Dose-Dependent Effects of Glyburide on Insulin Secretion and Glucose Uptake in Humans

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Objective: To examine the relationship between plasma glyburide concentrations (0, 50, 100, 200, 400, and 800 nM) and the insulin response and glucose metabolism during euglycemic (4.6 ± 0.1 mM) and hyperglycemic (11.6 ± 0.2 mM) conditions. Research Design and Methods: Nine healthy subjects participated in the study. Steady-state plasma glyburide concentrations were achieved by primed continuous intravenous infusion of glyburide. Results: During both euglycemia and hyperglycemia, glyburide enhanced insulin secretion and glucose disposal only to drug levels of 100–200 nM corresponding to an oral dose ≤10 mg. Conclusions: The data suggest that glyburide (and probably other sulfonylureas) operates within a narrow range of plasma concentrations (50–200 nM), which can be achieved with very low doses of the drug. It remains to be shown whether the threshold of maximal effect also in clinical practice is achieved with lower sulfonylurea doses than that currently used. *Diabetes Care* 14:724–27, 1991

The insulinotropic effect of sulfonylurea agents and their ability to enhance the stimulatory effect of glucose and other insulin secretagogues have been demonstrated both in vitro (1–4) and in vivo (5–13). However, little is known about the relationship between the sulfonylurea dose and its stimulatory effect on insulin secretion in vivo. There is even less information on the dose-response relationship between sulfonylurea and its clinical response, i.e., the blood glucose–lowering effect. If the treatment goal is not achieved, the doses are usually increased to 30 mg/day (glyburide) or 40 mg/day (glipizide) in the United States, whereas the maximum doses in Europe are somewhat lower (14 and 20 mg, respectively). However, this practice has little scientific support. Single-dose and short-term studies with glipizide have shown an increased blood glucose–lowering effect up to 10 mg (14,15). A long-term comparison of glyburide and glipizide showed little or no improvement in blood glucose control at doses >10 mg/day (11). Indeed, dose increase of glipizide from 15 to 25 mg/day resulted in increased rather than decreased blood glucose levels (14).

These findings infer that there may be a narrow range of plasma concentrations below the level at which sulfonylureas are ineffective and above the level at which there is little additional effect. Estimates from single- and multiple-dose studies infer that this concentration may be ~50–100 nM (10–12,14–16). This study was designed to test this hypothesis in an experimental setting by examining the relationship between the steady-state level of glyburide, attained by primed continuous intravenous infusion of the drug, and insulin release and glucose disposal during euglycemia and hyperglycemia in healthy volunteers.

RESEARCH DESIGN AND METHODS

Nine healthy volunteers (6 women, 3 men) participated in the study. They had a mean ± SD age of 26 ± 2 yr

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and their weight was 102 ± 16% of ideal. None of the subjects had a family history of diabetes or was taking any medication. Informed written consent was obtained from each subject, and the study protocol was approved by the Human Investigation Committee of the Yale University School of Medicine.

The subjects participated in six studies performed in random order at 1- to 2-wk intervals. All studies were performed at 0800 after the subjects had fasted overnight for 10–12 h. Catheters were inserted into an antecubital vein for infusion of test substances and were retrogradely inserted into a wrist vein for blood sampling. The hand was placed in a heated box (70°C) to ensure arterialization of venous blood. In the first study, the subjects received a continuous infusion of saline from 0 to 220 min. A +7 mM hyperglycemic clamp was performed during the 120- to 220-min period. The study thus consisted of a euglycemic (0–120 min) and a hyperglycemic (120–220 min) period. During the other five studies, a primed continuous infusion of glyburide (Boehringer Mannheim, Mannheim, Germany) was administered for 220 min to achieve steady-state plasma glyburide levels of 50, 100, 200, 400, and 800 nM. To achieve the glyburide concentration of 200 nM, the subjects received a bolus of 30 μmol/kg body wt glyburide followed by a continuous infusion of 170 nmol · kg^{-1} · min^{-1}. During the other protocols, the prime (8, 16, 60, and 120 μmol/kg body wt) and continuous infusion (40, 80, 340, and 680 nmol · kg^{-1} · min^{-1}) were adjusted accordingly.

Three basal samples for determination of plasma glucose and insulin were obtained before starting the glyburide-saline infusion; thereafter, glucose was measured at 5-min intervals, insulin at 10-min intervals, and glyburide at 15-min intervals throughout the study. The coefficient of variation of plasma glucose concentration during the euglycemic and hyperglycemic periods was <5% in all experiments.

