A Possible Mechanism of Insulin Resistance in the Rat Adipose Cell in Streptozotocin-induced Diabetes Mellitus

DEPLETION OF INTRACELLULAR GLUCOSE TRANSPORT SYSTEMS

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ABSTRACT The effects of insulin-dependent diabetes mellitus on glucose transport activity and on the concentrations of glucose transport systems in the plasma and low density microsomal membranes in adipose cells isolated from streptozotocin-induced diabetic rats have been examined. Glucose transport activity was assessed by measuring 3-*O*-methylglucose transport and the concentration of glucose transport systems estimated by measuring specific D-glucose-inhibitable cytochalasin B-binding. Basal glucose transport activity decreases from 0.19 to 0.12 fmol/cell per min with the induction of diabetes, but remains constant per unit cellular surface area and is accompanied by a constant 6 pmol of glucose transport systems/mg of membrane protein in the plasma membrane fraction. Maximally insulin-stimulated glucose transport activity decreases from 3.16 to 1.05 fmol/cell per min and from 0.26 to 0.12 amol/μm^2 per min, and is accompanied by a decrease from 25 to 15 pmol of glucose transport systems/mg of plasma membrane protein. These diminished effects of insulin on glucose transport activity and the concentration of glucose transport systems in the plasma membrane fraction are paralleled by a 45% decrease in the basal number of glucose transport systems per milligram of membrane protein in the low density microsomal membrane fraction, the source of those glucose transport systems appearing in the plasma membrane in response to insulin. Thus, the "insulin resistant" glucose transport of the adipose cell in the streptozotocin-induced diabetic rat appears to be the consequence of a depletion of glucose transport systems in the intracellular pool.

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INTRODUCTION

Kasuga et al. (1) and Kobayashi and Olefsky (2, 3) have demonstrated that streptozotocin-induced diabetes in the rat is associated with a marked reduction in insulin's ability to stimulate glucose transport in the isolated adipose cell in spite of increased insulin binding to its receptor. The latter investigators postulated that this effect occurs through an alteration in the number of functional glucose transport systems.

Recent studies in this laboratory (4, 5), and independently by Suzuki and Kono (6), have shown that insulin stimulates glucose transport in the isolated rat adipose cell primarily through a rapid, reversible, and hormone concentration-dependent translocation of glucose transport systems from a large intracellular pool to the plasma membrane. The present investi-

gations were undertaken to determine if a perturbation in this postulated mechanism of insulin action could explain the impairment in insulin's ability to stimulate glucose transport in cells from streptozotocin-induced diabetic rats.

METHODS

Animals. Two groups each of 40 male Sprague-Dawley rats (CD strain, Charles River Breeding Laboratories, Wilmington, Mass.) weighing 150–200 g were injected intravenously with either a freshly prepared solution of streptozotocin (ICN Pharmaceuticals, Cleveland, Ohio) in 0.1 mM citrate buffer, pH 4.5, 65 mg/kg (diabetic group), or buffer alone (control group). All animals were then maintained for 7 d with ad lib. feeding and monitoring of their urine glucose levels, body weight, and food and water consumption. On day 7, unfasted animals were killed between 9 and 11 a.m., and plasma was prepared in heparinized tubes and frozen for later analysis.

Isolated adipose cells and subcellular membrane fractions. The epididymal fat pads were removed, isolated adipose cells were prepared (7), and adipose cell size was determined (8). All incubations were carried out in Krebs-Ringer-bicarbonate-Hepes buffer, pH 7.4, 37°C, containing 10 mg bovine serum albumin/ml (4). The cells were then equally distributed in 15-ml volumes to 950-ml polypropylene jars containing 21 ml of incubation medium, and preincubated for 15 min. Insulin (crystalline zinc insulin, courtesty of Dr. Ronald E. Chance, Eli Lilly & Co., Indianapolis, Ind.) was added at final concentrations of 0 or 7.0 nM (1,000 μU/ml), incubation was continued for 15 min, and replicate 0.25-ml samples of cells were removed for determination of the rate of 3-*O*-methylglucose transport and the intracellular water space (calculated from steady-state uptake levels) (5). Plasma, high density microsomal, and low density microsomal membrane fractions were prepared by differential ultracentrifugation, equilibrium D-glucose-inhibitable cytochalasin B-binding was measured, and the concentrations of binding sites were calculated (4, 5). The specific 5′-nucleotidase, rotenone-insensitive NADH-cytochrome *c* reductase, and UDP-galactose:*N*-acetylglucosamine galactosyltransferase activities of each homogenate and membrane fraction were assayed (4–6).

