

β -Cell Insulin Secretory Response to Oral Hypoglycemic Agents Is Blunted in Humans *in Vivo* during Moderate Hypoglycemia

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Oral hypoglycemic agents bind to the ATP-sensitive potassium channel and lower glucose levels effectively in individuals with diabetes. Although the principle mechanism of action can also promote hypoglycemia, clinically profound hypoglycemia is rare. Decreased stimulation of insulin secretion by these agents at mild hypoglycemia could provide protection from more profound hypoglycemia. Sulfonylureas and meglitinides bind to both shared and unique sites on the ATP-sensitive potassium receptor/channel complex but have different pharmacokinetic profiles. To evaluate the differential ability of both sulfonylureas and meglitinides to stimulate insulin release at modest hypoglycemia, we evaluated dextrose infusion rates necessary to maintain plasma glucose after oral administration of repaglinide (1 mg) or glipizide (5 mg) at euglycemia and again at modest hypoglycemia. Healthy subjects with no family history of diabetes underwent four clamp studies, two performed while maintaining isoglycemia (glucose levels at the fasted value) and two while maintaining modest hypoglycemia of 2.78 mmol/liter (50 mg/dl) induced by

low-dose insulin infusion (3.6 pmol/kg·min). There was a marked decrease in the dextrose infusion rate with administration of either repaglinide or glipizide at hypoglycemia compared with drug administration at euglycemia ($P \leq 0.006$). This was accompanied by a reduction in C peptide secretion ($P \leq 0.001$). Although each drug demonstrated a unique pharmacokinetic profile, drug absorption was comparable at euglycemia and hypoglycemia. The mechanism accounting for the reduced dextrose requirement during hypoglycemia likely involves a markedly decreased insulin secretory response to the agents during hypoglycemia and suggests that at modest hypoglycemia, low glucose or other metabolite(s) or altered counterregulatory hormone levels are sufficient to inhibit insulin release in response to potent insulin secretagogues. These findings may help to explain the relatively low incidence of severe hypoglycemia with clinical administration of these drugs. (*J Clin Endocrinol Metab* 89: 4553–4557, 2004)

TYPE 2 DIABETES IS a progressive metabolic disorder, characterized by both insulin resistance in target tissues, mainly liver, muscle, and fat, and a defect in β -cell function. These different pathophysiological lesions are the primary targets of pharmacological intervention. The major goal of therapy is directed at optimizing glycemic control. In patients with diabetes, insulin secretion after a meal is both delayed and reduced (1). The sulfonylureas, which act by increasing insulin secretion, were the first class of oral therapy for type 2 diabetes, and insulin secretagogues remain among the most common agents prescribed to the patient with diabetes for use either alone or in combination with an insulin-sensitizing drug or with insulin itself. The use of insulin secretagogues to improve glycemic control in patients with type 2 diabetes increases the incidence of hypoglycemia; indeed, the hypoglycemic action of sulfonylureas serendipitously noted in 1942 (2) led to the development of this class of agents.

There now are three main classes of insulin secretagogues including the sulfonylureas, which are related to sulfon-

amide drugs and contain a sulfonylurea moiety with different attached side chains; the meglitinides, derived from benzoic acid; and the phenylalanine derivatives. Benzoic acid and phenylalanine derivatives are chemically unrelated to the sulfonylurea agents. Sulfonylureas and meglitinides induce insulin secretion by blocking the ATP-sensitive K^+ (K_{ATP}) channels on the β -cell via binding to the so-called sulfonylurea receptor (SUR), which is a subunit of the channel. Classical K_{ATP} channels are comprised of two subunits, the Kir6.2 subunit, which forms the K^+ -selective ion pore, and SUR (SUR1 and SUR2) subunits, where both classes of insulin secretagogues bind to promote closure of the channel. Closure of the K_{ATP} channel leads to depolarization of the plasma membrane, resulting in opening of voltage-dependent Ca^{2+} channels and Ca^{2+} influx. Ca^{2+} then binds to calmodulin, leading to the activation of insulin exocytosis in a manner similar to that seen after stimulation by glucose (3). Sulfonylureas and meglitinides regulate the K_{ATP} channel at more than one site, which include sites that are shared between the drugs and those that are unique to the specific drug (4, 5).

