Effect of Glimepiride on Insulin-Stimulated Glycogen Synthesis in Cultured Human Skeletal Muscle Cells

A comparison to glibenclamide

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OBJECTIVE — To examine the effect of glimepiride on insulin-stimulated glycogen synthesis in cultured human skeletal muscle cells in comparison with glibenclamide.

RESEARCH DESIGN AND METHODS — Myotubes derived from glucose-tolerant subjects were incubated with glimepiride or glibenclamide (0–100 μmol/l) for 4 h and with or without insulin (100 nmol/l) for 2 h, and subsequently glycogen synthesis was determined.

RESULTS — Glimepiride had no significant effect on basal glycogen synthesis; in contrast, glimepiride caused a dose-dependent increase of insulin-stimulated glycogen synthesis, with a maximal effect of 39.97 ± 8.4% (mean ± SEM, $n = 4$, $P < 0,02$). The time course of this glimepiride effect on insulin-stimulated glycogen synthesis showed a peak after 12 h incubation with a half maximal effect after 4 h. Preincubation of the myotubes with wortmannin (100 nmol/l), an inhibitor of phosphatidylinositol (PI)- 3 kinase, caused an inhibition of this glimepiride effect on insulin-stimulated glycogen synthesis. In contrast to glimepiride, incubation of myotubes with glibenclamide (0–100nmol/l), a second generation sulfonylurea, had no significant effect on basal or insulin-stimulated glycogen synthesis.

CONCLUSIONS — Incubation of cultured human skeletal muscle cells derived from glucose-tolerant subjects with glimepiride caused a dose-dependent increase of insulin-stimulated glycogen synthesis using therapeutic glimepiride concentrations. This glimepiride effect seems to be mediated via the PI3 kinase pathway. In contrast to glimepiride, glibenclamide had no significant effect on basal or insulin-stimulated glycogen synthesis. These results suggest that glimepiride, beside its well-known effect to stimulate insulin secretion, possess an insulin-sensitizing action in cultured human skeletal muscle cells in support of the concept of an extrapancreatic action of glimepiride.

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Several studies have examined the effect of sulfonylureas on peripheral glucose utilization in type 1 as well as in type 2 diabetic subjects. In type 1diabetic subjects, the majority of the studies failed to demonstrate any effect of sulfonlyureas on glucose utilization (1–5), whereas one short-term study revealed an increased glucose utilization due to chlorpropamide as well as glipizide treatment (2).

The effect of sulfonylureas on glucose utilization in impaired glucose tolerant and type 2 diabetic subjects was examined in six studies (1,6–10). All of these studies revealed that sulfonylureas increase peripheral glucose utilization by ~29%, ranging from nonsignificant (10%) to significant (52%). This improved peripheral insulin sensitivity was associated with a mean increase of plasma insulin levels averaging 33% in these studies (18–63%). Thus, the effect of sulfonylureas to increase peripheral glucose utilization is accompanied by an augmented insulin secretion of similar magnitude. Although, this does not exclude an extrapancreatic effect of sulfonylureas to improve peripheral glucose utilization, the above-mentioned studies were not designed to specifically to determine whether sulfonylureas have an intrinsic extrapancreatic mode of action to improve peripheral insulin action. Those studies would have to exclude interfering variables, such as nonstable concentrations of insulin, glucose, and free fatty acids as well as other metabolites and hormones known to influence peripheral insulin action. Consequently, based on the existing data, the evidence of an extrapancreatic action of sulfonylureas is rather indirect and based on studies not vigorously designed to answer this question.

