Effect of Recombinant Human Insulin-Like Growth Factor-I on Expression of Glucose Transporters, GLUT 2 and GLUT 4, in Streptozotocin-Diabetic Rat

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Received May 27, 1998    Accepted July 3, 1998

ABSTRACT—We investigated the effect of recombinant insulin-like growth factor-I (rhIGF-I) on the expression of glucose transporters, GLUT 2 in the liver and GLUT 4 in the heart, in streptozotocin (STZ)-diabetic rats by the reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry. GLUT 2 and its mRNA in the liver was elevated, whereas GLUT 4 and its mRNA in the heart were decreased in STZ-diabetic rats. A two-week treatment with rhIGF-I mostly restored the expression of GLUT 2 and GLUT 4 to normal rat level. We demonstrated that the effect of IGF-I on the expressions of GLUTs was almost the same as that of insulin.

Keywords: Glucose transporter, Reverse transcription-polymerase chain reaction (RT-PCR), Immunohistochemistry

In mammalian cells, the facilitative uptake of glucose is mediated by a group of specialized glucose transporters (GLUTs) (1–3). GLUT 2, expressed predominantly in the liver, increases its expression in streptozotocin (STZ)-diabetic rats and plays an important role in elevated hepatic glucose output, which is thought to be an essential factor for hyperglycemia in rats. On the other hand, peripheral insulin-sensitive tissues such as skeletal muscle and the heart express a unique transporter isoform, GLUT 4, which translocates from an intracellular storage site to the cell surface membrane under the influence of insulin (4). It has been reported that myocardial glucose transport was decreased as a consequence of lowered expression of GLUT 4 in STZ-diabetic rats. Recently the relation of myocardial dysfunction to the decrease in GLUT 4 was questioned (5). In this regard, hyperglycemia is the most important factor in the regulation of the expression of GLUT 4 and the pathogenesis of diabetic complications. By insulin treatment, the expression of GLUT 2 was decreased and the expression of GLUT 4 was restored to levels observed in nondiabetic normal rats (5–7). We have previously reported recombinant insulin-like growth factor-I (rhIGF-I) normalized both the overexpression of renal GLUTs 2 and 5 and renal function in STZ-diabetic rats (8). From our findings, it is suggested that rhIGF-I would improve the expression of GLUTs of other organs in the rat. In the present study, we compared the effect of rhIGF-I treatment with insulin on glucose transporter expression in liver (GLUT 2) and heart (GLUT 4) in STZ-diabetic rats.

RhiGF-I was obtained from Fujisawa Pharmaceutical Co., Ltd. (Osaka) and recombinant human insulin (HUMULIN R: 100 U/ml) and STZ were purchased from Shionogi Pharmaceutical Co., Ltd. (Osaka) and Sigma Chemical Co. (St. Louis, MO, USA), respectively. Male Sprague-Dawley rats (Charles Rivers Japan, Inc., Hino), aged, 6 weeks, were used. The protocol for the experiments followed the Regulations of Animal Experiments Agreement of Fujisawa Pharmaceutical Co., Ltd. STZ was administered intravenously (60 mg/kg). Ten rats treated with vehicle were regarded as normal. Blood glucose was measured by a glucose oxidase method (Glucose B-test; Wako Pure Chemicals, Osaka). After three weeks, STZ-diabetic rats were divided into three groups of ten rats: saline control, insulin and rhIGF-I, so that each group would have similar characteristics in terms of blood glucose and body weight. Insulin (6 U/day/rat) or rhIGF-I (3.2 mg/day/rat) were administered subcutaneously for two weeks by using ALZET mini-osmotic pumps (Model 2ML2; Alza, Palo Alto, CA, USA).
After two weeks of treatment, rats were anesthetized with ether and blood was collected from the abdominal aorta; then the liver and heart were taken for mRNA and immunohistochemical analyses. For mRNA analysis, tissues were frozen in liquid N₂ and stored at −80°C. Tissues were homogenized in TRIzol Reagent (Gibco BRL, Gaithersburg, MD, USA) by a Polytron homogenizer (Kinematica AG, Lucerne, Switzerland). Total RNA was extracted according to Gibco’s protocol. One microgram of total RNA was applied for the reverse transcription-polymerase chain reaction (RT-PCR) with one step RT-PCR (RT-PCR high-Plus.; Toyobo, Osaka), which was performed according to Toyobo’s protocol (9). Pairs of sense and antisense oligonucleotide primers for GLUT 2, GLUT 4 and actin were as follows: for sense 5'-TTTCA GACAGCTGGCATCAGCC-3', 5'-GGTCCATCCAT GAGTTATGTGTC-3' and 5'-TCAACACCCCCACGCT GTACG-3', respectively, and for antisense, 5'-GAGGAA GTCCCGCAATGTACTGG-3', 5'-CTAGAGAGAAGGG TGTCCGTGCG-3' and 5'-CAGGAGGAAGCGCTGGA AGAAG, respectively. PCR products for GLUT 2, GLUT 4 and actin were 392, 343 and 421 bp, respectively, and they were checked for the respective nucleotide sequence. The PCR products were subjected to electrophoresis. Then band intensity was determined by a FluorImmmer (Model FSI; Molecular Dynamics, Sunnyvale, CA, USA), and photos of the band were taken by Electronic Trans-illuminators (FASII; Ultra Lum, Inc., Carson, CA, USA). The intensity ratio (GLUT/Actin) of each band was calculated and compared statistically. Data are represented as the mean ± S.E.M. Effects were assessed by Student’s t-test or multiple comparisons by Dunnett’s test.