Although hypoglycemia is the major adverse event associated with the use of either drug class, and symptomatic low glucose levels are common, it is somewhat surprising that the incidence of major hypoglycemic events is infrequent and occur in only 0.5–1% of treated patients (6). To determine the

Abbreviations: AUC, Area under the curve; K_{ATP} , ATP-sensitive K^+ ; NS, not significant; SUR, sulfonylurea receptor.

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effects of these agents on insulin secretion *in vivo* in humans at a modest level of hypoglycemia where additional insulin secretion would be undesirable, we compared the glucose infusion rates induced by repaglinide or glipizide under euglycemic and hypoglycemic conditions.

Subjects and Methods

Subjects

The study was approved by the Institutional Review Board of the Joslin Diabetes Center (Boston, MA), and the investigations were carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all individuals before participation. Five subjects with no family history of diabetes completed participation in the study and are included in the analysis. One subject relocated after completing only the isoglycemic clamps and has not been included in any analysis. All subjects were healthy as judged by medical history, were normotensive as determined by office blood pressure, and were nonsmokers. None of the subjects were on any prescribed or over-the-counter medications on a regular basis.

Study design

The study was conducted in a double-blind, crossover design involving four interventional visits. Each subject underwent two isoglycemic and two hypoglycemic clamp studies. During each pair of studies, subjects were randomly given either 1 mg repaglinide (Novo Nordisk, Bagsvaerd, Denmark) or 5 mg glipizide (Mylan Pharmaceuticals, Greensboro, NC) orally, administered with one cup of diet caffeine-free Coca Cola.

Isoglycemic clamp studies

Subjects were studied at the Joslin Diabetes Center Clinical Research Center after an overnight fast. Intravenous lines were placed for the infusion of test substances and collection of blood samples. The hand bearing the blood sampling catheter was placed into a box heated to 70°C to ensure arterialization of venous blood (7). Catheters were kept patent by a slow infusion of isotonic saline. Basal glucose levels were determined by the average of three readings over 15 min before the administration of the study drug (either repaglinide or glipizide). To avoid clamping at a glucose level above basal, thereby inducing insulin secretion, or at a level below basal, which could cause relative hypoglycemia within an individual, blood glucose was maintained at the basal level, termed isoglycemia, by evaluation of plasma glucose at 5-min intervals and adjusting a variable rate 20% glucose infusion as previously described (8). Samples for hormone and substrate levels including glucose, insulin, C peptide, and drug levels were obtained at intervals throughout the clamp study. Subjects returned 1 month after the first isoglycemic clamp for an identical procedure, receiving either repaglinide or glipizide, whichever was not administered first.

Hypoglycemic clamp studies

Two months after the second isoglycemic clamp, subjects were readmitted after an overnight fast for the first of paired randomized hypoglycemic studies. Intravenous catheters were inserted and maintained as described above. After the collection of baseline blood samples for hormones and substrates, a primed (1.4 pmol/kg·min for 5 min followed by 0.72 pmol/kg·min for 5 min) continuous infusion of insulin aspart (Novolog 0.36 pmol/kg·min) (Novo Nordisk) was administered. Blood glucose was evaluated at 5-min intervals throughout the insulin infusion and allowed to fall to 2.78 mmol/liter at which time a variable rate 20% glucose infusion was initiated and adjusted to maintain the modestly hypoglycemic target. After 60 min of stable blood glucose at 2.78 mmol/liter, the study drug (repaglinide or glipizide) was administered orally with 8 oz of diet, caffeine-free Coca Cola. Blood glucose was maintained at the modestly hypoglycemic target for an additional 4 h after study drug administration, but drug levels were followed for an additional hour in case of delayed absorption. Because subjects were maintained at hypoglycemia for 1 h before administration of repaglinide or glipizide, steady-state levels of glucose infusion necessary to prevent

further decrease in plasma glucose were calculated. Glucose infusion rates above this level were calculated and reported as the amount necessary to maintain the target glucose in the presence of the test drug. Hypoglycemic clamp procedures were repeated 1 month later with subjects receiving either repaglinide or glipizide, whichever was not administered first.

