Studies on the mechanism of action of sulphonylureas in type II diabetic subjects: glicludone

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ABSTRACT. The mechanism of action of sulphonylureas is not completely understood. In the present study we evaluated the effects of glicludone, a second-generation compound, on several metabolic parameters in 22 patients with untreated newly-diagnosed type II (noninsulin-dependent) diabetes mellitus. After either 1 or 6 months of treatment with glicludone plus isocaloric diet we observed: 1) a significant decrease in fasting plasma glucose and glycemic profile after oral glucose load; 2) unchanged fasting and postglucose plasma insulin levels; 3) no change in fasting C-peptide levels but a significant increase in C-peptide concentrations after glucose challenge; 4) a significant increase in glucose disappearance rate from plasma following iv insulin injection; 5) an increase in the insulin-induced reduction of plasma levels of free-fatty acids; 6) no change in plasma C-peptide levels following iv insulin injection; 7) a significant increase in specific insulin binding to monocytes. After 6 but not 1 month of glicludone therapy we also found an increase in the activity of hexokinase in circulating mononuclear leukocytes. These results suggest that the hypoglycemic effect of glicludone occurs through either an increased beta cell response to glucose stimulus or an enhanced insulin sensitivity. The latter effect seems to depend on both receptor and postreceptor mechanisms.

INTRODUCTION
Sulphonylureas have been extensively used in the last three decades in the treatment of type II diabetes mellitus (1). Their precise mechanism of action is still controversial, but the majority of authors agree that these drugs enhance B cell secretion in response to glucose (2-7). Indeed, also in the absence of increased plasma insulin levels, the effect of sulphonylurea treatment on pancreatic B cell can be appreciated by the evaluation of plasma levels of C-peptide (8). The increased effect of glucose stimulus on insulin secretion is easily appreciated after a short term period of treatment with sulphonylureas (3-7). On the contrary, several studies which evaluated the long term effect of these drugs did not report increased plasma insulin (9-15). These studies supported the concept that sulphonylureas also possess extrapancreatic effects. Later on, these effects were actually appreciated in vitro and seem to occur at either receptor or postreceptor level, including an increased activity of intracellular enzymes (16-24). Studies performed in vitro indicated that sulphonylureas can affect not only glucose metabolism but free fatty acids (FFA) metabolism too (25, 26). However, this effect, which would occur through an extrapancreatic mechanism and would be related to a potentiation of cellular effect of insulin, was not extensively evaluated in vivo.

If sulphonylureas possess an effect of improvement of insulin action, this might involve other biological effects of the hormone. For example, sulphonylureas might increase the insulin effect of inhibition of pancreatic B cell (27, 28). If present, this effect could in part explain the lower than expected plasma insulin levels in some patients chronically treated with sulphonylureas.

Aim of the present study was to evaluate the short and long term effect of glicludone, a second-generation sulphonylurea, on 1) basal and glucose-stimulated B cell secretion in vivo; 2) insulin-mediated glucose disposal in vivo; 3) basal and insulin-suppressed plasma FFA levels; 4) insulin-inhibited B cell secretion in vivo; 5) insulin binding to receptors in vitro; 6) activity of some intracellular enzymes of glucose metabolism in vitro.

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MATERIALS AND METHODS

Subjects
Twenty-two patients (17 males, 5 females) with untreated newly-diagnosed type II diabetes mellitus were studied after informed consent was obtained. They were aged 49 ± 9 yr (mean ± SD) and had a body mass index (BMI) of 31.4 ± 7.4 kg/m². Fourteen patients were obese (BMI ≥ 27 kg/m²). All subjects had normal hepatic and renal function and no concomitant disease. The diagnosis of type II diabetes was made on the basis of fasting plasma glucose levels repeatedly > 140 mg/dl (7.8 mM) in the absence of ketosis (29).

Study protocol
At study entry, patients were placed on a weight-maintaining diet (50% carbohydrate, 30% lipid, 20% protein) that was followed for the entire study and did not result in significant changes in body weight (BMI 31.1 ± 6.9 kg/m² after 1 month, and 31.6 ± 7.2 kg/m² after 6 months of treatment, respectively). After 2-3 weeks, on three not consecutive days, patients underwent an oral glucose tolerance test, an insulin tolerance test and a withdrawal of mononuclear leucocytes to study insulin binding to receptors and intracellular enzymes of glucose metabolism. The tests were repeated 1 and 6 months after the patients had taken 30 to 120 mg/day of gliquidone (Glurenor®, Guidotti, Pisa, Italy) in 2 doses, at breakfast and dinner.

Oral glucose tolerance test (OGTT)
A 75 g oral glucose tolerance test was carried out with standard technique at 08:00 am after an overnight fast. Blood was drawn at times 0, 30, 60, 90, 120, 180, 240 min for the measurement of plasma glucose (gluco-oxidase method, BMI, Milan, Italy), insulin (30), and C-peptide (31).

Insulin tolerance test (ITT)
A 0.1 U/kg body weight insulin tolerance test was carried out at 08:30 am after an overnight fast according to the procedure previously described (32). Blood was drawn at times 0, 3, 6, 9, 12, 15, 20, 30, 40, 50, 60 min for the measurement of plasma glucose (gluco-oxidase method, BMI, Milan, Italy), insulin (30), and C-peptide (31).

Mononuclear leukocytes for insulin binding and enzyme activities
Three-hundred ml of venous blood was drawn at 08:00-08:30 h after an overnight fast and used to achieve a suspension of leukocytes according to Boyum (33). Plasma and red blood cells were re-infused into the patient. Approximately 1/3 of mononuclear leukocytes were used, as previously described (34), for measuring the maximum specific insulin binding to 10⁷ monocytes. The remaining cells were used for enzyme studies. For this purpose, the cells were suspended in an extraction solution (30 mM potassium phosphate, 10 mM dextrose, 0.3 M sucrose, 5 mM 2-mercaptoethanol, pH 7.4), sonicated and centrifuged. On the supernatant (cytosol), the following enzymatic activities were measured by a spectrophotometer as previously described (35): hexokinase, 6-phosphofructokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase. The enzyme activities were expressed as nmol/min/mg protein. Protein content of cellular extracts were assayed according to Lowry (36).

Calculations and statistics
Area under the plasma glucose curve, and insulin and C-peptide incremental areas after oral glucose load were calculated by a geometric method (37). As previously described, glucose disappearance rate from plasma in the period 3-15 min after iv insulin injection was calculated and used as an index of in vivo insulin action on glucose metabolism (32). The period 3-15 min was chosen because a) 3 minutes following iv insulin injection all insulin is distributed even in its remote compartment and begin to exert its metabolic effect (38) and b) within this period of time no counterregulatory response does occur (32). Maximum percent decreases under basal of plasma FFA and C-peptide after iv insulin administration were calculated and used as indexes of insulin effect on FFA metabolism and insulin-inhibited B cell secretion, respectively. Student’s t test for paired data and analysis of variance (39) were used for statistical purposes.

RESULTS
Figure 1 reports plasma glucose, insulin and C-peptide levels before and after treatment with gliquidone. Before treatment, fasting plasma glucose levels averaged 9.3 ± 0.7 mM (mean ± SE). After 1 month of treatment there was a significant reduction of fasting plasma glucose (8.1 ± 0.6 mM, p < 0.01), glycemic profile after oral glucose load (ANOVA, p < 0.01), and area under the plasma glucose curve (11.7 ± 0.8 vs 13.7 ± 1.2 mM/min, p < 0.05). The same was noticed after 6 months of treatment, without any substantial difference in plasma glucose levels at fasting and following oral