Alanine and Terbutaline in Treatment of Hypoglycemia in IDDM

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OBJECTIVE — To test the hypothesis that, in contrast to administration of glucose or glucagon, administration of the amino acid Ala or of the β_2-adrenergic agonist terbutaline produces sustained glucose recovery from hypoglycemia.

RESEARCH DESIGN AND METHODS — We developed a model of clinical hypoglycemia using subcutaneous injection of insulin (0.15 U/kg) in patients with IDDM. In comparison with nondiabetic subjects, patients with IDDM exhibited reduced glucagon ($P = 0.0001$), epinephrine ($P = 0.0060$), and pancreatic polypeptide ($P = 0.0001$) responses to hypoglycemia. In addition to placebos, the following were administered during hypoglycemia (2 h after insulin injection) in IDDM patients: oral glucose, 10 and 20 g; subcutaneous glucagon, 1.0 mg; oral Ala, 40 g; oral terbutaline, 5.0 mg; and subcutaneous terbutaline, 0.25 mg.

RESULTS — Glucose (10 and 20 g) and glucagon raised plasma glucose ($P = 0.0163$, 0.0060, and 0.0001, respectively) from 3.0–3.3 mM to peaks of 5.4 ± 0.4, 6.8 ± 0.7, and 11.8 ± 0.8 mM within 30, 45, and 60 min, respectively, but the responses were transient. Oral Ala raised glucose levels ($P = 0.0401$) to 4.0 ± 0.4 mM within 30 min; glucose levels then rose gradually to a 6-h value of only 7.1 ± 0.9 mM. Oral terbutaline raised glucose levels ($P = 0.0294$) to 4.3 ± 0.3 mM within 30 min; glucose levels then rose substantially. In contrast, subcutaneous terbutaline raised glucose levels ($P = 0.0249$) to 3.7 ± 0.1 mM within 15 min; the levels plateaued at 5.0 mM from ~60–150 min and then parallelled the placebo curve.

CONCLUSIONS — Ala and terbutaline produce sustained glucose recovery from hypoglycemia in IDDM and are therefore potentially useful agents for the treatment of mild or moderate iatrogenic hypoglycemia, or the prevention of iatrogenic hypoglycemia, when food intake is not anticipated over the following several hours.

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IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; EPI, EPINEPHRINE; NE, NOREPINEPHRINE; NEFA, NONESTERIFIED FATTY ACID; BMI, BODY MASS INDEX; β-OHB, β-HYDROXYBUTYRATE; ANOVA, ANALYSIS OF VARIANCE.

Iatrogenic hypoglycemia is the limiting factor in the management of IDDM (1). It causes recurrent physical morbidity, and some mortality, and the fear of hypoglycemia often causes psychological morbidity. Clearly, new approaches to its prevention and treatment are needed.

Hypoglycemia in IDDM is the result of absolute or relative therapeutic insulin excess, compromised glucose counterregulatory mechanisms (particularly reduced glucagon and EPI secretory responses), or probably most commonly an interplay of both (1). We (this issue, B.V. Wiethop and P.E. Cryer, p. 1124–30) have shown that administration of the amino acid Ala (which stimulates glucagon secretion) and of the β_2-adrenergic agonist terbutaline (which almost assuredly exerts direct glycemic actions and also stimulates sympathetic neural NE release and raises NEFA levels, potential indirect glycemic actions) both raise plasma glucose concentrations substantially in insulin-infused, initially euglycemic patients with IDDM. Thus, Ala and terbutaline represent potential new approaches to the treatment, and perhaps the prevention, of iatrogenic hypoglycemia. Clearly, however, these first need to be tested in a clinically appropriate model of insulin-induced hypoglycemia in patients with IDDM. We report herein the development of such a model, and the glycemic responses to conventional treatments (oral glucose and subcutaneous glucagon) and to oral Ala and oral and subcutaneous terbutaline. We hypothesized that Ala and terbutaline, in contrast to glucose and glucagon, produce sustained recovery from hypoglycemia in IDDM.