Analytic methods

Plasma glucose was determined using a YSI Y2300 glucose analyzer (YSI Inc., Yellow Springs, OH). Insulin and C peptide levels were measured by RIA (Diagnostic Systems Laboratories, Webster, TX). Area under the curve (AUC) for glucose infusion, insulin, and C peptide were calculated by the triangulation method (9).

Drug level assays were performed at Novo Nordisk. In brief, serum samples were assessed for repaglinide or glipizide by high-turbulence liquid chromatography tandem mass spectrometry using online solid phase extraction (turbochromatography), reversed phase chromatography, and positive multiple reaction monitoring mode tandem mass spectrometry [mass to charge ratio for glipizide, 446.0 (*r*) 321.3, and repaglinide (internal standard), 453.8 (*r*) 229.8, respectively]. A calibration graph was generated based on blank serum samples spiked with the respective drug in the concentration range 1.00–100 ng/ml using weighted linear least squares regression (1/*x*), and the concentration of repaglinide or glipizide in the subject sample was calculated using the peak area ratio of analyte to internal standard. The method performance was performed with coassaying quality control samples in duplicate at three concentration levels (3.0, 10.0, and 80.0 ng/ml). Results of at least one quality control sample at each concentration level and at least four of six fell within 85–115% of their nominal concentration. The analysis was carried out on a PE Sciex API 3000 mass spectrometer using a turbolonspray interface (PerkinElmer Life and Analytical Sciences, Boston, MA). The high-turbulence liquid chromatography system consisted of an HP 1100 series isocratic pump, an HP 1100 series binary pump, a valve interface module (VIM), and a CTC PAL autosampler (Hewlett Packard, Palo Alto, CA).

Statistical analysis

Subject characteristics are expressed as mean \pm SD, and data results are expressed as means \pm SE. Statistical analysis was performed for paired data using two-tailed Student's *t* tests with *P* values < 0.05 considered significant. Statistical analyses were carried out with the StatView program (StatView; SAS Institute Inc., Cary, NC).

Results

Five subjects including two males and three females completed the study. Baseline characteristics included two men and three women, age 27.6 ± 4.2 yr, weight 64.2 ± 11.8 kg, body mass index 22.9 ± 3.3 kg/m², glycosylated hemoglobin $5.0 \pm 0.2\%$, fasting glucose 4.6 ± 0.34 mmol/liter, and fasting insulin 18.0 ± 6.0 pmol/liter.

Isoglycemic studies

Isoglycemic clamps maintained glucose levels throughout the study $3.0 \pm 0.7\%$ below the mean fasting value, with isoglycemic clamps for repaglinide maintained $1.6 \pm 0.9\%$ and glipizide $4.7 \pm 0.8\%$ below the fasting value (between-groups comparison with baseline, *P* = NS) (Fig. 1).

Dosing of the two insulin secretagogues was selected based on doses predicted to cause similar early insulin response (15–30 min) in healthy volunteers (10) and to be a proportional 25% of the maximum Food and Drug Administration-approved dosing in patients with type 2 diabetes. As expected, at euglycemia, the peak level of repaglinide was earlier, and in a narrower interval (40–60 min) than for glipizide (90–190 min). The drug AUC achieved by 1 mg repaglinide was significantly

lower than with 5 mg glipizide ($1,222 \pm 170$ vs. $41,678 \pm 4,623$ ng/ml·min, repaglinide vs. glipizide, $P = 0.0004$). These data demonstrate on a milligram basis, a nearly 40-fold difference in drug AUC for a 5-fold higher dose of glipizide (Fig. 2).

