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Decreased GLUT 4 mRNA Levels did not Related with Degree of Hyperglycemia in Skeletal Muscles of Streptozotocin-induced Diabetic Rats

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=ABSTRACT=

In our previous study (Kim et al, 1991), GLUT 4 protein content correlated negatively with plasma glucose levels in skeletal muscles of STZ-induced diabetic rats. Thus, in this study, to confirm whether expression of GLUT 4 correlate negatively with degree of hyperglycemia, we measured levels of GLUT 4 mRNA in red and white gastrocnemius muscles in STZ-induced mild and severe diabetic rats.

Rats were randomly assigned to control, mild, and severe diabetic groups, and the diabetes was induced by intraperitoneal administration of STZ. The experiment was carried out 10 days after STZ administration. Gastrocnemius red and white muscles were used for the measurement of GLUT 4 expression.

Plasma glucose levels of mild and severe diabetic rats were increased compared to control rats (control, mild, and severe diabetes; 6.4±0.32, 9.4±0.68, and 22.0±0.58 mmol/L, respectively). Plasma insulin levels of mild and severe diabetic rats were decreased compared to control rats (control, mild, and severe diabetes; 198±37, 114±14, and 90±15 pmol/L, respectively). GLUT 4 mRNA levels of gastrocnemius red muscles in mild and severe diabetic rats were decreased compared to control rats (64±1.2% and 71±2.0% of control, respectively), but GLUT 4 mRNA levels in gastrocnemius white muscles were unaltered in diabetic rats.

In summary, GLUT 4 mRNA levels were decreased in STZ-induced diabetic rats but did not correlated negatively with degree of hyperglycemia, and this result suggest that the regulatory mechanisms of decreased GLUT 4 mRNA levels are hypoinsulinemia and/or other metabolic factor but not hyperglycemia. And regulation of GLUT 4 expression in STZ-induced diabetes between red and white enriched skeletal muscles may be related to a fiber specific gene regulatory mechanism.

Key Words: Glucose transporter 4 mRNA, Skeletal muscles, Streptozotocin, Diabetes

INTRODUCTION

Animal model of diabetes mellitus can be induced by streptozotocin(STZ) injection. The STZ-induced diabetic rats show hypoinsulinemia and hyperglycemia, and have peripheral insulin resistance (Nishimura et al, 1989).

It is well known that post-receptor phase is a main cause of insulin resistance (Kolterman et al, 1983; Nishimura et al, 1989). Especially glucose transport is rate limiting for glucose metabolism in skeletal muscle (Fink et al, 1992). Currently six facilitated diffusion glucose transporter genes and one pseudogene have been identified and nomenclature refer to them in the order of cloning. Among them, GLUT

4 gene is expressed primarily in adipocytes and skeletal muscles in which glucose transport is rapidly and markedly enhanced in response to insulin (Fukumoto et al, 1989; Birnbaum, 1989; Charron, 1989; James et al, 1989). It's regulation may be a major determinant of insulin responsiveness in adipocytes and skeletal muscles.

Insulin-stimulated glucose transport is reduced in insulin responsive tissue by decrease in number, intrinsic activity, and translocation from a large intracellular pool to the plasma membrane of GLUT 4 protein. Garvey et al (1988) and Kahn (1992) reported GLUT 4 mRNA and protein levels were markedly decreased in adipocytes of patient with NIDDM and Dohm (1988) found GLUT 4 levels of skeletal muscles were decreased in human with insulin resistance.

Relative role of hyperglycemia and hypoinsulinemia in the regulation of GLUT 4 have been studied. In our previous study (Kim et al, 1991), cytochalacin B binding GLUT 4 protein levels in skeletal muscles correlated inversely with plasma glucose levels in STZ-induced diabetic rats. Kahn et al (1992) found that GLUT 4 mRNA levels correlated negatively with fasting plasma glucose in skeletal muscles of human with insulin-dependent diabetes mellitus. But Burcelin et al (1993) reported that hyperglycemia did not affect the decrease in GLUT 4 mRNA but insulin played an important role.

Thus, in this study, to confirm whether expression of GLUT 4 correlate negatively with degree of hyperglycemia, we measured levels of GLUT 4 mRNA in red and white gastrocnemius muscles in STZ-induced mild and severe diabetic rats.

MATERIALS AND METHODS

Experimetal animals

Female Sprague-Dawley rats were used at body weight ranging from 180 to 230 g. Rats were randomly assinged to control, mild and severe diabetic groups. Mild and severe diabetic rats were induced by a single peritoneal injection of 40 and 60 mg/kg BW of STZ dissolved in citrate buffer(pH 4.5), respectively. Control rats were injected with the same amounts of normal saline.

The induction of diabetes was confirmed by measurement of urine glucose using urine strip on the second day of STZ injection. The rats were anesthetized with intraperitoneal injection of pentothal sodium(40 mg/kg BW) following 15~16 hours fasting on the tenth day after STZ injection. 5 ml of arterial blood was drawn from abdominal aorta, and centrifuged, and the plasma was stored at $-75^{\circ}C$ until glucose and insulin analysis. Plasma glucose was measured by colorimetric glucose oxidase method, and plasma insulin was determined by radioimmunoassay. Red and white gastrocnemius muscles were rapidly removed and frozen in liquid nitrogen for GLUT 4 mRNA analysis.

