Gliclazide Treatment of Streptozotocin Diabetic Rats Restores GLUT4 Protein Content and Basal Glucose Uptake in Skeletal Muscle

Nieves Pulido, Ana Suarez, Benito Casanova, Remedios Romero, Esther Rodriguez, and Adela Rovira

This study examined whether the treatment of streptozotocin (STZ)-diabetic rats with gliclazide (5 mg/kg body weight twice daily orally) increases muscle glucose uptake. Rats were treated (group G, n = 10) or untreated (group D, n = 11) for 12 days. Normal rats served as controls (group C, n = 11). At the end of the treatment, both basal and insulin-stimulated glucose uptake by the perfused hindquarters were measured. In gastrocnemious muscles, the protein content of GLUT4 and the insulin binding and tyrosine kinase activity of partially purified solubilized insulin receptors were measured. Group G had a lower mean glycemic value during the treatment period than group D (mean ± SEM, 17 ± 0.6 *v* 19.7 ± 0.5 mmol/L, $P < .05$), without differences in serum insulin levels. Basal glucose uptake by the hindquarters was significantly higher in group G versus group D (2.8 ± 0.3 *v* 1.3 ± 0.2 μmol/g/h, $P < .05$), and was not different versus group C (3.6 ± 0.2 μmol/g/h). Insulin-stimulated glucose uptake was higher ($P < .05$) in group C compared with the two groups of diabetic rats. Glucose uptake at 10^{-7} mol/L insulin was higher in group G than in group D (9.2 ± 0.6 *v* 7.0 ± 0.6 μmol/g/h, $P < .05$). Both insulin binding and tyrosine kinase activity were similar in muscle insulin receptors from both groups of diabetic rats. The GLUT4 protein content was higher in group G than in group D (95 ± 10 *v* 57 ± 7 arbitrary units [AU]/μg protein, $P < .05$) and similar to that of group C (113 ± 13 AU/μg protein). In conclusion, gliclazide has a glucose-lowering effect in STZ-diabetic rats that could be attributed to an increase in muscle glucose clearance by a post–insulin receptor mechanism, probably related to a normalization of GLUT4 content.

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SULFONYLUREAS have been used for several decades in the treatment of non–insulin-dependent diabetic (NIDDM) subjects. Their acute hypoglycemic action involves stimulation of the rate of insulin secretion. However, plasma insulin levels in NIDDM patients treated for a long time with these drugs often return to pretreatment values while maintaining good glycemic control.[1,2] This fact suggests that sulfonylureas also have extrapancreatic effects. Numerous studies have observed that sulfonylureas enhance insulin-mediated glucose utilization by muscle tissue and cultured muscle cells[3,4] by a mechanism distal to the insulin receptor.[5,6] In addition, our group and others have reported a direct effect of sulfonylureas on glucose uptake by rat skeletal muscle.[7,8]

On the other hand, it is known that mature skeletal muscle expresses the two glucose transporters GLUT1 and GLUT4, and the latter is decreased in streptozotocin (STZ)-diabetic rats.[9] In this study, we examined the effect and mechanism of action of gliclazide, a second-generation sulfonylurea, on glucose uptake by perfused hindquarters from STZ-diabetic rats.

MATERIALS AND METHODS

Animals

Male Wistar rats with an initial body weight of 150 to 175 g were used in all of the experiments. Diabetes was induced by intravenous injection (65 mg/kg) of a freshly prepared solution of STZ (Sigma, St Louis, MO). Diabetic rats with serum glucose between 11 and 22 mmol/L 3 days after injection of STZ were selected and randomly separated into two groups: group D, left untreated for 12 days (n = 11), and group G, treated with gliclazide 5 mg/kg (Servier, Madrid, Spain) dissolved in 0.02N NaOH and administered orally twice per day (9 AM and 8 PM) for 12 days (n = 10). Nondiabetic rats served as a control (group C, n = 11).

Analytical Methods

Serum glucose levels were determined every 2 days and on the day of the perfusion (day 13) by a glucose oxidase method (Beckman Glucose Analyzer 2; Beckman Instruments, S.A. Madrid, Spain). Serum immunoreactive insulin was determined on days 0 and 13 with a rat insulin radioimmunoassay (Novo Research Institute, Copenhagen, Denmark).

Hindquarters Perfusion

Rats were fasted 12 hours before perfusion. Hindquarter preparations were perfused as previously described[7] for a total of 90 minutes: 30 minutes in the absence of insulin (basal), 30 minutes with 10^{-9} mol/L insulin, and another 30 minutes with 10^{-7} mol/L insulin. Glucose was assayed in the perfusion medium every 5 minutes to calculate glucose uptake.