Likewise, repaglinide was associated with significantly lower insulin secretion when compared with glipizide as assessed by endogenous insulin (AUC insulin $15,390 \pm 2,124$ vs. $27,180 \pm 3,906$ pmol/liter·min, $P = 0.05$, repaglinide vs. glipizide) with a similar trend for C peptide (AUC C peptide 699 ± 80 vs. 904 ± 104 ng/ml·min, $P = 0.09$, repaglinide vs. glipizide) (Fig. 3). Glucose infusion rates required for maintaining euglycemia were lower with repaglinide in a magnitude similar to the insulin levels (total clamp dextrose infusate 410 ± 95 vs. 815 ± 21 mg dextrose/kg) (Fig. 4). Taken together, insulin and C peptide production, glucose use to maintain isoglycemia in response to the oral agents, and drug levels indicate that 1 mg repaglinide has a lower bioequivalence than 5 mg glipizide. However, the much greater drug AUC for glipizide induced a much less dramatic (about 2-fold) increase in insulin secretion. Thus, on a milligram per milligram basis, repaglinide is relatively more potent than glipizide.

Hypoglycemic studies

Both repaglinide and glipizide were administered at similar levels of hypoglycemia (2.90 ± 0.12 vs. 3.00 ± 0.07 mmol/liter repaglinide vs. glipizide, $P = \text{NS}$). Hypoglycemic clamps maintained glucose levels throughout the study close to the targeted level (2.85 ± 0.03 vs. 2.87 ± 0.04 mmol/liter, repaglinide vs. glipizide, $P = \text{NS}$) (Fig. 1). In addition, exogenous insulin levels achieved to maintain hypoglycemia were similar between the two study days (210 ± 42 vs. 204 ± 30 pmol/liter, $P = \text{NS}$, repaglinide vs. glipizide).

There was a dramatic reduction in the glucose infusion necessary to maintain the target glucose of 2.78 mmol/liter for each agent (total clamp dextrose infusate 47.5 ± 27.5 and 138.2 ± 21.7 mg dextrose/kg, repaglinide and glipizide, compared with that at euglycemia, $P < 0.006$ for both) (Fig. 4). C peptide levels remained below baseline levels throughout insulin infusion with both drugs (Fig. 3). The C peptide AUC tended to be lower after repaglinide administration than after glipizide (24.16 ± 3.31 vs. 33.76 ± 2.98 nmol/liter·min, $P = 0.055$) with the difference approaching significance, sug-

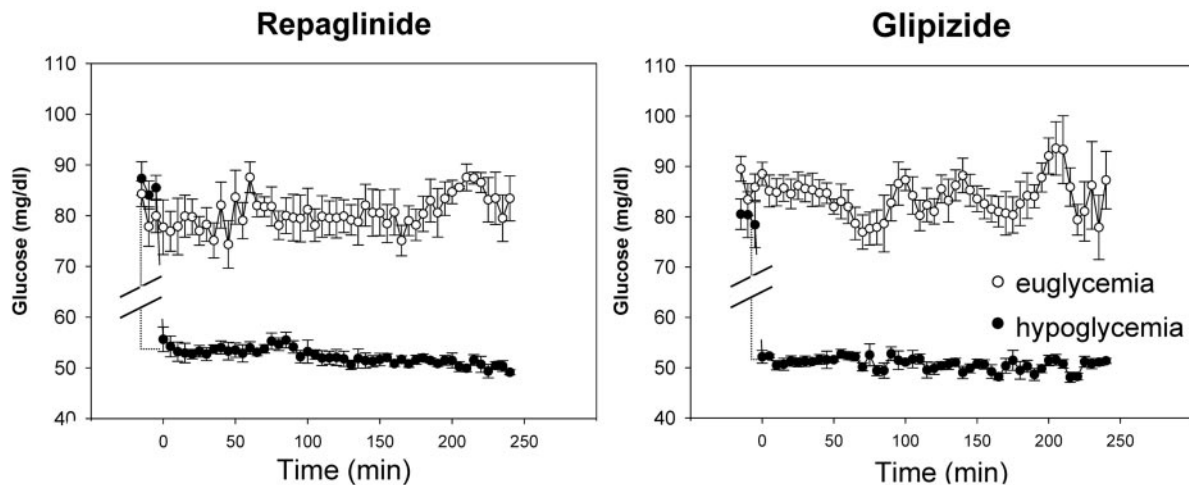


FIG. 1. Glucose levels achieved during euglycemic (○) and hypoglycemic (●) clamps after repaglinide or glipizide administered at time 0 min (conversion factor for SI units, 1 mg/dl = 0.05551 mmol/liter).