The mean plasma insulin concentration was averaged from values measured during the euglycemic (0–120 min) and hyperglycemic (120–220 min) periods, whereas the mean plasma glyburide concentration was calculated from the entire study period (0–220 min). The glucose infusion rate during the euglycemic and hyperglycemic period was determined by calculating the mean value observed during the 20- to 120-min and 140- to 220-min periods. The glucose infusion rate was corrected for urinary glucose losses during the hyperglycemic period.

Plasma glucose concentration was determined by a glucose oxidase method on a Beckman Glucose Analyzer II (Fullerton, CA). Plasma insulin was determined with a double-antibody radioimmunoassay (17). Plasma glyburide concentration was determined by liquid chromatography (18).

All data are means ± SE. Significance of difference from the previous drug concentration on the dose-response curve was tested with analysis of variance and corrected for multiple comparisons with a Biomedical data processing computer program (Los Angeles, CA).

RESULTS

The mean plasma glucose concentrations during the euglycemic and hyperglycemic periods were 4.6 ± 0.1 and 11.6 ± 0.2 mM. During the euglycemic period, increasing the plasma glyburide concentration to 108 ± 14 nM resulted in a dose-dependent increase in the plasma insulin concentration (35 ± 7, 71 ± 14, and 135 ± 35 pM; $P < 0.001$). Thereafter, the dose-response curve flattened, and the increase from 108 ± 14 to 178 ± 20 nM in the plasma glyburide concentration was associated with only a small increase in the plasma insulin concentration (to 156 ± 28 pM; NS).

During the hyperglycemic period, there was a significant increase in the plasma insulin response to a plasma glyburide concentration of ~100 nM ($P < 0.001$). Further increase of the glyburide concentration was not accompanied by any further increase in plasma insulin concentration (Fig. 1). During both the euglycemic and hyperglycemic periods, the rate of glucose uptake was

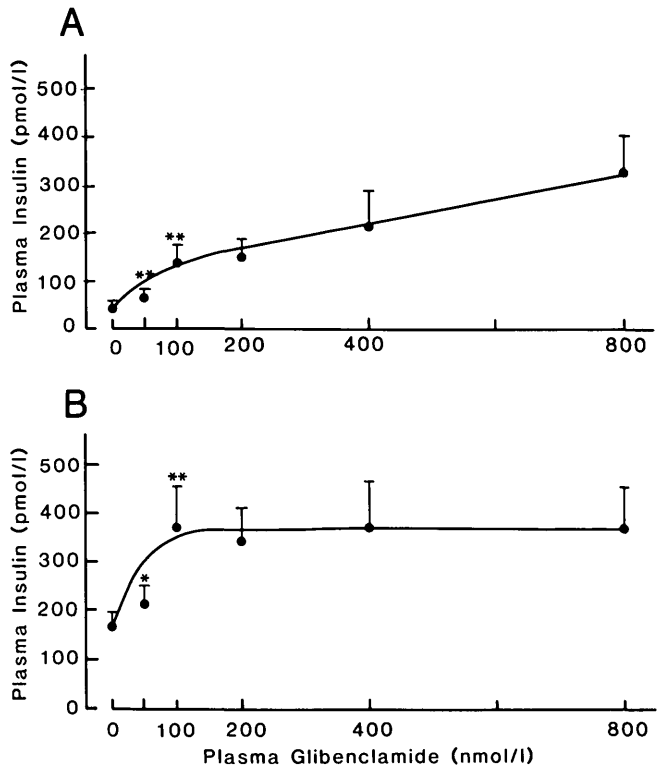

FIG. 1. Dose-response curves relating plasma insulin response during euglycemic (*A*) and hyperglycemic (*B*) periods to plasma glibenclamide (glyburide) concentration in 9 healthy subjects. Values are means ± SE. *$P < 0.05$, **$P < 0.01$, compared with previous level.