Glimepiride is a recently introduced sulfonylurea that has some interesting pharmacokinetic and pharmacodynamic properties (11,12). The molecular mechanism of action (13–17) as well as the clinical efficacy of glimepiride (18–25) have recently been described. One advantage of this drug is, due to its pharmacokinetic properties, that it can be taken only once daily. Furthermore, this compound is of special interest in the treatment of insulin resistance, because its

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Abbreviations: AAS, amphotericin B, penicillin, and streptomycin; FBS, fetal bovine serum; IRS, insulin receptor substrate; PI, phosphatidylinositol; SUR, insulin receptor substrate.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

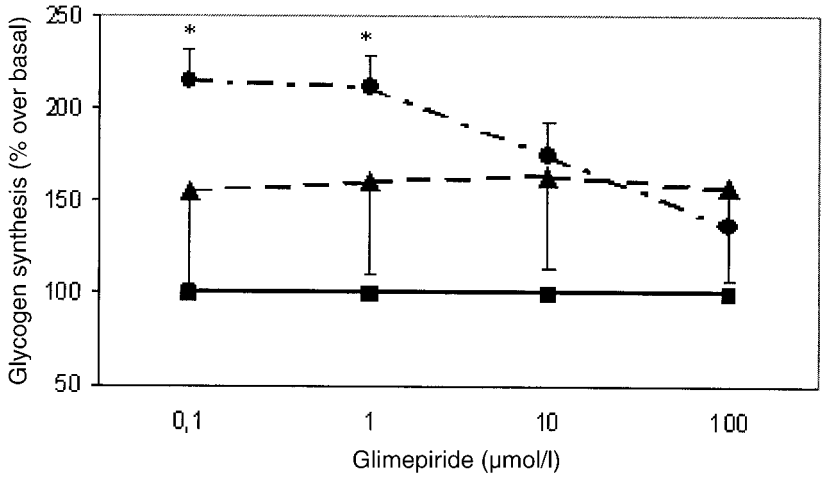

Figure 1—*Effect of glimepiride on insulin-stimulated glycogen synthesis (dose response). The effect of glimepiride on insulin-stimulated glucogen synthesis in human myotubes was determined afterincaubation (4 h) with the indicated concentrations of glimepiride.* ^{14}C *incorporation into glycogen in the basal state was set at 100%.* ■*, basal;* ▲*, insulin;* ●*, insulin/glimepiride.* $*P < 0.02$ *(n = 4).*

antihyperglycemic potency is of similar magnitude as sulfonylureas of the second generation, i.e., glibenclamide, although the insulinotropic action of glimepiride is less pronounced than that of glibenclamide, as has been demonstrated in animal models of insulin resistance (16). These data implicate that glimepiride may have an intrinsic extrapancreatic activity. We therefore performed this study, indicating evidence for an in vivo insulin-sensitizing action of glimepiride.

The aim of the current study was to further examine the molecular mechanism by which glimepiride enhances insulin action. Thus, we investigated the effect of glimepiride on insulin-stimulated glycogen synthesis in an in vitro cell system relevant to human insulin action, the cultured human skeletal muscle cell. The results suggest that glimepiride, by increasing insulin-stimulated glycogen synthesis, possess an insulin-sensitizing action in cultured human skeletal muscle cells.

RESEARCH DESIGN AND METHODS

Subjects

Skeletal muscle cells were derived from six normal weight, healthy, and nondiabetic offspring of type 2 diabetic subjects (aged 25.8 ± 3.35 years, BMI 23.78 ± 1.71 kg/m^2, 3 men and 3 women). The study was approved by the local ethical committee, and informed written consent had been obtained from all subjects before the biopsy.

Materials

Cell culture reagents were purchased from Life Technologies (Gaithersburg, MD). Human insulin (Huminsulin) was from Lilly (Giessen, Germany). D-[^{14}C] glucose (250–360 mCi per mmol/l) was supplied by NEN-DuPont (Boston, MA). Glimepiride, glibenclamide, rilmakalim, and Hoechst Marion Roussel (HMR) 1098 were a gift from Aventis Pharma (Frankfurt, Germany). All other reagents were purchased from Sigma (St. Louis, MO).

Methods

Primary cultures were grown from satellite cells obtained from percutaneus biopsies performed on the lateral portion of the vastus lateralis, as previously described (26–28). All experiments were performed on subcultured cells (first and second pass). All cells were cloned for 10 days in a 1:1 mixture of α-modified Eagle's medium (MEM) and nutrient mixture HAM's F-12 supplemented with 20% fetal bovine serum (FBS), 1% chicken embryo extract, and 0.2% AAS (amphotericin B, penicillin, and streptomycin). When myoblasts reached 80–90% confluence, the cells were fused for 4 days in α-MEM with 2% FBS and 0.2% AAS according to Henry et al. (27).