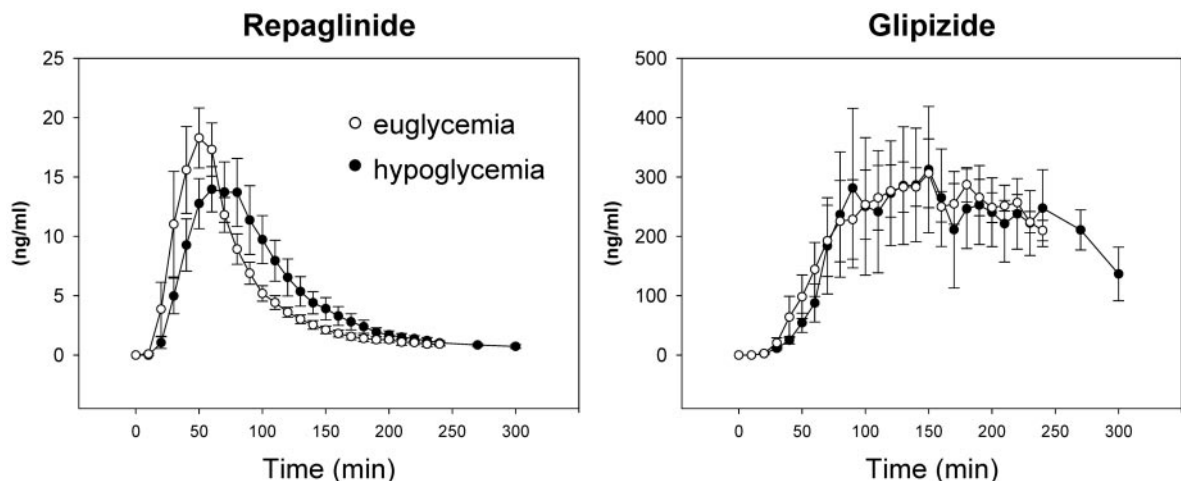


FIG. 2. Serum levels of repaglinide or glipizide (nanograms per milliliter) after administration at euglycemia (○) and hypoglycemia (●) ($P = \text{NS}$ euglycemia vs. hypoglycemia).

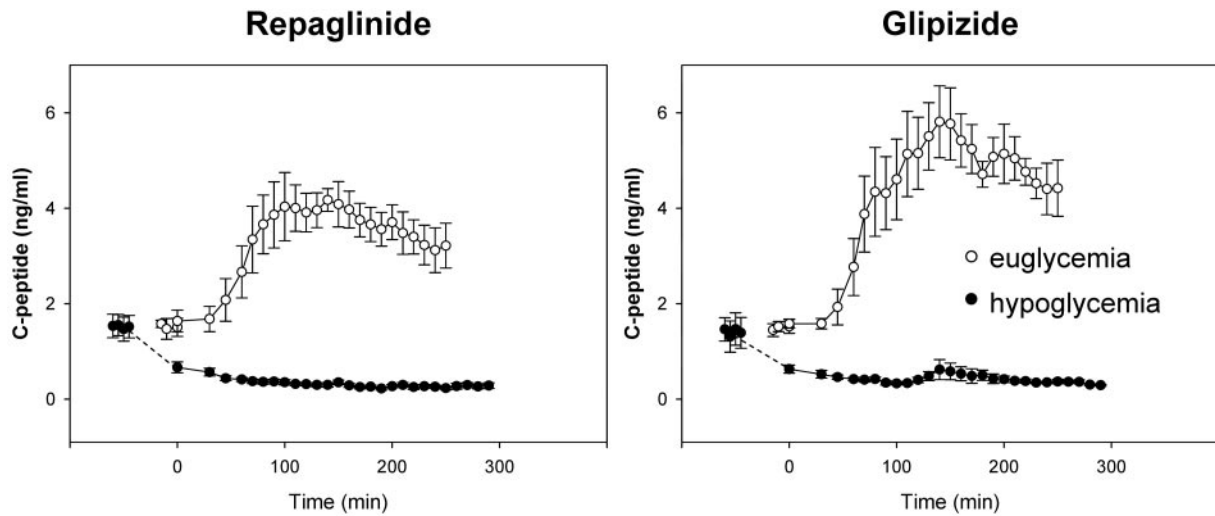


FIG. 3. C peptide levels (nanograms per deciliter) after administration of repaglinide or glipizide at euglycemia (○) and hypoglycemia (●) ($P < 0.006$ euglycemia vs. hypoglycemia for both repaglinide and glipizide) (conversion factor for SI units, 1 ng/ml = 0.331 nmol/liter).