RESEARCH DESIGN AND METHODS

— Eight nondiabetic subjects and 9 patients with IDDM consented to participate in these studies, which were approved by the Washington University Human Studies Committee and conducted at the Washington Uni-

versity General Clinical Research Center. The mean ± SD ages and BMIs of the nondiabetic subjects (6 women and 2 men) were 23.1 ± 2.8 yr and 21.2 ± 3.0 kg/m^2, respectively. The mean ages and BMIs of the IDDM patients were 29.1 ± 5.7 yr and 23.4 ± 3.5 kg/m^2, respectively. For the latter group, the mean duration of IDDM was 8.9 ± 1.9 yr and the mean GHb level (in an assay with an upper limit if normal of 6.3%) was 11.5 ± 1.8%. The patients were selected for the absence of overt atherosclerotic disease; hypertension and diabetic nephropathy; and neuropathy and proliferative retinopathy. All participated in the placebo limb (see below); 6 participated in the two oral glucose limbs and the glucagon limb, and 6 participated in the oral and subcutaneous terbutaline limbs and the oral Ala limb. Nondiabetic subjects participated only in the placebo limb.

Experimental protocol

Nondiabetic subjects were studied, after a 12- to 14-h overnight fast, as General Clinical Research Center outpatients. Patients with IDDM were admitted to the General Clinical Research Center the day before study and also studied after an overnight fast. Their diabetes was managed with regular insulin the day before study; near euglycemia was maintained overnight with intravenous insulin (3).

At ~0700 regular insulin (Novolin R, Novo-Nordisk, Bagsvaerd, Denmark), 0.15 U/kg, was injected subcutaneously (and intravenous insulin was discontinued in the patients). Arterialized venous blood samples were obtained, through an indwelling needle in a hand vein with the hand kept in a 65–75°C box, at 15-min intervals from −0.5 through 8.0 h. Blood pressures and heart rates were also measured at these time points. Two hours after insulin injection, all subjects received an oral solution and a subcutaneous injection. The oral solutions were sweetened (saccharin) water (placebo), water containing 10 or 20 g of glucose, sweetened water containing 5.0 mg of terbutaline sulfate (Geigy Pharmaceuticals, Ardsley, NY), or sweetened water containing 40 g of Ala (Sigma, St. Louis, MO). The subcutaneous injections were saline (placebo), 1.0 mg of glucagon (Eli Lilly, Indianapolis, IN), or 0.25 mg of terbutaline sulfate. Thus, the interventions, in random sequence, were placebos in both nondiabetic subjects and in the patients with IDDM and, in the patients, 10 g oral glucose; 20 g oral glucose; 1.0 mg subcutaneous glucagon; 5.0 mg oral terbutaline; 0.25 mg subcutaneous terbutaline; and 40 g oral Ala.

Analytical methods

Plasma glucose concentrations were determined with a glucose oxidase method on a glucose analyzer (Beckman, Fullerton, CA). Plasma free insulin (4), free C-peptide (4), glucagon (5), pancreatic polypeptide (6), growth hormone (7), and cortisol (8) were measured with radioimmunoassays. Plasma NE and EPI were measured with a single isotope derivative (radioenzymatic) method (9). Terbutaline, added to plasma in concentrations up to 100 μg/L, did not alter measured NE or EPI concentrations (data not shown). Serum NEFAs were determined with an enzymatic method (10). Microfluorometric methods were used to measure blood Ala (11), lactate (12), and β-OHB (13) levels.

Statistical analysis

A repeated-measures ANOVA was used to identify treatment effects. Data are presented as the mean ± SE except where the SD is specified.

RESULTS

Comparison of responses of nondiabetic subjects and IDDM patients

After subcutaneous insulin injection, plasma glucose concentrations fell to similar nadirs (3.4 ± 0.2 mM) in the nondiabetic subjects and IDDM patients (Fig. 1). Increments in plasma free insulin were similar; free insulin concentrations did not differ significantly after adjustment for higher baseline levels in the patients (Fig. 2). C-peptide levels were unmeasurable throughout in the patients, and fell to unmeasurable levels in the nondiabetic subjects (Fig. 2). Compared with the nondiabetic subjects, the IDDM patients displayed reduced glucagon (P = 0.0001), EPI (P = 0.0060), and pancreatic polypeptide (P = 0.0001) responses to hypoglycemia (Fig. 2). Peak glucagon levels were 154 ± 14 and 94 ± 7 ng/L, peak EPI levels were 2220 ± 850 and 980 ± 260 pM, and peak pancreatic polypeptide levels were 55 ± 6 and 19 ± 3 pM, respectively. NE responses (Fig. 2) were also reduced (P = 0.0067) in the patients. Growth hormone and cortisol levels were similar as were NEFA, β-OHB, and Ala levels (data not shown). A prominent increase in blood lactate (from 715 ± 50 to 1150 ± 180 μM) in the nondiabetic subjects was not seen in the IDDM patients (P = 0.0265) (data not shown). No differences in heart rates were observed, but systolic (P = 0.0026), diastolic (P = 0.0018), and mean (P = 0.0085) blood pressures (data not shown) were slightly lower in the nondiabetic subjects after insulin injection.