Analytical procedures. Protein was determined by the Coomassie Brilliant Blue method described by Bradford (9) (Bio-Rad Protein Assay, Bio-Rad Laboratories, Richmond, Calif.) using crystalline bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) as the standard. Urine glucose was monitored using reagent strips (Labstix, Ames Co., Elkart, Ind.). Plasma glucose and insulin were determined using a glucose analyzer (Glucose Analyzer 2, Beckman Instruments, Inc., Fullerton, Calif.) and by radioimmunoassay against a human insulin standard (Immo Phase, Corning Medical, Corning Glass Works, Medfield, Mass), respectively. Comparisons were made using a *t* test of statistical significance and differences accepted as significant at the $P \leq 0.05$ level.

RESULTS

General characteristics. Some pertinent general characteristics of the experimental animals are illustrated in Table I. The streptozotocin-treated rats drank more water and consumed more food, but gained weight less rapidly than the control animals. At killing, plasma glucose levels were markedly increased in the diabetic rats. Although plasma insulin levels were similar, they were inappropriately low in the streptozotocin-treated rats for the plasma glucose levels observed. The failure to observe decreased plasma insulin levels in the streptozotocin-treated rats may be explained by the submaximal dose of streptozotocin employed here, the strain and body weight of the animals, and/or their death in the postprandial state. Mean epididymal adipose cell size and intracellular water space were 38 and 41% smaller, respectively, in the streptozotocin-treated, than control, animals.

TABLE I
General Characteristics of Control and Streptozotocin-induced Diabetic Rats

Parameter	Experimental group	
	Control	Diabetic
Water intake, ml/rat/24 h	31±2 (31)	113±5 (94)
Food consumption, g/rat/24 h	15.7±0.6 (17)	25.5±1.0 (17)
Body weight gain, g/rat/24 h	7.3±0.2 (40)	2.9±0.1 (119)
Plasma glucose, mg/dl	145±4 (9)	477±11 (20)
Plasma insulin, μU/ml	43±3 (17)	46±3 (21)*
Adipose cell size, μg lipid/cell	0.114±0.005 (3)	0.071±0.005 (3)
Adipose intracellular water, pl/cell	2.3±0.1 (3)	1.4±0.1 (3)
Adipose cellular protein recovered, pg/cell in:		
Homogenate	524±50 (3)	342±12 (3)
Plasma membrane fraction	34.5±3.8 (3)	19.3±3.8 (3)
High density microsomal membrane fraction	18.1±3.0 (3)	8.7±2.3 (3)
Low density microsomal membrane fraction	16.2±2.5 (3)	8.7±0.9 (3)

Water intake, food consumption, and body weight gain were measured during 7 d after treatment. Plasma glucose and insulin were measured at killing. Isolated adipose cells were prepared and cell size, intracellular water, and the recovery of cellular protein measured as described in Methods. Results are the means±SEM of the indicated numbers of observations.
* Difference between diabetic and control animals is not statistically significant at the $P \leq 0.05$ level.