Insulin Binding and Muscle Insulin Receptor Kinase Activity

After hindquarters perfusion, gastrocnemius muscles were quickly removed, frozen under liquid nitrogen, and stored at −70°C. Insulin receptors were partially purified by affinity chromatography with wheat-germ agglutinin Sepharose (Pharmacia, Uppsala, Sweden). These insulin receptor preparations were used to measure [^{125}I]-insulin binding and tyrosine-specific protein kinase activity as previously described.[10] The amount of protein used in these assays was 15 μg as estimated by the Bradford dye method (Bio-Rad Laboratories, Richmond, CA).

Analysis of Glucose Transporter Protein GLUT4

The insulin-sensitive glucose transporter protein (muscle/fat GLUT4) content was measured by Western blot analysis on the solubilized material obtained from homogenates of gastrocnemius muscles, as previously described.[10]

From the Department of Endocrinology, Fundación Jiménez Díaz, Universidad Autónoma de Madrid, Madrid, Spain.

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Address reprint requests to Adela Rovira, MD, Department of Endocrinology, Fundación Jiménez Díaz, Avda. Reyes Católicos 2, 28040 Madrid, Spain.

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Statistical Analysis

Results are given as the mean ± SEM. Student's *t* test for unpaired data was used to evaluate the statistical significance of differences between diabetic rat groups. ANOVA tests were performed between both groups of diabetic rats and group C.

RESULTS

General Characteristics of the Animal Groups

Nonfasting serum glucose (12 hours after the last dose of gliclazide) throughout the treatment period was significantly lower ($P < .05$) in group G (17.0 ± 0.6 mmol/L) versus group D (19.7 ± 0.5 mmol/L). On the day of the perfusion, group G also had a mean fasting serum glucose value lower ($P < .05$) than group D (16 ± 0.6 *v* 19.9 ± 0.8 nmol/L). Nonfasting serum insulin at the beginning of treatment was similar in groups G and D (1.17 ± 0.17 *v* 0.84 ± 0.19 nmol/L, P = NS), and fasting serum insulin on the day of the perfusion was again similar in both groups of diabetic rats (1.00 ± 0.16 *v* 0.84 ± 0.17 nmol/L, P = NS). The body weight of the rats at the time of perfusion was not different in group G compared with group D (162 ± 8 *v* 164 ± 7 g, P = NS) (Table 1).

Hindquarter Perfusion

Basal glucose uptake was twofold increased in group G compared with group D (2.8 ± 0.3 *v* 1.3 ± 0.2 μmol/g/h, $P < .05$) and similar compared with group C (3.6 ± 0.2 μmol/g/h). No significant differences in glucose uptake at the insulin concentration of 10^{-9} mol/L were observed between either group of diabetic rats (5.6 ± 0.3 *v* 4.7 ± 0.8 μmol/g/h). At 10^{-7} mol/L insulin, glucose uptake was higher in group G than in group D (9.2 ± 0.6 *v* 7 ± 0.6 μmol/g/h, $P < .05$). Compared with group C, both groups of diabetic rats showed a lower glucose uptake in the presence of insulin (Fig 1 and Table 2).

^{125}I-Insulin Binding to Solubilized Insulin Receptor

The maximal specific ^{125}I-insulin binding to partially purified insulin receptors obtained from skeletal muscle and its displacement by increasing concentrations of unlabeled insulin were similar in both groups of diabetic rats. Binding affinity (ID_{50}) was also similar in both groups of diabetic rats (group D, 1.7 ± 0.1, and group G, 2.1 ± 0.1 × 10^{-9} mol/L, P = NS). Scatchard analysis of the binding data showed that the binding capacity of insulin receptors of both high and low affinity was unchanged in group G compared with group D (Fig 2).