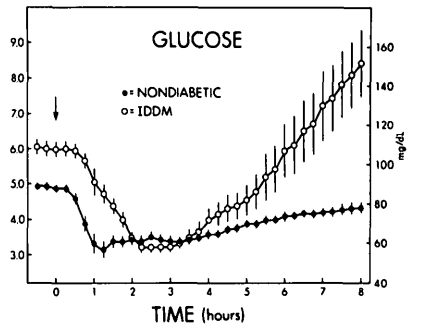

Figure 1—*Mean ± SE plasma glucose concentrations before and after subcutaneous injection of insulin (0.15 U/kg, arrow) in nondiabetic subjects and previously overnight insulin-infused IDDM patients.*

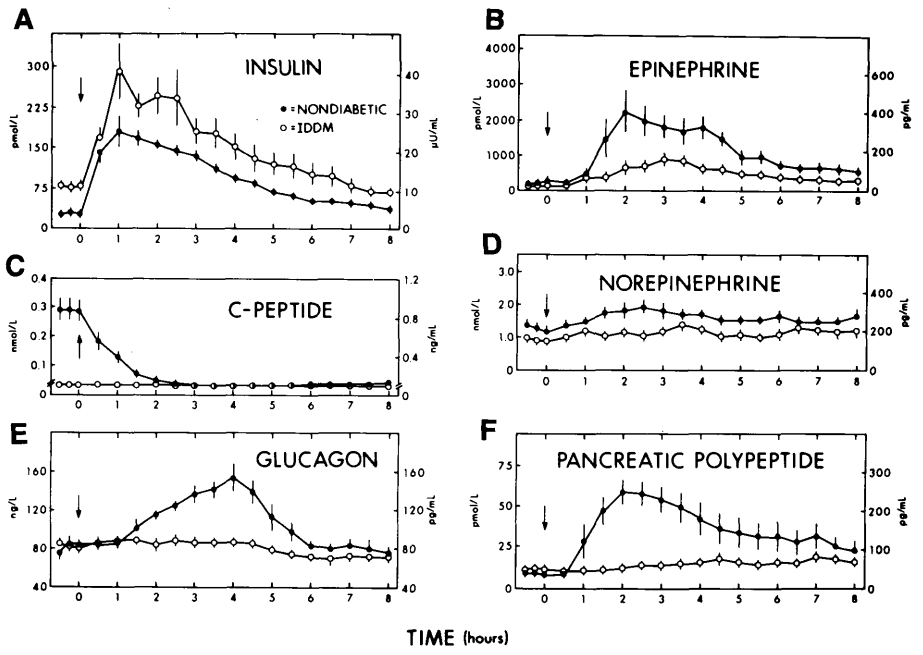

Figure 2—*Mean ± SE plasma free insulin (A), EPI (B), free C-peptide (C), NE (D), glucagon (E), and pancreatic polypeptide (F) concentrations before and after subcutaneous injection of insulin (0.15 U/kg, arrow) in nondiabetic subjects (●) and previously overnight insulin-infused IDDM patients (○).*

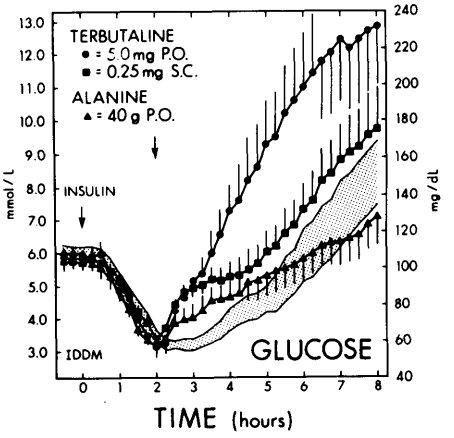

Figure 4—*Mean ± SE plasma glucose concentrations before and after subcutaneous injection of insulin (0.15 U/kg, left arrow) in previously overnight insulin-infused IDDM patients with interventions at 120 min (right arrow) with the following: oral and subcutaneous placebos (▥); 5.0 mg oral terbutaline; 0.25 mg subcutaneous terbutaline; or 40 g oral Ala, in random sequence.*