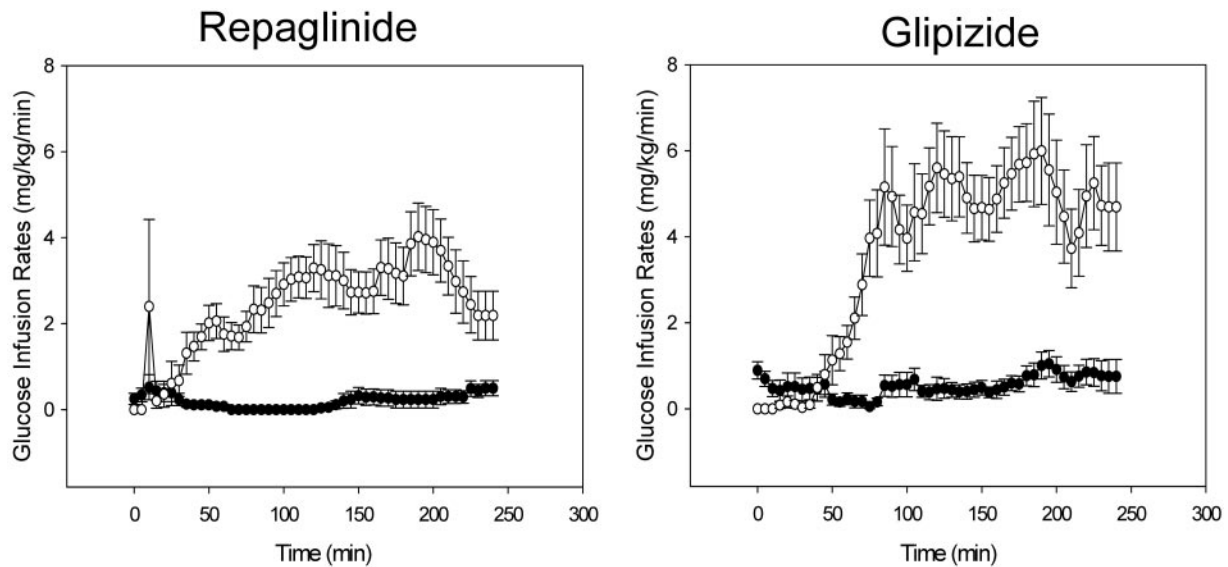


FIG. 4. Glucose infusion rates (milligrams per kilogram per minute) administered after ingestion of repaglinide or glipizide to maintain euglycemia (○) and hypoglycemia (●) ($P < 0.006$ euglycemia vs. hypoglycemia for both repaglinide and glipizide).

gesting lower stimulation of insulin production. For both agents, the C peptide levels were dramatically lower than when the drugs were administered at euglycemia ($P = 0.0009$ for repaglinide and $P = 0.001$ for glipizide). However, given the suppression of C peptide below baseline and the relatively greater stimulation of insulin production at baseline with glipizide, no relative difference in insulin secretion at hypoglycemia could be demonstrated. Thus, when administered at hypoglycemia, each drug causes markedly less insulin release than when administered at euglycemia.

One possible mechanism by which these drugs might induce less insulin secretion at hypoglycemia would be whether the drugs were poorly absorbed when administered at relatively low glucose. However, both drugs were comparably absorbed when administered at moderate hypoglycemia as when administered at euglycemia (AUC drug 1,301 \pm 182 and 34,513 \pm 17,042 ng/ml·min, $P = 0.4$ repaglinide and $P = 0.7$ glipizide

compared with administration at euglycemia, respectively). Although there was a tendency for a modestly reduced peak level ($P = 0.08$) and later peak with repaglinide when administered at hypoglycemia than at euglycemia, no kinetic difference was demonstrated with glipizide (Fig. 2). Thus, during hypoglycemia, low levels of cellular energy substrates (glucose or other metabolic products) or altered counterregulatory hormone levels are sufficient to inhibit insulin production in response to potent insulin secretagogues.