Assays for glycogen synthesis were modified from the methods recently described (26). Fused cells in six-well dishes were washed three times with HEPES-buffered saline solution (20 mmol/l HEPES, 140 mmol/l NaCl, 5 mmol/l KCl, 2.5 mmol/l $MgSO_4$, 1 mmol/l CaC_{l2}, and 0.1 BSA, ph 7.4) and then incubated in the same buffer with different concentrations of glimepiride or glibenclamide (0–100 µmol/l) for 4 h and subsequently with 100 nmol/l insulin for 2 h and D-glucose/D-[14C]glucose (5 mmol/l final con-

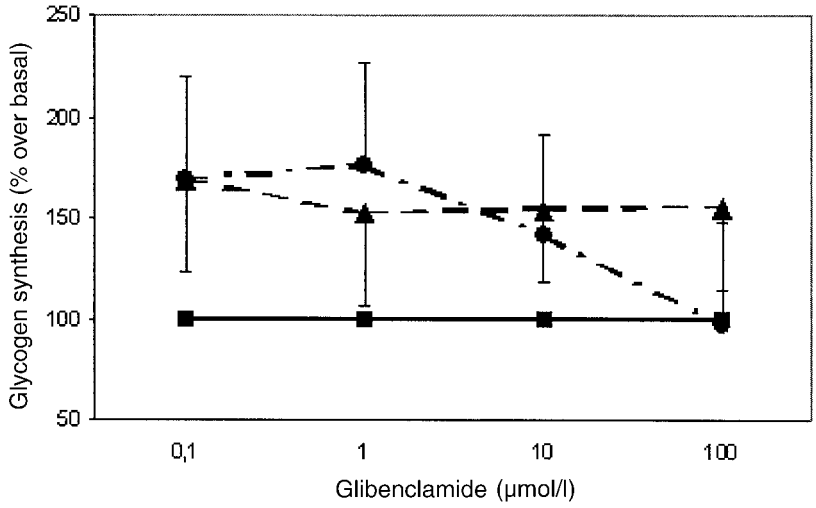

Figure 2—*Effect of glibenclamide on insulin-stimulated glycogen synthesis (dose response). The effect of glibenclamide on insulin-stimulated glycogen synthesis in human myotubes was determined after incubation (4 h) with the indicated concentrations of glibenclamide.* ^{14}C *incorporation into glycogen in the basal state was set at 100%.* ■*, basal;* ▲*, insulin;* ●*, insulin/glibenclamide.* $P > 0.05$ *at all glibenclamide concentrations (n = 4).*

centration, 0.3 μCi/well) for 1 h. During the incubation period temperature was at 37°C and CO_2 levels at 5%. The cells were then washed three times with ice-cold PBS and lysed in 30% KOH. The extracts were heated for 30 min at 95°C and cooled on ice. Glycogen was precipitated with 95% ethanol and centrifuged for 5 min at 5,000*g*. The resulting pellet was washed once. Radioactivity was determined by liquid scintillation counting.

Statistical methods

ANOVA and *t* tests were performed. The level of significance was 5%.

RESULTS

Glimepiride did not significantly alter glycogen synthesis in the basal state (data not shown). In contrast, glimepiride dose-dependently increased insulin-stimulated glycogen synthesis with a maximal effect of a 39.97 ± 8.4% (mean ± SEM, $n = 4$, $P < 0{,}02$) increase using a glimepiride concentration of 0.1 μmol/l. The time course of this glimepiride effect on insulin-stimulated glycogen synthesis showed a peak after 12 h of incubation with a half maximal effect after 4 h (data not shown).

Interestingly, as shown in Fig. 2, incubation of myotubes with glibenclamide under the same incubation conditions had no significant effect on insulin-stimulated glycogen synthesis. Using higher concentrations, both sulfonylureas show a decreased effect on insulin-stimulated glycogen synthesis, presumably due to a toxic effect (Fig. 1 and 2).