Comparisons of the responses of patients with IDDM to oral glucose, subcutaneous glucagon, and placebos

Compared with placebos, 10 g oral glucose ($P = 0.0163$), 20 g oral glucose ($P = 0.0060$), and 1.0 mg subcutaneous glucagon ($P = 0.0001$) produced prompt but transient increments in plasma glucose concentrations from hypoglycemic levels (Fig. 3). After 10 g oral glucose, plasma glucose increased from 3.3 ± 0.2 mM to a peak of 5.4 ± 0.4 mM 30 min later. However, glucose levels fell after 60 min. After 20 g oral glucose, plasma glucose increased from 3.2 ± 0.2 mM to a peak of 6.8 ± 0.7 mM 45 min later. Again, levels fell after 60 min. After subcutaneous glucagon, plasma glucose increased markedly from 3.0 ± 0.2 mM to a peak of 11.8 ± 0.8 mM 60 min later. Glucose levels started to fall after 90 min.

No differences in plasma free insulin, free C-peptide, pancreatic polypeptide, or NE concentrations were observed among the treatment groups (Fig. 4). Plasma glucagon increased to 400 ng/L after glucagon injection (Fig. 4). Hypoglycemia elicited small increments in plasma EPI; the levels tended to decrease below control (placebo) levels after the treatments (Fig. 4). Similarly, increased growth hormone levels fell after the treatments (data not shown). Those following 20 g of glucose ($P = 0.0217$) and glucagon ($P = 0.0174$) were significant statistically. Serum NEFA ($P = 0.0338$) and blood β-OHB ($P = 0.0085$) fell below control values after glucagon administration (data not shown). Blood β-OHB levels were also lower after 20 g of glucose (data not shown).

No significant differences were noted in heart rates or in systolic, diastolic, or mean blood pressures among the treatment groups (data not shown).

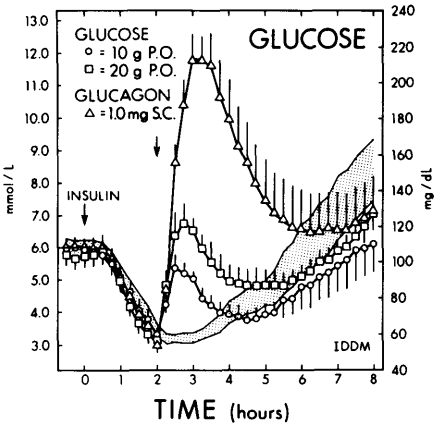

Figure 3—*Mean ± SE plasma glucose concentrations before and after subcutaneous injection of insulin (0.15 U/kg, left arrow) in previously overnight insulin-infused IDDM patients with interventions at 120 min (right arrow) with the following: oral and subcutaneous placebos (▥); 10 g oral glucose; 20 g oral glucose; or 1.0 mg subcutaneous glucagon, in random sequence.*