Discussion

Insulin secretagogues are widely used in the treatment of type 2 diabetes either alone or as part of combination therapy. Although symptomatic hypoglycemic events are relatively common, severe hypoglycemia is more uncommon despite use by patients who frequently omit glucose moni-

toring and have irregular eating and exercise habits. In humans *in vivo*, pretreatment with glucose has been demonstrated to potentiate insulin secretion in response to subsequent glucose challenge (11). However, it has not been demonstrated *in vivo* that preceding hypoglycemia could attenuate subsequent insulin response to glucose. *In vitro*, stimulation of insulin release from cultured islets is lower when cells are incubated in low-glucose medium, and repaglinide does not stimulate insulin release in the complete absence of glucose (4). Moreover, the incidence of severe hypoglycemia has been suggested to be lower with repaglinide compared with sulfonylureas in clinical trials (0.57% for repaglinide and 1.01% for sulfonylureas, respectively) (6). There are multiple potential explanations for this phenomenon including altered pharmacokinetic or pharmacodynamic properties between the drugs. In addition, in practice, insulin secretagogues are prescribed with instruction to be administered with food intake and to be withheld during periods of fasting. Administration with food intake is especially true for repaglinide, which is generally recommended to be taken 15–30 min before a meal. These recommendations may contribute to the relatively low incidence of severe hypoglycemia for both secretagogues and may provide some degree of additional protection for repaglinide.

This study evaluated the effects of repaglinide and glipizide on insulin secretion and glucose use at euglycemia and modest hypoglycemia, when additional insulin secretion would be undesirable. Differences in kinetics including onset and half-life make it impossible to have absolutely bioequivalent drug dosing between agents. However, for a 5-fold higher dose of glipizide on a milligram basis, there was a nearly 40-fold higher area under the plasma drug concentration curve but a much less dramatic, 2-fold increase in insulin secretion. Thus, on a milligram per milligram basis, repaglinide is relatively more potent than glipizide.

When administered at hypoglycemia, both drugs were well absorbed. In humans, drug kinetics have been poorly studied during modest hypoglycemia. Although one cannot extrapolate to any other class of medications because patients with diabetes often require multiple drugs, it is reassuring that at least two chemically dissimilar agents are well absorbed during modest hypoglycemia.

Although differences might exist between real world hypoglycemia in patients who take insulin secretagogues and our controlled hypoglycemia conditions, at relative hypoglycemia, both drugs caused less insulin secretion, assessed by both glucose infusion rates necessary to maintain plasma glucose and C peptide levels compared with insulin secretion at euglycemia. This dramatic reduction in insulin secretion would be protective *in vivo* for patients taking insulin secretagogues without hyperglycemia such as may occur with improving glycemic control and without monitoring with irregular dietary or exercise habits. Our data suggest that both glipizide and repaglinide are weak insulin secretagogues in the face of hypoglycemia and that severe hypoglycemia in patients on combined insulin and insulin secre-

tagogue therapies may be predominantly due to the insulin therapy with minimal contribution by the secretagogue. These findings of reduced insulin secretion induced by the drugs appear consistent with both short-acting insulin secretagogues; however, it is not known whether these findings may apply similarly to the long-acting sulfonylureas. Finally, it is possible that the insulin infusion itself rather than the hypoglycemia was responsible for suppression of β -cell secretory response because some studies suggest that insulin itself is a negative regulator of β -cell function (12).

Subjects experienced mild classical symptoms consistent with low glucose levels throughout the hypoglycemic clamps, suggesting that counterregulatory response is not eliminated by either drug; however, counterregulatory hormone levels were not formally assessed.

In conclusion, at modest hypoglycemia, low glucose or other metabolite(s) or altered counterregulatory hormone levels are sufficient to inhibit insulin production in response to these potent insulin secretagogues. These findings may help to explain the relatively low incidence of severe hypoglycemia with clinical administration of these drugs.

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