For immunohistochemistry, a frozen section was prepared according to Marcus et al. (10). Slices of the liver or heart were embedded and frozen in Tissue-Tek OCT medium (Miles, Inc., Elkhart, IN, USA), and sectioned to 5-μm thickness by a Leitz cryostat (Leica Instruments GmbH, Nussioch, Germany). Sections were fixed with acetone and kept at −80°C. Rabbit anti-GLUT 2 and 4 were purchased from Transformation Research, Inc. (Framingham, MA, USA). The sections were washed with phosphate-buffered saline (PBS), blocked with 10% normal goat serum and incubated in anti-GLUT 2 or 4 (1:200 dilution) antibody, at 4°C overnight. The sections were then washed with PBS and incubated in fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (1:150 dilution; Jackson Immuno-Research Laboratories, Inc., West Grove, PA, USA) at room temperature for 30 min. The sections were then washed again with PBS and fixed with DABCO (1,4-diazabicyclo-2,2,2-octane) in glycerol for observation by a laser scan confocal microscope (MRC-1024; Bio-Rad Microscience Ltd., Hertfordshire, England). The pictures were printed by Fujix Pictography 3000 (Fuji Film, Tokyo).

Blood glucose and body weight changes in STZ-diabetic rats, pre- and post-2-week treatment with insulin or rHGF-I, are shown in Fig. 1. Blood glucose and body weight in STZ-controls were significantly (P < 0.001) different from those of normal rats. By treatment with insulin or rHGF-I, blood glucose was restored to normal rat level. Significant (P < 0.01) body weight gain was

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**Fig. 1.** Blood glucose and body weight in STZ-diabetic rats pre- and post-2-week treatment with insulin or rHGF-I and normal rats. A: Blood glucose and B: Body weight; ○, normal rat; ●, STZ-diabetic rat (saline control); ▲, STZ-diabetic rat (insulin, 6 U/day/rat); □, STZ-diabetic rat (rHGF-I, 3.2 mg/day/rat). Data represent the mean ± S.E.M. of ten rats. ***P < 0.001, compared with normal rat (Student’s t-test) and **P < 0.01, compared with the saline control (Dunnett’s multiple comparison procedure).
achieved by treatment with these drugs compared with STZ-saline rats, especially when rhIGF-I was used.

Expression of GLUT 2 in the livers of the STZ-controls was significantly (P<0.01) increased compared with that in normal rats detected by RT-PCR (Fig. 2). By treatment with insulin or rhIGF-I, the expression of GLUT 2 was significantly (P<0.05) reduced to the normal rat level compared with that in STZ-controls. On the other hand, expression of GLUT 4 in the STZ-controls was greatly (P<0.01) reduced compared with that in normal rats. Treatment with insulin or rhIGF-I restored GLUT 4 expression to the normal rat level. The immunolocalization site of GLUT 2 protein in the liver of normal rat was in the sinusoidal membrane of the hepatocytes (Fig. 3A). GLUT 2 protein expression level was increased in the STZ-controls (Fig. 3B) compared with the normal rat, and it was decreased by treatment with insulin (Fig. 3C) or rhIGF-I (Fig. 3D). The site of expression of GLUT 4 protein in the heart was the plasma membrane in normal rats (Fig. 3E), whereas the expression level decreased strikingly in the STZ-controls (Fig. 3F). By treatment with insulin (Fig. 3G) or rhIGF-I (Fig. 3H), the expression level was restored to the normal rat level.