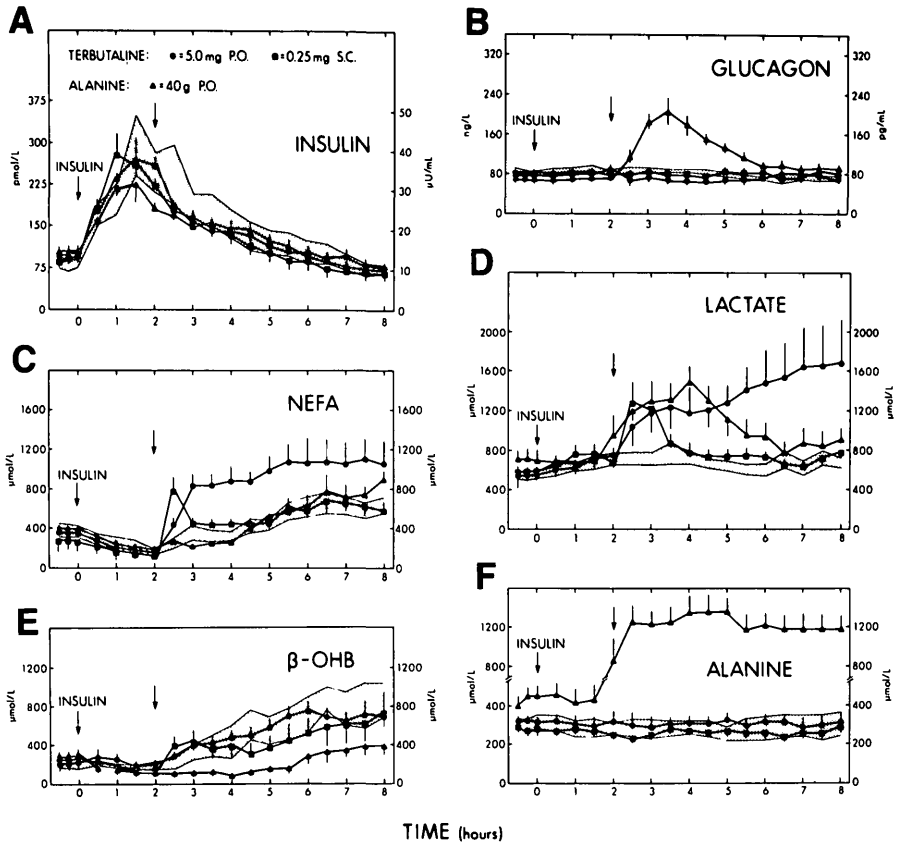

Figure 5—*Mean ± SE plasma free insulin (A) and glucagon (B), serum NEFA (C), and blood lactate (D), β-OHB (E), and Ala (F) concentrations before and after subcutaneous injection of insulin (0.15 U/kg, left arrow) in previously insulin-infused IDDM patients with interventions at 120 min (right arrow) with the following: oral and subcutaneous placebos (▥); 5.0 mg oral terbutaline; 0.25 mg subcutaneous terbutaline; or 40 g oral Ala, in random sequence.*

Comparisons of responses of IDDM patients to oral and subcutaneous terbutaline and oral Ala and placebos

Compared with placebos, oral terbutaline ($P = 0.0294$), subcutaneous terbutaline ($P = 0.0249$), and oral Ala ($P = 0.0401$) produced sustained increments in plasma glucose concentrations from hypoglycemic levels (Fig. 5). After oral terbutaline, plasma glucose increased from 3.1 ± 0.3 to 4.3 ± 0.3 mM at 30 min and then increased progressively. After subcutaneous terbutaline, plasma glucose increased from 3.2 ± 0.1 to 3.7 ± 0.1 mM at 15 min, plateaued at 5.0 mM at ~60 min, and then parallelled the placebo curve. After oral Ala, plasma glucose increased from 3.2 ± 0.2 to 4.0 ± 0.4 mM at 30 min and then rose gradually thereafter. The final glucose concentration was 7.1 ± 0.8 mM.

No differences in plasma free insulin, free C-peptide, pancreatic polypeptide, or NE concentrations were observed among the treatment groups (Fig. 6). Plasma glucagon increased ($P = 0.0019$) following Ala ingestion. Oral and subcutaneous terbutaline suppressed plasma EPI ($P = 0.0159$ and 0.0325, respectively) (Fig. 6) and growth hormone ($P = 0.0039$ and 0.0134, respectively) (Fig. 7). Oral terbutaline produced sustained increments in NEFA ($P = 0.0255$) and lactate ($P = 0.0077$) levels; subcutaneous terbutaline produced transient increments in NEFA ($P = 0.0144$) and lactate ($P = 0.0331$) levels (Fig. 7). β-OHB levels were suppressed ($P = 0.0076$), lactate levels rose transiently ($P = 0.0269$), and Ala levels were increased ($P = 0.0008$) after Ala ingestion (Fig. 7).

No significant differences were noted in heart rates or in systolic, diastolic, or mean blood pressures among the treatment groups (data not shown).

CONCLUSIONS— We developed a model of clinical insulin-induced hypoglycemia in IDDM patients, and used that model to demonstrate that oral and subcutaneous terbutaline and oral Ala, in contrast to oral glucose and subcutaneous glucagon, produce sustained recovery from hypoglycemia in IDDM.