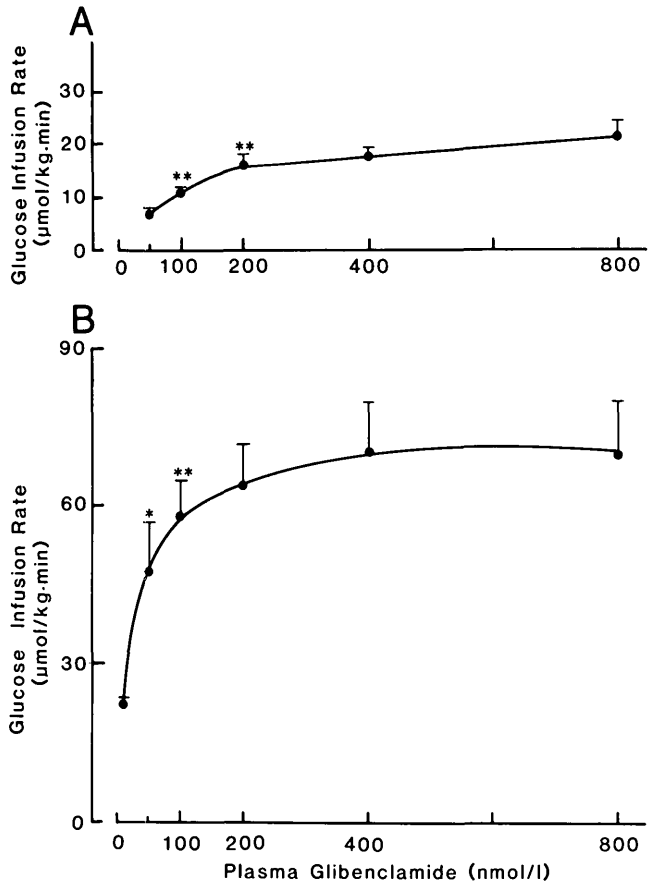

FIG. 2. Dose-response curves relating glucose infusion rate during euglycemic (*A*) and hyperglycemic (*B*) periods to plasma glibenclamide (glyburide) concentration in 9 healthy subjects. Values are means ± SE. *$P < 0.05$, **$P < 0.01$, compared with previous level.

enhanced after an increase in the plasma glyburide level but only to a drug concentration of ~200 nM (Fig. 2).

CONCLUSIONS

Despite the fact that the established clinical practice of the use of sulfonylurea agents assumes a dose-response relationship between the drug and its effect, this has never been proved. This study provides novel information on the dose-response relationship between plasma glyburide and its effect on insulin secretion and glucose disposal in healthy individuals. Only one previous study has examined the effect of two different glyburide concentrations on the insulin response to intravenous glucose (20). The plasma glucose was allowed to fall during the study, which makes interpretation of the results difficult. However, doubling of the infused glyburide dose was associated with only a 20% increase in the plasma insulin response. The dose-response curve of this study, including five different concentrations of glyburide in addition to a control study without sulfonylurea, indicates that increasing the plasma glyburide concentration to >100 nM has little (euglycemia) or no (hyperglycemia) effect on insulin secretion and glucose disposal. Accordingly, it seems likely that glyburide (and other sulfonylureas) operates within a narrow range of plasma concentrations. Because there is evidence to suggest that sulfonylureas stimulate insulin secretion via receptorlike mechanisms in the β-cell (21), it is possible that saturation of these receptorlike structures is already achieved at rather low plasma concentrations of glyburide.

Under hyperglycemic conditions, plasma glyburide levels >200 nM did not evoke any additional stimulation of insulin secretion or glucose disposal rate. In clinical practice, however, a drug level of 200 nM is usually far exceeded in many non-insulin-dependent diabetes mellitus (NIDDM) patients who do not become euglycemic on low doses of glyburide. Assuming a distribution volume of 10 L and a plasma protein binding of 95–99% (14), the maximum effective concentration (200 nM) of plasma glyburide would correspond to a maximum effective oral dose <5 mg. Even though the bioavailability of the glyburide formulation used in the U.S. is incomplete (21), it is probably >50%, signifying that the maximum effective oral dose of glyburide would be <10 mg. It is possible that the maximum dose would be higher in NIDDM patients than in healthy volunteers, but note that there are no controlled studies in diabetic subjects that show an increased effect of glyburide or glipizide in doses >10–15 mg.

Under euglycemic conditions, the plasma glyburide concentration had to be raised eightfold (from 100 to 800 nM) to achieve the same insulin response that was achieved by 100 nM of glyburide under hyperglycemic conditions. In other words, the plasma glucose concentration during hyperglycemia enhances the stimulatory effect of sulfonylurea on insulin secretion. In healthy subjects, maximum stimulation of insulin secretion by glucose and arginine may promote insulin levels of 2800–3500 pM (22). These data indicate that the maximum insulinotropic effect of sulfonylurea plus glucose saturates at much lower insulin levels, ~350–430 pM. This represents an experimental study in healthy subjects and may be difficult to generalize to chronic treatment in NIDDM subjects. However, the data demonstrate that in healthy subjects the maximum acute effect of sulfonylurea is achieved at lower doses than we previously thought.

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