Preincubation of the myotubes with wortmannin (100 nmol/l), an inhibitor of PI3 kinase, caused an inhibition of the effect of glimepiride to increase insulin-stimulated glycogen synthesis (Fig. 3).

Incubation of myotubes with rilmakalim in a concentration of 0.1 μmol/l (a potassium-channel opener) and with HMR 1098 in a concentration of 1 μmol/l (a potassium-channel closing agent) caused no change in the effect of glimepiride on insulin-stimulated glycogen synthesis (data not shown).

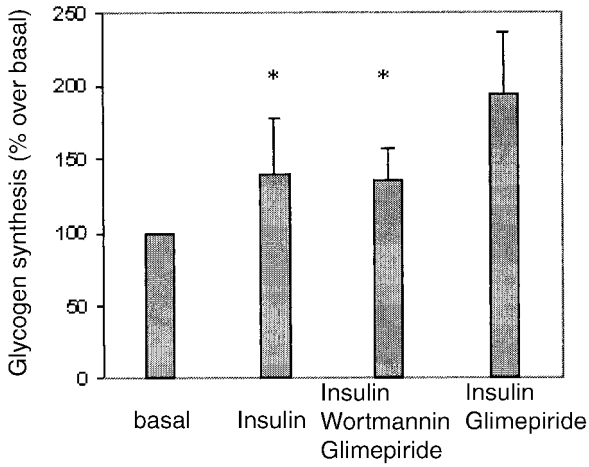

Figure 3—*Effect of wortmannin on glimepiride-stimulated glycogen synthesis. The effect of wortmannin (100 nmol/l) on insulin-stimulated glycogen synthesis in human myotubes was determined after incubation (2 h) with glimepiride (1 μmol/l). ^{14}C incorporation into glycogen in the basal state was set at 100%* (n = *4*). *P < *0.02.*

CONCLUSIONS — This study demonstrates that glimepiride increases insulin-stimulated glycogen synthesis in cultured human skeletal muscle cells. This finding is consistent with other studies demonstrating a similar effect of glimepiride on glycogen synthesis and glucose uptake in rat diaphragm and 3T3L1 adipocytes (30–32). The effect of glimepiride is insulin dependent, since we could not detect an effect of glimepiride under basal conditions.

The dose of glimepiride required to increase insulin-stimulated glycogen in vitro is in the range of the therapeutic concentration reached following intake of 3 mg glimepiride in subjects with type 2 diabetes.

Concerning the molecular mechanism by which glimepiride may stimulate insulin-induced glycogen synthesis, it has been shown that glimepiride causes tyrosine phosphorylation of insulin receptor substrate (IRS)-1 and -2 as well as association of PI3 kinase with IRS-1 and -2 to a greater extent than glibenclamide and tolbutamide, which was ineffective (30). The results of the current study, that wortmannin inhibited the effect of glimepiride to stimulate glycogen synthesis, is in good agreement with these data and further suggests that glimepiride acts upstream PI3 kinase. Thus, our finding that, in contrast to glimepiride, glibenclamide did not stimulate insulin-induced glycogen synthesis might be due to the different capability of the two sulfonylureas to activate the insulin signal transduction pathway distal to the insulin receptor, on which glimepiride has no direct effect (33), and upstream PI3 kinase.

In human myotubes the insulin receptor substrate SUR-2a is expressed (34), and sulfonylureas inhibit the ATP-dependent potassium channel. Recently it has been shown that these channels regulate the intracellular Ca concentrations via Ca/calmodulin (35), which possibly influence the insulin-signaling cascade, including glycogen synthesis. To exclude an effect via this receptor, we incubated the myotubes with glimepiride and rilmakalim (a potassium-channel opener) as well as with glimepiride and HOE 1098 (a potassium-channel closing agent). However, there was no effect of these agents on insulin-stimulated glycogen synthesis. These results suggest that the effect of glimepiride on insulin-stimulated glycogen synthesis is independent of the SUR receptor.

In summary, we have shown that incubation of human skeletal muscle cells with glimepiride increases insulin-stimulated glycogen synthesis in a dose-dependent manner. This effect seems to be mediated via the PI3 kinase pathway. In contrast, glibenclamide had no significant effect on basal or insulin-stimulated glycogen synthesis. These results suggest that glimepiride, beside its well-known effect to stimulate insulin secretion, possess an insulin-sensitizing action in cultured human skeletal muscle cells in support of the concept of an extrapancreatic action of glimepiride.