To maximize the clinical relevance of the model, we produced hypoglycemia with insulin injected subcutaneously, the route by which the hormone is currently replaced in IDDM, and studied patients with IDDM. To characterize the model, we first compared the responses of these patients with IDDM with those of nondiabetic individuals. Compared with nondiabetic subjects, the patients, with a mean duration of IDDM of only ~9 yr, displayed markedly reduced glucagon, EPI, and pancreatic polypeptide secretory responses to comparable degrees of hypoglycemia (3.4 ± 0.2 mM). These data reaffirm and extend the fact that IDDM is a multihormone deficiency state (1,14). Insulin deficiency is the proximate cause of clinical IDDM, selectively deficient glucagon responses to plasma glucose decrements develop early in the course of IDDM, and selectively deficient EPI responses develop in the majority of patients with relatively longstanding IDDM. In the nearly universal setting of deficient glucagon responses, the deficient EPI responses compromise glucose counterregulation critically and result in a high frequency of severe iatrogenic hypoglycemia, the clinical syndrome of defective glucose counterregulation (1). These de-

ranged hormonal patterns, and their clinical impact, have been discussed elsewhere (1,14). The fact that pancreatic polypeptide responses to hypoglycemia are also reduced in patients with relatively longstanding IDDM, even in the absence of classical diabetic autonomic neuropathy (14,15), is less widely appreciated. Unlike deficient glucagon and EPI responses, deficient pancreatic polypeptide secretion presumably has no direct clinical impact. However, reduced EPI, pancreatic polypeptide and neurogenic symptom responses to hypoglycemia in the absence of classical diabetic autonomic neuropathy define a previously unrecognized form of functional and selective adrenomedullary, parasympathetic, and sympathetic failure, respectively. We have termed this hypoglycemia-associated autonomic failure (14, 15).

Before applying this model of clinical insulin-induced hypoglycemia to the study of novel treatments, we thought it important to define the temporal patterns and the magnitude of the glycemic responses to standard treatments. Intravenous glucose, which was not studied, and subcutaneous glucagon, which was, are widely used (generally, but not invariably [16], by physicians and family members, respectively) in the emergency treatment of hypoglycemia sufficiently severe to preclude oral treatment. In the model, glucagon injection caused a prompt (15 min) but transient (2–3 h) increase in plasma glucose from hypoglycemic levels. The 1.0-mg dose used produced substantial hyperglycemia (nearly 12.0 mM); the dose could be reduced to minimize this (17) but that would increase the risk of recurrent hypoglycemia shortly thereafter.

Oral glucose, in one form or another, is the standard treatment of less severe hypoglycemia, but the appropriate dose has not been well-defined (18,19). In the model, 10 g of glucose orally produced a prompt but rather small (to 5.4 ± 0.4 mM) and brief (1 h) increment in plasma glucose from rather mild hypoglycemia (3.3 ± 0.2 mM). This provides a narrow margin for error even in the treatment of mild hypoglycemia, and might well be inadequate in the treatment of more severe hypoglycemia. Twenty grams of oral glucose raised plasma glucose to 6.8 ± 0.7 mM. Thus, this dose increased the margin of error but did not result in hyperglycemia and would, therefore, seem preferable in the short-term treatment of hypoglycemia. Again, however, the response was transient. In the absence of food, or additional glucose, ingestion the risk of recurrent hypoglycemia would be high. Thus, it would seem reasonable to seek treatments that produce sustained recovery from hypoglycemia.