Measurement of GLUT 4 mRNA levels

Expression of GLUT 4 gene in gastrocnemius muscles were determined by RNA slot blot hybridization using a GLUT 4 cDNA probe which was kindly donated by Yong-ho Ann (Biochemistry Department, Yonsei Medical College). Total RNA was extracted by using the acid-guanidium thiocyanate-phenol-chloroform method (Chomczynski & Sacchi, 1987). Ten microgram of total RNA was dissolved in 10 μl of DEPC(diethy pyrocarbonate)-treated water, and then 20 μl of formamide, 7 μl of 37% formaldehyde, and 2 μl of 20×SSC (175.3 g NaCl and 88.2 g sodium citrate/l, pH 7.0)were added to RNA solution. The tubes containing RNA solution were incubated at $68^{\circ}C$ for 15 minute, and two volumes of 20×SSC were added. The RNA solution was blotted on a reinforced nitrocellulose membrane(S&S) using BioRad slot blot appratus. After baking the memebrane at $80^{\circ}C$ for 2 hours in a dryoven, the membrane was prehybridized in a solution containing 5×SSC, 50% formamide, 1× Denhardt' solution (5 g Ficoll, 5 g polyvinylpyrrolidine and 5 g bovine serum albomin/500 ml), 0.1% SDS (sodium dodecyl sulfate), 25 mM KPO_4,

200 μg/ml salmon sperm DNA at 42°C for 18~24 hours, and hybridized with ^{32}P-labelled GLUT 4 probe for 18~24 hours at 42°C in a hybridization solution(10% dextran sulfate was added to a prehybridization solution). The cDNA of GLUT 4 was labeled with α-^{32}P-dATP using Nick translation kit(Boerhinger Manheim). The membrane was rinsed at 42°C for 15 minutes, one time in 2×SSC and 0.1% SDS, two times 0.5×SSC and 0.1% SDS and one time 0.1×SSC and 0.1% SDS. The membrane was exposed to X-ray film with intensifying screen at −75°C. Equal loading of RNA was measured with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe. Quantification of GLUT 4 mRNA expressed in muscles from each groups were measured by densitometery(Toyo, DMU 33C), and corrected with the GAPDH expression.

Statistical analysis

All values were presented as the mean±S.E. Student's t test was performed for statistical analysis.

RESULTS

Body weight was increased by 2% for 10 days in control rats, but reduced in mild and severe diabetic rats by 5% and 11%, respectively(Table 1). Plasma glucose levels of control rats were 6.4±0.32 mmol/L, and those of mild and severe diabetic rats were 9.4±0.68 and 22.0±0.58 mmol/L, respectively. Plasma insulin levels of control rats were 198 ±37 pmol/L, and those of mild and severe diabetic rats were 114±14 and 90±15 pmol/L, respectively (Fig. 1).

GLUT 4 mRNA levels in gastrocnemius red muscles were decreased in 64±1.2% and 71±2.0% of control in mild and severe diabetic rats, respectively. In gastrocnemius white muscles, the levels of GLUT 4 mRNA were not different between control and diabetic rats(Fig. 2).

Table 1. Changes of body weight(g) in control and diabetic rats after injection

Groups	n	STZ administration	
		Before	After 10 days
Control	6	202±6.3	206±7.0
Diabetes			
mild	5	195±6.5	186±16.9
severe	7	201±7.9	179±4.5*

Values are mean±S.E., n indicates number of cases. *p < 0.05 vs. before STZ administration.

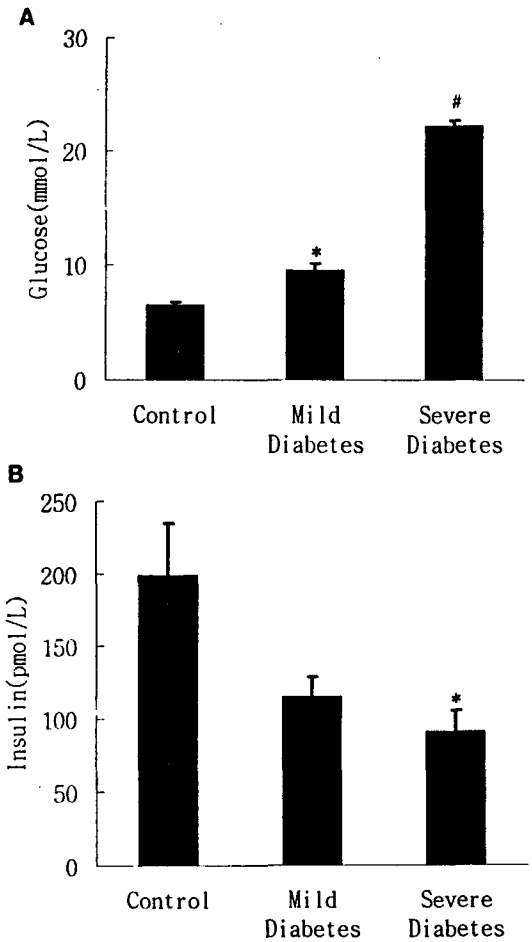

Fig. 1. Concentration of the fasting plasma glucose(A) and insulin(B) in the experimental animals. Values represents the means±S.E.
**p<0.05 vs control. #p<0.05 vs mild diabetes.*