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References

1. Simonson DC: Effects of glyburide on *in vivo* insulin-mediated glucose disposal. *Am J Med* 89 (Suppl. 2A):44S–50S, 1990
2. Pontiroli AE, Alberetto M, Bertoletti A, Baio G, Pozza G: Sulfonylureas enhance *in vivo* the effectiveness of insulin in type 1 (insulin dependent) diabetes mellitus. *Horm Metabol Res* 16:167–170, 1984
3. Pernet A, Trimble ER, Kuntschen F, Assal JP, Hahn C, Renold AE: Sulfonylureas in insulin-dependent (type I) diabetes: evidence for an extrapancreatic effect in vivo. *J Clin Endocrinol Metab* 61:247–251, 1985
4. Keller U, Müller R, Berger W: Sulfonylurea therapy fails to diminish insulin resistance in type I-diabetic subjects. *Horm Metabol Res* 18:599–603, 1986
5. Leblanc H, Thote A, Chatellier G, Passa P: Effect of glipizide on glycemic control and peripheral insulin sensitivity in type 1 diabetics. *Diabete Metab* 16:93–97, 1990
6. Schulz B, Ratzmann KP, Heinke P, Besch W: A stimulatory effect of tolbutamide on the insulin-mediated glucose uptake in subjects with impaired glucose tolerance (IGT). *Exp Clin Endocrinol* 82:222–231, 1983
7. Kolterman OG, Olefsky JM: The impact of sulfonylurea treatment upon the mechanism responsible for the insulin resistance in type II diabetes. *Diabetes Care* 7 (Suppl. 1):81–88, 1984
8. Greenfield MS, Doberne L, Rosenthal M, Schulz B, Widstrom A, Reaven GM: Effect of sulfonylurea treatment on in vivo insulin secretion and action in patients with non-insulin-dependent diabetes mellitus. *Diabetes* 31:307–312, 1982
9. Kolterman OG: Longituinal evaluation of the effects of sulfonylurea therapy in subjects with type II diabetes mellitus. *Am J Med* 79:23–33, 1985
10. Ward G, Harrison LC, Proietto J, Aitken P, Nankervis A: Gliclazide therapy is associated with potentiation of postbinding insulin action in obese, non-insulin-dependent diabetic subjects. *Diabetes* 34: 241–245, 1985
11. Langtry HD, Balfour JA: Glimepiride: a review of its use in the management of type 2 diabetes mellitus. *Drugs* 55:563–584, 1998
12. Rosenkranz B, Profozic V, Metelko Z, Mrzljak V, Lange C, Malerczyk V: Pharmacokinetics and safety of glimepiride at clinically effective doses in diabetic patients with renal impairment. *Diabetologia* 39:1617–1624, 1996
13. Kramer W, Müller G, Geisen K: Characterization of the molecular mode of action of the sulfonylurea, glimepiride, at β-cells. *Horm Metab Res* 28:464–468, 1996
14. Müller G, Geisen K: Characterization of the molecular mode of action of the sulfonylurea, glimepiride, at adipocytes. *Horm Metab Res* 28:469–487, 1996
15. Ashcroft FM: Mechanism of the glycaemic effects of sulfonylureas. *Horm Metab Res* 28:456–463, 1996
16. Müller G, Satoh Y, Geisen K: Extrapancreatic effects of sulfonylureas: a comparison between glimepiride and conventional sulfonylureas. *Diabetes Res Clin Pract* 28 (Suppl. 28):S115–S137, 1995
17. Clark HE, Matthews DR: The effect of glimepiride on pancreatic beta-cell function under hyperglycaemic clamp and hyperinsulinaemic, euglycaemic clamp conditions in non-insulin-dependent diabetes mellitus. *Horm Metab Res* 28:445–450, 1996
18. Rosenstock J, Samols E, Muchmore DB, Schneider J: Glimepiride, a new once-daily sulfonylurea. *Diabetes Care* 19: 1194–1199, 1996