Overexpression of GLUT 2 in the liver of STZ-diabetic rats and the decrease in its expression by insulin treatment has been reported by several workers (6, 7). In our present study, overexpression of GLUT 2 in STZ-control rats was confirmed by both RT-PCR and immunohistochemistry. The effect of insulin on expression of GLUT 2 was also verified. Moreover, by treatment with rhIGF-I, the expression of GLUT 2 was decreased as well. On the other hand, expression of GLUT 4 in the heart was significantly decreased in the STZ-controls. This observation agrees with that reported by Garvey et al. (5). Glucose uptake in the heart was mediated by GLUT 4 and expression of GLUT 4 was decreased in STZ-diabetic rats (5). In fact, in vivo $[^{1}H]^{-2}$-deoxyglucose (2-DOG) uptake in the heart, diaphragm and gastrocnemius isolated from STZ-diabetic rat were decreased compared with those of normal rats. On the other hand, $[^{1}H]^{-2}$-DOG uptake in the same tissues isolated from two-week rhIGF-I-treated STZ-diabetic rat increased remarkably (T. Asada et al., unpublished data). These results, our previous report (8), and one other report (11) indicate that the blood glucose level might regulate the expression of GLUTs. Therefore, the blood glucose lowering effect of insulin and rhIGF-I

Fig. 2. RT-PCR analyses of GLUT 2 and GLUT 4 expression of normal rat, STZ-diabetic rat (saline control), STZ-diabetic rat (insulin, 6 U/day/rat) and STZ-diabetic rat (rhIGF-I, 3.2 mg/day/rat). A: Total RNAs isolated from liver and heart were used in the one-step RT-PCR assay, and actin was used as a reference. PCR products were electrophoresed on an agarose gel, visualized by adding ethidium bromide and photographed under u.v. lighting. B: Fluorescence intensity was analyzed by the FluorImager; the intensity ratio (GLUT/Actin) of each group of rats was calculated and compared statistically. Bars represent the mean±S.E.M. of ten rats. $^{##} P<0.01$, compared with normal rat (Student's t-test) and $^{*} P<0.05$, compared with the saline control (Dunnett's multiple comparison procedure).
might restore the level of GLUT 2 and 4 expression to the normal rat level. However, the possibility remains that a change in GLUTs expression to the normal rat level would decrease the blood glucose level (12).

Insulin binds insulin receptor which then undergoes autophosphorylation and is able to phosphorylate insulin receptor substrate 1 (IRS-1). Then IRS-1 binds p85 subunits of phosphatidylinositol (PI) 3-kinase and activates PI-3 kinase to translocate GLUT 4 from the intracellular pool to the plasma membrane. After translocation, GLUT 4 transports glucose from the blood to peripheral tissue (muscle, adipocytes, heart, etc.), subsequently reducing blood glucose (13). In a similar manner, upon IGF-I binding, the IGF-I receptor undergoes autophosphorylation and is able to phosphorylate IRS-1. IGF-I receptors are present in the skeletal muscle in which IGF-I stimulates glucose uptake as efficiently as insulin by promoting GLUT 4 translocation (14).

As Scheiwiller et al. (15) reported that plasma IGF-I concentration was very low in STZ-diabetic rats, it is reasonable to treat STZ-diabetic rats with IGF-I. They showed that the growth retardation of STZ-diabetic rats was improved by treatment with rhIGF-I. In agreement with Scheiwiller et al., we also showed that considerable body weight gain was achieved by treatment with rhIGF-I.

We previously demonstrated that overexpression of renal GLUTs 2 and 5, and renal dysfunction in STZ-diabetic rats were restored by treatment with rhIGF-I or insulin (8). In the present study, we showed the increase of GLUT 2 expression in the liver and decrease of GLUT 4 expression in the heart in STZ-diabetic rats were restored to normal rat levels by treatment with rhIGF-I, which were similar to the actions of insulin.

Taken together, the effect of IGF-I on abnormal GLUTs expression in STZ-diabetic rats was almost the same as that of insulin.

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