Glucose transport activity. Glucose transport activity in the intact adipose cell in the absence or presence of 7.0 nM (1,000 μU/ml) insulin is illustrated in Fig. 1. Basal 3-*O*-methylglucose transport is some-

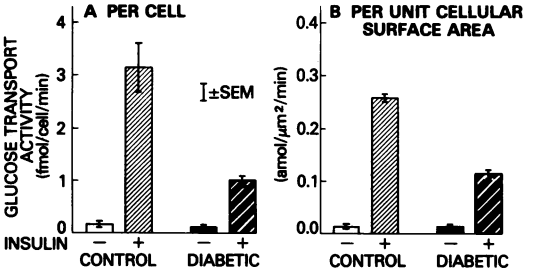

FIGURE 1 Glucose transport activity per cell (A) and per unit cellular surface area (B) in the intact adipose cell. Isolated cells were prepared from control and streptozotocin-induced diabetic rats (mean cell sizes of 0.114 and 0.071 μg lipid/cell, respectively), incubated in the presence of 0 or 7.0 nM (1,000 μU/ml) insulin, and sampled for measurement of 3-*O*-methylglucose transport as described in Methods. Values per unit cellular surface area were obtained from those per cell by assuming a total lipid density of 0.9 g/ml in converting cell size to volume and a spherical geometry in converting cell volume to surface area. Results are the means±SEM of the individual mean values obtained from triplicate samples in three experiments.

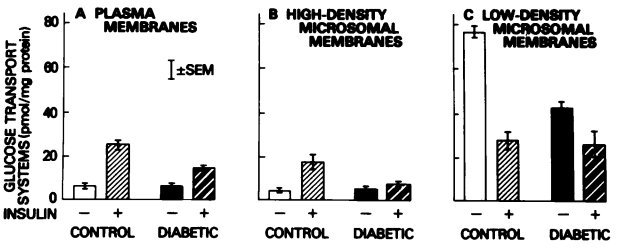

FIGURE 2 Concentration of glucose transport systems in the plasma membrane (A), high density microsomal membrane (B), and low density microsomal membrane (C) fractions of the adipose cell. Membrane fractions were prepared from the isolated cells described in Fig. 1 and the concentrations of glucose transport systems determined using a D-glucose-inhibitable cytochalasin B-binding assay as described in Methods. Results are the means±SEM of the individual values obtained in three experiments.

what decreased in cells from the diabetic, compared with control, rats when expressed per cell (Fig. 1A), but this difference disappears when expressed per unit cellular surface area (Fig. 1B). Maximally insulin-stimulated 3-*O*-methylglucose transport, on the other hand, is decreased in cells from the diabetic animals by 67% when expressed per cell (Fig. 1A), and by 55% when expressed per unit cellular surface area (Fig. 1B).

Subcellular distribution of glucose transport systems. The distribution of glucose transport systems among the plasma, high-density microsomal, and low-density microsomal membrane fractions prepared from basal or maximally insulin-stimulated adipose cells is illustrated in Fig. 2. While the numbers of glucose transport systems per milligram of membrane protein in the plasma membrane fraction are similar in basal cells from the diabetic and control rats, the increase in their number in response to insulin is decreased by 53% in cells from the diabetic animals (Fig. 2A). A similar relationship is observed in the high density microsomal membrane fraction (Fig. 2B), a fraction thought to be contaminated by plasma membranes (unpublished observations). In contrast, the number of glucose transport systems per milligram of membrane protein in the low density microsomal membrane fraction prepared from basal cells of the diabetic animals is decreased by 45%. Furthermore, although the absolute numbers of glucose transport systems per milligram of membrane protein in the low density microsomal membrane fraction of maximally insulin-stimulated cells from the diabetic and control rats are similar, their net disappearance in response to insulin is decreased by 67% in the former (Fig. 2C).

These alterations in the distribution of glucose transport systems are not accompanied by significant alterations in the relative distributions of membrane marker enzyme activities (not illustrated). Furthermore, although the yields of protein in each fraction and homogenate of cells from the diabetic, compared to control, rats are decreased (Table I), they are roughly proportional to the surface area of the original intact cells.