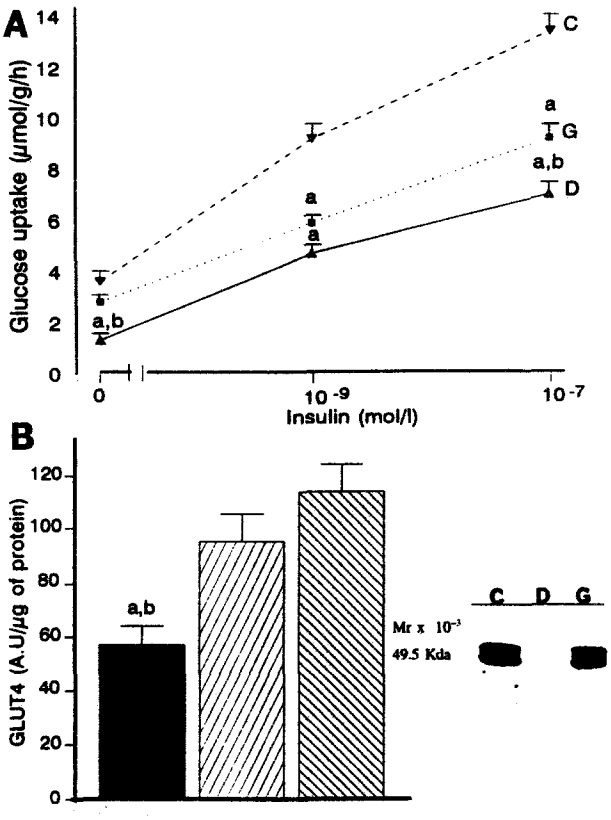

Fig 1. (A) Glucose uptake by hindquarters in the absence and presence of insulin for groups D (n = 11), G (n = 10), and C (n = 11). $^{a}P < .05$ *v* group C, $^{b}P < .05$ *v* group G. (B) Left: Mean ± SEM GLUT4 content in skeletal muscle from group D (■, n = 6), group G (◣, n = 8), and group C (▨, n = 10). $^{a,b}P < .05$ *v* groups G and C. Right: Autoradiogram showing GLUT4 from gastrocnemius muscles. The 45-kd band was scanned, and results are expressed as AU/μg protein.

Insulin Receptor Tyrosine Kinase Activity

The tyrosine kinase activity of partially purified insulin receptors from skeletal muscle, determined as the ability to phosphorylate their own β-subunit and also the exogenous substrate Poly Glu4:Tyr1, was found to be similar in both groups of diabetic rats (Fig 2). No significant differences in the kinase activity of insulin receptors after stimulation by insulin during muscle perfusion or after further in vitro stimulation with insulin 10^{-7} mol/L were found between the diabetic groups.

Table 1. General Characteristics of Animals

Group	Body Weight (g)	Glucose (mmol/L) Days 0-12	Glucose (mmol/L) Day 13	Insulin (nmol/L) Day 0	Insulin (nmol/L) Day 13
D	164 ± 7* (11)	19.7 ± 0.5*† (70)	19.9 ± 0.8*† (11)	0.84 ± 0.19 (6)	0.84 ± 0.17 (6)
G	162 ± 8* (10)	17.0 ± 0.6* (82)	16 ± 0.6* (10)	1.17 ± 0.17 (9)	1.0 ± 0.16 (9)
C	185 ± 2 (11)	6.1 ± 0.2 (17)	5.5 ± 0.3 (8)	—	1.17 ± 0.16 (11)

NOTE. D, untreated diabetic rats: G, gliclazide-treated diabetic rats; C, control rats. Values are mean ± SEM. In parentheses are the numbers of assays.

*P < .05 *v* group C.

†P < .05 *v* group G.

Table 2. Glucose Uptake by Skeletal Muscle in the Absence and Presence of Insulin in the Three Groups (μmol/g/h)

Group	Basal	Insulin 10^{-9} mol/L	Insulin 10^{-7} mol/L
D	1.3 ± 0.2*†	4.7 ± 0.8*	7.0 ± 0.6*†
G	2.8 ± 0.3	5.6 ± 0.3*	9.2 ± 0.6*
C	3.6 ± 0.2	9.2 ± 0.6	13.4 ± 0.9

*$P < .05$ *v* group C.

†$P < .05$ *v* group G.