19. Dills DG, Schneider J: Clinical Evaluation of glimepiride versus glyburide in NIDDM in a double-blind comparative study. *Horm Metab Res* 28:426–429, 1996
20. Draeger KE, Wernicke-Panten K, Lomp HJ, Schüler E, Robkamp R: Long-term treatment of type 2 diabetic patients with the new oral antidiabetic agent glimepiride (Amaryl): a double-blind comparison with glibenclamide. *Horm Metab Res* 28:419–425, 1996
21. Riddle MC: Combined therapy with a sulfonylurea plus evening insulin: safe, reliable, and becoming routine. *Horm Metab Res* 28:430–433, 1996
22. Goldberg RB, Holvey SM, Schneider J: A dose-response study of glimepiride in patients with NIDDM who have previously received sulfonylurea agents: the Glimepiride Protocol #201 Study Group. *Diabetes Care* 19:849–856, 1996
23. Robkamp R, Wernicke-Panten K, Draeger E: Clinical profile of the novel sulphonylurea glimepiride. *Diabetes Res Clin Pract* 31 (Suppl. 31):S33–S42, 1996
24. Campbell RK: Glimepiride: role of a new sulfonylurea in the treatment of type 2 diabetes mellitus. *Ann Pharmacother* 32: 1044–1052, 1998
25. Massi-Benedetti M, Herz M, Pfeiffer C: The effects of acute exercise on metabolic control in type II diabetic patients treated with glimepiride or glibenclamide. *Horm Metab Res* 28:451–455, 1996
26. Krützfeld J, Kausch C, Volk A, Klein HH, Rett K, Häring HU, Stumvoll M: Insulin signaling and action in cultured skeletal muscle cells from lean healthy humans with high and low insulin sensitivity. *Diabetes* 49:992–998, 2000
27. Henry RR, Abrams L, Nikoulina S, Ciaraldi TP: Insulin action and glucose metabolism in nondiabetic control and NIDDM subjects: comparison using human skeletal muscle cell cultures. *Diabetes* 44:936–946, 1995
28. Marynissen G, Smets G, Klöppel G: Internalisation of glimepiride and glibenclamid in the pancreatic B-cell. *Acta Diabetol* 29:113–114, 1992
29. Kawamori R, Morihima T, Kubota M, Ineda K, Kamada T: Influence of oral sulfonyklurea agents on hepatic glucose uptake. *Diabetes Res Clin Pract* 28 (Suppl.1):109–113, 1995
30. Müller G: The molecular mechanism of the insulin-mimetic/sensitizing activity of the antidiabetic sulfonylurea drug Amaryl. *Mol Med* 6:907–933, 2000
31. Müller G, Wied S: The sulfonylurea drug, glimepiride, stimulates glucose transport, glucose transporter translocation, and dephosphorylation in insulin resistent rat adipocytes in vitro. *Diabetes* 42:1852–1876, 1993
32. Müller G, Geisen K: Characterisation of the molecular mode of action of the sulfonylurea, glimepiride, at adipocytes. *Horm Metab Res* 28:469–487, 1996
33. Takada Y, Takata Y, Iwanishi M, Imamura T, Sawa T, Morioka H, Ishihara H, Ishiki M, Usui I, Temaru R, Urakaze M, Satoh Y, Inami T, Tsuda S, Kobayashi M: Effect of glimepiride (HOE 490) on insulin receptors of skeletal muscle from genetically diabetic KK-Ay mouse. *Eur J Pharmacol* 308: 205–210, 1996
34. Reimann F, Gribble FM, Ashcroft FM: Differential response of K(ATP) channels containing SUR2A or SURB subunits to nucleotides and pinacidil. *Mol Pharmacol* 58:1318–1325, 2000
35. Olbrich HG, Mueller M, Lindner S, Henke B, Zarse M, Riehle M, Oremek G, Mutschler E: Glimpepiride inhibits the rilmakalim induced decrease in intracellular free calcium and contraction of isolated heart muscle cells from guinea pigs to a lesser extent than glibenclamide. *Int J Cardiol* 72:53–63, 1999