DISCUSSION

The results of the present investigations demonstrate that the isolated adipose cell's resistance to insulin at the glucose transport level in the streptozotocin-induced diabetic rat is accompanied by (*a*) a markedly diminished stimulatory effect of insulin on the concentration of glucose transport systems in the plasma membrane fraction (Fig. 2A), closely paralleling the markedly decreased stimulatory effect of insulin on 3-*O*-methylglucose transport in the intact cell expressed per unit cellular surface area (Fig. 1B), and (*b*) a marked reduction in the basal concentration of glucose transport systems in, and a corresponding decrease in their disappearance in response to insulin from, the low density microsomal membrane fraction (Fig. 2C), the cell's intracellular pool from which glucose transport systems are translocated to the plasma membrane. These alterations occur without significant changes in the membrane species comprising those fractions examined here, as reflected in the distributions of marker enzyme activities (not illustrated). Thus, the "insulin resistant" glucose transport of the adipose cell in this animal model of insulin-dependent diabetes mellitus can be explained by a decrease in the number of glucose transport systems appearing in the plasma membrane in response to insulin as the consequence of a depletion of these glucose transport systems in the cell's intracellular pool.

The total number of glucose transport systems in the intact adipose cell can, at present, only be estimated since methods are not available for determining their number in the original homogenate and, therefore, the extent of their recovery in each fraction. The observed recovery of protein in the plasma membrane fraction of cells from both the diabetic and control animals, however, is proportional to the surface area of the intact cells (Table I). A correction for the 30% decrease in the surface area of cells from the diabetic, compared to control, rats must, therefore, be introduced in estimating the total number of glucose transport systems in the plasma membrane of the intact cell from the diabetic animals. Since the concentrations of glucose transport systems in the plasma membrane fraction of basal cells from the diabetic and control rats are similar (Fig. 2A), then their total number per cell in the plasma membrane of basal cells from the diabetic rats would appear to be decreased by roughly 30%. Since the concentration of glucose transport systems in the plasma membrane fraction of insulin-stimulated cells from the diabetic animals is decreased by 40% (Fig. 2A), then their total number per cell in the plasma membrane would appear to be decreased by roughly 60%. These estimates closely correlate with the decreased basal and insulin-stimulated glucose transport activities per cell actually observed (Fig. 1A).

Since the adipose cell's triglyceride stores comprise a large, central lipid droplet occupying at least 95% of the cell's volume, its cytoplasm is confined to a thin shell adjacent to the plasma membrane and is, therefore, also proportional to the cell's surface area (Table I). Thus, the total number of glucose transport systems in the intact cell's intracellular pool can be estimated in a fashion similar to that just used in estimating their total number in the intact cell's plasma membrane. Since the concentration of glucose transport systems in the low density microsomal membrane fraction of basal cells from the diabetic rats is decreased by 45% (Fig. 2C), then their total number per cell in the intracellular pool would appear to be decreased by roughly 60%. This latter decrease closely correlates with the decrease in their estimated total number per cell in the plasma membrane in the insulin-stimulated state. The mechanism of the translocation process and the factors which determine the residual number of glucose transport systems left in the intracellular pool following attainment of the steady-state maximal response to insulin remain to be determined.

The specific process through which the intracellular pool of glucose transport systems in the adipose cell from diabetic rats is reduced remains to be explored. Nevertheless, the possibility that this reduction occurs at the level of net protein synthesis may be implied by (*a*) a markedly decreased maximal capacity for glucose metabolism (1–3), (*b*) a decreased intracellular water space per cell (Table I) (3), a reflection of the cell's cytoplasmic mass, and (*c*) a decreased recovery of total protein per cell in the homogenate (Table I).

The adipose tissue mass per se probably plays a minor role in total systemic glucose homeostasis. Nevertheless, the alteration in the adipose cell's intracellular pool of glucose transport systems reported here may reflect a more general systemic impairment in insulin action, and thus a potential explanation for the apparent in vivo insulin resistance in the streptozotocin-induced diabetic rat and, perhaps, in newly diagnosed and/or ketoacidotic insulin-dependent diabetes mellitus in man.

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