The critical glucose counterregulatory defect in IDDM is a selectively deficient glucagon response to falling plasma glucose levels (1). The fact that this defect is selective, i.e., that the glucagon secretory response to other stimuli is largely, if not entirely (this issue, B.V. Wiethop and P.E. Cryer, p. 1124–30), intact, is fundamental. Based on this pathophysiology, we reasoned that an agent that would stimulate glucagon secretion would be potentially effective in the treatment of hypoglycemia. Therefore, we tested Ala, a potent stimulator of glucagon secretion, and showed that it raised plasma glucagon and glucose concentrations in insulin infused, initially euglycemic patients with IDDM (this issue, B.V. Wiethop and P.E. Cryer, p. 124–30). Parenthetically, it did not raise plasma glucose levels in nondiabetic individuals because of increased insulin secretion in response to the amino acid, very small increments in plasma glucose, or both (this issue, B.V. Wiethop and P.E. Cryer, p. 1124–30). In the model, Ala ingestion raised plasma glucagon concentrations and produced sustained glucose recovery. Plasma glucose concentrations increased from hypoglycemic (3.2 ± 0.2) to euglycemic (4.0 ± 0.4) levels within 30 min. They then increased to ~5.0 mM over the next 2 h. Notably, substantial hyperglycemia did not occur. The plasma glucose concentration 6 h after Ala ingestion was only 7.1 ± 0.8 mM. Thus, it would appear that oral Ala might be an appropriate treatment for mild hypoglycemia (including asymptomatic mild hypoglycemia detected by self glucose monitoring) when food intake is not anticipated over the next several hours. The simplest example of the latter would be an overnight fast, but other scenarios could be envisioned. Because of the relatively slow onset of the glycemic response to Ala, it is reasonable to speculate that the addition of a more rapid-acting agent, such as 10 g of glucose, to Ala would produce a preferable glycemic response. Glucose might also improve the taste of Ala. Finally, it could be reasoned that the message of these Ala data might be to use a high protein (perhaps with carbohydrate) treatment of mild to moderate hypoglycemia when food intake is not anticipated over the next several hours. However, we studied the effect of the amino acid, not of protein.

A selectively deficient EPI response to falling plasma glucose levels compromises glucose counterregulation critically, in the setting of deficient glucagon responses, in IDDM (1,14,15). Based on this pathophysiology, we reasoned that a β_2-adrenergic agonist would be potentially effective in the treatment of hypoglycemia. Therefore, we tested terbutaline and showed that it raised plasma glucose concentrations in insulin infused, initially euglycemic patients with IDDM (this issue, B.V. Wiethop and P.E. Cryer, p. 1124–30). It did not raise plasma glucose levels substantially in nondiabetic individuals because of increased insulin secretion in response to the agonist, small increments in plasma glucose, or both (this issue, B.V. Wiethop and P.E. Cryer, p. 1124–30). In the model, terbutaline also produced sustained recovery from hypoglycemia. The oral dose of 5.0 mg increased plasma glucose concentrations from hypoglycemic (3.1 ± 0.3 mM) to euglycemic (4.3 ± 0.3 mM) levels within

30 min, but then produced rather substantial hyperglycemia. Subcutaneous terbutaline (0.25 mg) increased plasma glucose concentrations more quickly (from 3.2 ± 0.1 to 3.7 ± 0.1 mM within 15 min). It did not produce substantial hyperglycemia. Glucose levels plateaued between 5.0 and 6.0 mM between 1 and 2.5 h and then rose in parallel with the placebo curve. Thus, the glycemic response to subcutaneous terbutaline was superior to that of oral terbutaline in that the former produced a more prompt but still sustained increase in plasma glucose concentrations.

Terbutaline did not produce subjective side-effects or significant changes in blood pressure. Heart rates tended to be higher after oral terbutaline, but this was not significant statistically. However, terbutaline exhibited metabolic effects in addition to those on glucose metabolism. It increased both NEFA and lactate levels in the model, as it did in our earlier study of insulin infused, initially euglycemic patients with IDDM (2).

Although one could envision use of subcutaneous terbutaline as an emergent treatment for severe hypoglycemia, it was, in the dose tested, a less potent glycemic agent than glucagon. It might be useful as a treatment of mild or moderate hypoglycemia when food intake is not anticipated over the next several hours, as discussed for Ala. Theoretically, parenteral terbutaline might be used as a component of future closed-loop insulin administration systems, if perfected insulin replacement did not prevent hypoglycemia in IDDM.

In summary, we have developed a model of clinical insulin-induced hypoglycemia using subcutaneous insulin injection in patients with IDDM. Using this model, we have shown that oral glucose and subcutaneous glucagon produce prompt but transient glucose recovery, whereas oral Ala and oral and subcutaneous terbutaline produce sustained glucose recovery from hypoglycemia. Thus, Ala and terbutaline are potentially useful agents for the treatment of mild or moderate iatrogenic hypoglycemia, or the prevention of iatrogenic hypoglycemia, when food intake is not anticipated over the following several hours.

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