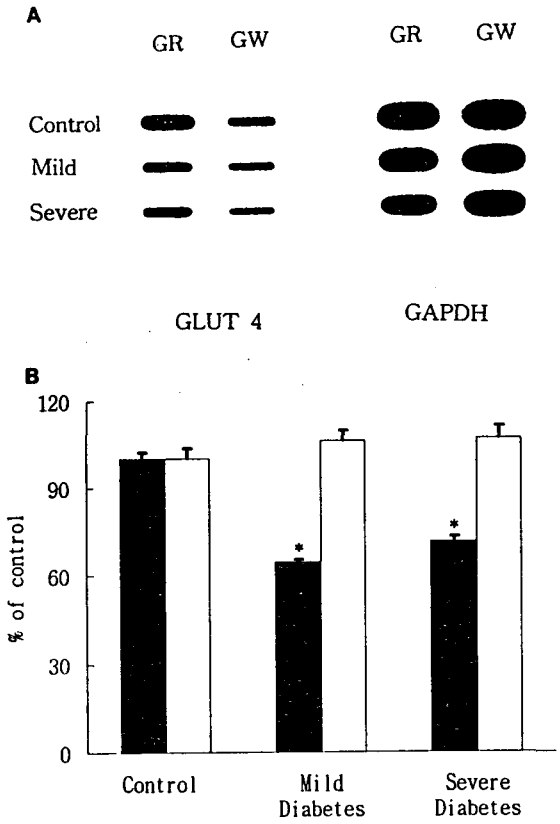

*Fig. 2. Slot blot analysis(A) and percent expression(B) of glucose transporter 4 (GLUT 4) in the experimental animals. GR (closed bar) and GW (open bar) represents gastrocnemius red and gastrocnemius white muscle, respectively. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was measure for equal loading in each groups *p<0.05 vs control.*

DISCUSSION

Skeletal muscle is responsible for more than 70~75% of insulin mediated glucose uptake (Defronzo, 1988) and glucose transport is rate limiting for skeletal muscle glucose metabolism in normal and STZ-induced diabetic rats (Ziel et al, 1988). Thus, the defect in function of GLUT 4 protein and the expression of GLUT 4 in skeletal muscle could potentially explain the insulin resistance in glucose uptake.

In this study, the expression of GLUT 4 in the gastrocnemius red muscle were decreased in diabetic rats compared to control rats and this result agree with others (Garvey et al, 1988; Neufer et al, 1993; Kahn et al, 1992). And in the relationship between GLUT 4 expression and degree of hyperglycemia, GLUT 4 expression was only slightly higher in severe diabetic rats than in mild diabetic rats. This result is in accord with Burcelin et al's (1993). They reported GLUT 4 expression was not modified by hyperglycemia but rapidly affected by an acute decrease of the plasma insulin levels. But Kahn et al (1992) found that in skeletal muscles of insulin-dependent diabetic patients, GLUT 4 mRNA levels correlated negatively with fasting plasma glucose but not with fasting plasma insulin levels. In our result, plasma glucose levels in severe diabetic rats were 2.5-fold higher than in mild diabetic rats, but plasma insulin levels were only slightly lower in severe diabetic rats. Thus, these results suggest that the regulatory mechanisms of decreased GLUT 4 mRNA levels in red skeletal muscles are hypoinsulinemia and/or other metabolic factor but not hyperglycemia.

In our previous study (Kim et al, 1991), GLUT 4 protein content, unlike GLUT 4 expression in this study, correlated negatively with plasma glucose levels in skeletal muscles of STZ-induced diabetic rats. In that study, plasma insulin levels were 2-fold higher in mild diabetic rats than in severe diabetic rats. The difference of plasma insulin levels maybe possible explain the disparity in protein content and gene expression of GLUT 4 in mild and severe diabetic rats.

The expression of GLUT 4 in the gastrocnemius white muscles, unlike gastrocnemius red muscles, were not different between control and diabetic rats. The result is in accord with others (Richardson et al, 1991) in which GLUT 4 mRNA levels were found to decrease in red muscles with no significant alteration in white muscles in STZ-induced diabetic rats. The disparity in the regulation of GLUT 4 expression in STZ-induced diabetes between red and white enriched skeletal muscles suggests that the hormonal control of substrate utilization for energy

production may be related to a fiber specific gene regulatory mechanism.

In summary, GLUT 4 mRNA levels were decreased in STZ-induced diabetic rats but did not correlated negatively with degree of hyperglycemia, and this result suggest that the regulatory mechnisms of decreased GLUT 4 mRNA levels are hypoinsulinemia and/or other metabolic factor but not hyperglycemia. And regulation of GLUT 4 expression in STZ-induced diabetes between red and white enriched skeletal muscles may be related to a fiber specific gene regulatory mechanism.

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