum/milk solution) for 1 h at room temperature. Each incubation was followed by 6 washings with PBS. Cell fluorescence was analyzed with an Axiohot 2 microscope (Zeiss, Jena, Germany).

**Quantitative analysis of nuclear and cytoplasmic glucokinase in cultured hepatocytes**

Distribution of nuclear and cytoplasmic glucokinase in cultured hepatocytes was quantitated by analyzing nuclear and cytoplasmic fluorescence intensities in images of cells stained with immunofluorescence technique, since glucokinase leaks from the nuclei into the aqueous isolation media during subcellular fractionation (MiWA et al., 1990).

Images of stained cells were recorded on an Axiohot 2 fluorescence microscope equipped with a cooled CCD camera ($1317 \times 1035$ pixels, Photometrics CH 250). Nuclear and cytoplasmic fluorescence intensities in images of stained cells were analyzed using an NIH image.

**Phosphorylation of glucose in cultured hepatocytes**

The phosphorylation of glucose was estimated by the release of $^3$H₂O from $[2^{-3}H]$ glucose in cultured hepatocytes. Cells were incubated for 1 h at 37°C in

**Fig. 3.** Effect of glucose on glucokinase distribution in hepatocytes from Wistar (A, C, E) and GK (B, D, F) rats. Cells were incubated for 30 min in MEM containing the indicated concentrations of glucose, and then stained for glucokinase by the indirect immunofluorescence method. Bar = 25 μm

**Fig. 4.** Effect of fructose and sorbitol on glucokinase distribution in hepatocytes from Wistar (A, C) and GK (B, D) rats. Cells were incubated for 30 min in MEM containing 5 mM glucose plus either 1 mM fructose (A, B) or 1 mM sorbitol (C, D), and then stained for glucokinase by the indirect immunofluorescence method. Bar = 25 μm
MEM with the desired sugars plus \([2-\text{H}]\) glucose (5 \(\mu\text{Ci}\)). \(^{1}\text{H}\)O released in the medium was determined as described previously (MIWA et al., 1991).

**Protein assay**

Protein was assayed by the method by LOWRY et al. (1951) with bovine serum albumin as a standard.

**RESULTS AND DISCUSSION**

Hepatocytes from OLETF and LETO rats were incubated with various concentrations of glucose. At 5 mM glucose, immunofluorescence staining of glucokinase was present predominantly in the nuclei of hepatocytes from both strains (Fig. 1A, B). Hepatocytes from LETO rats, when incubated with high concentrations of glucose, showed a decrease in the nuclear glucokinase immunofluorescence and an increase in that in the cytoplasm (Fig. 1C, E). In contrast, in hepatocytes from OLETF rats, marked immunofluorescence was still observed in the nuclei under the same conditions (Fig. 1D, F). These results indicate that the glucose-induced translocation of glucokinase is impaired in OLETF rats.

Next, we examined the effect of a lower concentration (1 mM) of either fructose or sorbitol, which is known to increase glucose phosphorylation by glucokinase (VAN SCHAFTINGEN and VANDERCAMMEN, 1989; FILLAT et al., 1993) on glucokinase translocation. When hepatocytes from LETO rats were incubated in MEM containing 5 mM glucose plus either 1 mM fructose or 1 mM sorbitol, the nuclear immunofluorescence was markedly decreased; conversely, the cytoplasmic immunofluorescence was increased (Fig. 2A, C). In contrast, in hepatocytes from OLETF rats, the cytoplasmic immunofluorescence was increased, but the nuclear immunofluorescence was still evident (Fig. 2B, D). The results indicate that fructose- or sorbitol-induced translocation of glucokinase is also impaired in OLETF rats.

We also examined glucokinase translocation by sugars in hepatocytes from GK and Wistar rats. Hepatocytes from both rats were incubated with various concentrations of glucose, 5 mM glucose plus 1 mM fructose, or 5 mM glucose plus 1 mM sorbitol. As shown in Figures 3 and 4, the extent of glucokinase translocation was less marked in GK rats than in Wistar rats. Quantitative analysis of nuclear and cytoplasmic intensities of stained cells clearly showed that cytoplasmic intensity was significantly lower in GK rats than in Wistar rats (Fig. 5). Furthermore, the rate of glucose phosphorylation was also lower in GK rats than in Wistar rats (Fig. 6). These
results indicate that both sugar-induced glucokinase translocation and glucose phosphorylation are impaired in GK rats.

The present study indicates that sugar-induced glucokinase translocation is impaired in hepatocytes of diabetic rats and suggests that impaired glucokinase translocation is involved both in the accelerated hepatic glucose output and in the suppressed hepatic glucose uptake in diabetic rats, thereby contributing to their hyperglycemia. It is reasonable to speculate that hyperglycemia in type 2 diabetes would be improved by stimulating the glucokinase translocation from the nucleus to the cytoplasm. It seems likely, therefore, that the control of glucokinase translocation might be a new approach to the normalization of hyperglycemia in type 2 diabetes.

Van Schaftingen et al. (1994) identified a 68-kDa regulatory protein of glucokinase in the liver that binds to glucokinase and competitively inhibits its activity with respect to glucose. Fructose 1-phosphate, the product of fructose phosphorylation by ketohexokinase, acts to release glucokinase from the regulatory protein.

An immunohistochemical study in our laboratory was first to show that the regulatory protein is distributed predominantly in the nuclei of hepatocytes and that some of it is translocated from the nucleus to the cytoplasm in response to high concentrations of glucose (Toyoda et al., 1995b). We therefore suggested that the regulatory protein may participate in the regulation of glucokinase translocation between the nucleus and the cytoplasm (Toyoda et al., 1997b). It was recently reported that the glucokinase regulatory protein is necessary for the import of glucokinase into the nucleus, whereas a nuclear export signal sequence in glucokinase is involved in the glucokinase translocation from the nucleus to the cytoplasm (Shiota et al., 1999). The mechanism by which glucokinase translocation is impaired in diabetic rats remains to be clarified.

REFERENCES


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