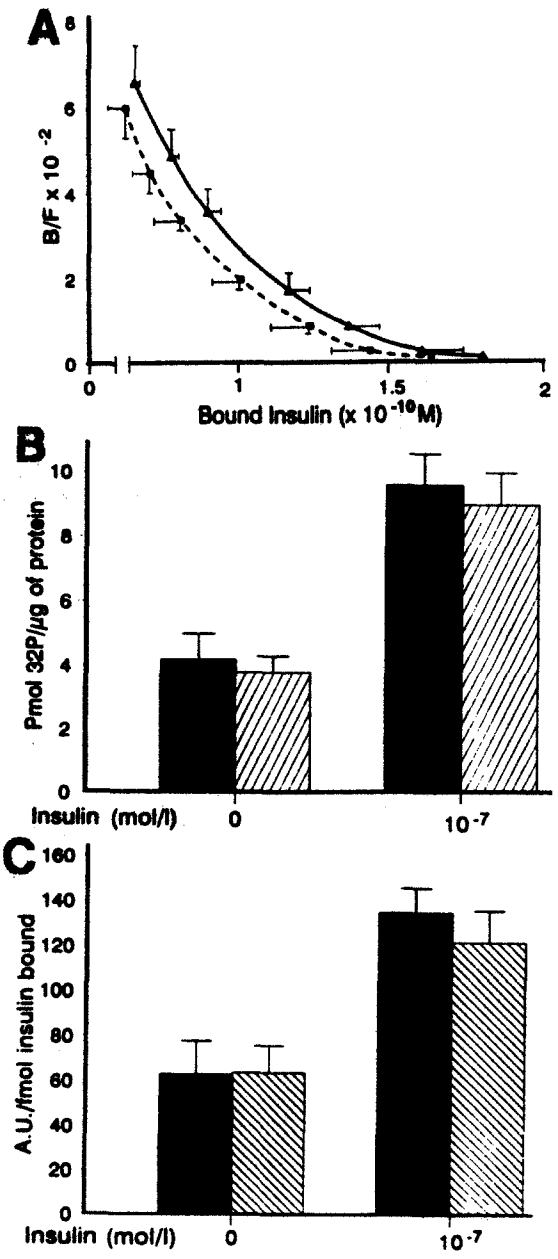

Fig 2. (A) Scatchard plot of the binding data from group D (■—■, n = 8) and group G (▲—▲, n = 7). (B) Phosphorylation of exogenous substrate Poly Glu4:Tyr1 by wheat-germ agglutinin–purified muscle insulin receptors from group D (■, n = 9) and group G (▨, n = 7) in the absence and presence of 10^{-7} mol/L insulin. Data are expressed as pmol of ^{32}P incorporated into exogenous substrate per µγ of receptor protein. (C) Autophosphorylation of the β-subunit of muscle insulin receptor from group D (■, n = 5) and group G (▧, n = 5) in the absence and presence of 10^{-7} mol/L insulin. Results are expressed as AU per amount of insulin receptor preparation that binds 1 fmol insulin (mean ± SEM).

Glucose Transporter Protein GLUT4

To study the effect of gliclazide treatment on the insulin-sensitive glucose transporter (GLUT4), Western blot analyses were performed using a polyclonal antisera specific for the carboxyl-terminal peptide of GLUT4. Figure 1 shows the approximately 45-kd band corresponding to GLUT4 obtained by autoradiography from gastrocnemius muscles of the three groups of rats. Densitometry of the band revealed a lower quantity ($P < .05$) of GLUT4 in group D (57 ± 7 arbitrary units [AU]/µg protein) versus group G (95 ± 10 AU/µg protein) and group C (113 ± 13 AU/µg protein). No significant differences between group G and group C were found.

DISCUSSION

STZ-diabetic rats treated with gliclazide showed a moderate but significant decrease in the mean glycemia in either the nonfasting state or the fasting state compared with diabetic rats left untreated. Amelioration of the glycemia was not associated with an increase in serum insulin. Therefore, the glucose-lowering effect of gliclazide in these diabetic rats could not be attributed to an insulin-stimulatory effect of the sulfonylurea. The hindquarter perfusion experiments showed that treatment with gliclazide produced a normalization of basal glucose uptake, which was twofold higher than in diabetic rats left untreated. Thus, in accordance with a previous study,[11] diabetes induced by STZ produces a detrimental effect in basal glucose disposal by skeletal muscle, and this defect can be corrected by sulfonylurea treatment.

Glucose uptake by skeletal muscle is mediated by the glucose transporters GLUT1 and GLUT4. Their relative importance in basal glucose transport in skeletal muscle is unclear due to the fact that although GLUT1 is mainly present at the surface of cells, its concentration is very low relative to that of GLUT4, which has a preferential intracellular location in non–insulin-stimulated conditions.[12] It has been shown that STZ-diabetic rats have an important decrease in GLUT4 protein content in skeletal muscle, which is clearly seen 7 days after the induction of diabetes.[13,14] This defect is reversed with insulin therapy.[9,13]

In this study, the normalization of basal glucose uptake by skeletal muscle in diabetic rats treated with gliclazide was accompanied by the restoration of muscle GLUT4 protein content to normal levels. This effect was not due to an increase in serum insulin levels or to changes in insulin receptor kinase activity. A secondary effect to reduction in glycemia cannot be excluded although the decrease from the mean glucose level of 362 to 292 mg/dL was modest. It is thus possible that sulfonylureas can regulate the expression of GLUT4 in the skeletal muscle of insulinopenic diabetic rats at a step distal to the insulin receptor. However, normalization of GLUT4 protein levels was not enough to normalize insulin sensitivity in skeletal muscle, as demonstrated in the hindquarter perfusion studies. Diabetic rats treated with gliclazide showed an increase in maximal insulin-stimulated glucose uptake compared with untreated diabetic rats, but it was still lower than in nondiabetic rats, thus indicating a defective functional activity of the glucose transporter.

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