Glycemic Actions of Alanine and Terbutaline in IDDM

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OBJECTIVE— To test the hypothesis that the amino acid Ala and the β2-adrenergic agonist terbutaline raise plasma glucose concentrations substantially, and do so through different mechanisms, in IDDM patients.

RESEARCH DESIGN AND METHODS— We administered these (Ala: 20 and 40 g, orally; terbutaline: 2.5 and 5.0 mg orally and 0.25 mg subcutaneously) and placebos in random sequence to 6 nondiabetic subjects and 6 insulin-infused, initially euglycemic IDDM patients, each studied on six different occasions. Inhaled terbutaline, 0.4 mg, was also tested on a seventh occasion in IDDM patients.

RESULTS— Ala administration raised plasma glucagon ($P = 0.0219$), C-peptide ($P = 0.0014$), and insulin ($P = 0.0094$), with no significant change in plasma glucose, in nondiabetic subjects. In patients with IDDM it raised glucagon ($P = 0.0001$), but not C-peptide or insulin, and plasma glucose rose to 8.3 ± 0.3 (Ala 20 g, $P = 0.0006$) and 10.0 ± 1.0 mM (Ala 40 g, $P = 0.0094$). Catecholamine levels were unchanged. Terbutaline ingestion raised plasma glucose minimally (e.g., to 6.3 ± 0.3 mM, $P = 0.0133$) in nondiabetic subjects but substantially, to 10.2 ± 1.0 (terbutaline 2.5 mg, $P = 0.0078$) and 14.0 ± 0.6 mM (terbutaline 5.0 mg, $P = 0.0001$), in IDDM patients; subcutaneous terbutaline raised plasma glucose (to a peak of 10.3 ± 0.7 mM, $P = 0.0017$) with an initial effect within 10 min, but inhaled terbutaline did so more slowly. In addition to its direct glycemic actions, terbutaline stimulated sympathetic neural norepinephrine release ($P = 0.0151$) and increased nonesterified fatty acid levels ($P = 0.0104$), potential indirect glycemic actions. Glucagon levels were unchanged; insulin levels increased in the nondiabetic subjects.

CONCLUSIONS— These data demonstrate substantial glycemic responses to Ala and terbutaline, through different mechanisms, in IDDM patients. Thus, Ala and terbutaline represent potential new approaches to the treatment, and perhaps the prevention, of iatrogenic hypoglycemia in IDDM.

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

Iatrogenic hypoglycemia is a major problem for people with IDDM (1). Clearly, new approaches to its prevention and treatment are needed. Although the pursuit of perfected methods of insulin replacement is intuitively attractive, the recent observation of recurrent hypoglycemia despite feedback-regulated insulin secretion after successful pancreas transplantation in IDDM patients (2) suggests that this alone might not be sufficient. We have elected to pursue methods to compensate for defective glucose counterregulation (1) in IDDM.

Based on the physiology of glucose counterregulation (3) and its pathophysiology in IDDM (1), coupled with the known glucagon-releasing and glycemic effects of amino acids (4–6) and direct and indirect glycemic effects of adrenergic agonists (7), we hypothesized that administration of the amino acid Ala and of the β_2-adrenergic agonist terbutaline would raise plasma glucose concentrations substantially in people with IDDM but minimally in nondiabetic individuals (because the latter, but not the former, can secrete insulin). Oral Ala has been reported to raise plasma glucose levels further in hyperglycemic (insulin withdrawn) patients with IDDM (8). Intravenous salbutamol, a β_2-adrenergic agonist, has been reported to precipitate diabetic ketoacidosis (9) and stimulate glucose production (10), and terbutaline has been reported to raise plasma glucose levels somewhat in nondiabetic pregnant women (11).

To test these hypotheses, we studied the metabolic effects of oral Ala (20 and 40 g), oral (2.5 and 5.0 mg) and subcutaneous (0.25 mg) terbutaline, and placebos in nondiabetic subjects and insulin-infused, initially euglycemic IDDM patients. Inhaled terbutaline (0.4 mg) was also tested in the latter patients.

RESEARCH DESIGN AND METHODS

— Six nondiabetic subjects and 6 IDDM patients gave their written consent to participate in this study,

which was approved by the Washington University Human Studies Committee and conducted at the Washington University General Clinical Research Center. The mean ± SD ages and BMIs of the nondiabetic subjects (3 women and 3 men) were 27.3 ± 4.5 yr and 24.3 ± 2.9 kg/m^2, respectively. The mean ages and BMIs of the diabetic subjects (2 women and 4 men) were 25.6 ± 5.5 yr and 23.2 ± 3.5 kg/m^2, respectively. For the latter group, the mean duration of IDDM was 14.8 ± 7.9 yr (range 6–23 yr), and the mean GHb level (in an assay with an upper limit, if normal, of 6.3%) was 11.2 ± 1.7% (range 9.1–13.8%). The patients were selected for the absence of overt atherosclerotic disease; hypertension and diabetic nephropathy; and neuropathy and proliferative retinopathy. Three had background retinopathy.

Experimental protocol

All subjects were studied on six separate occasions in random sequence with administration of both oral and subcutaneous placebos, oral Ala 20 g, oral Ala 40 g, oral terbutaline 2.5 mg, oral terbutaline 5.0 mg, or subcutaneous terbutaline 0.25 mg. The IDDM subjects were also studied on a seventh occasion with administration of inhaled terbutaline (0.4 mg). Ala (Sigma, St. Louis, MO) was dissolved in ~240 ml of water; a noncaloric sweetener (saccharin) was added. The preparation was kept at 4°C before its administration. Terbutaline sulfate (Brethine, Geigy Pharmaceuticals, Ardsley, NY) preparations were obtained from our hospital pharmacy. All subjects and patients received an oral solution and a subcutaneous injection on all occasions. The oral solutions were sweetened water alone (placebo) or sweetened water containing Ala or terbutaline. The subcutaneous injections were saline (placebo) or terbutaline.

All studies were performed after a 12- to 14-h overnight fast. Nondiabetic subjects came to the Washington University General Clinical Research Center early on the morning of study. Patients with IDDM took their last dose of intermediate or long-acting insulin 48 h before study and used regular insulin to manage their diabetes thereafter. They were admitted to the Washington University General Clinical Research Center the evening before study, and kept nearly euglycemic with intravenous insulin, using modifications of a published algorithm (12), overnight. After plasma glucose levels were shown to be stable at a given insulin infusion rate before the study, that insulin infusion rate was fixed and continued throughout the ~7-h study.

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 −30, −15, and 0 min and at 10, 20, 30, 45, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min after placebo or drug administration. Heart rates and blood pressures were also recorded at those time points. Subjects were asked to report any symptoms that occurred, and were observed by a physician investigator and a research nurse throughout.

Analytical methods

Plasma glucose concentrations were determined with a glucose oxidase method on a glucose analyzer (Beckman, Fullerton, CA). Plasma free insulin (13), free C-peptide (13), glucagon (14), pancreatic polypeptide (15), growth hormone (16), and cortisol (17) were measured with radioimmunoassays. Plasma NE and EPI were measured with a single isotope derivative (radioenzymatic) method (18). Terbutaline, added to plasma in concentrations up to 100 μg/L, did not alter NE or EPI concentrations measured with this assay (data not shown). Serum NEFAs were determined with an enzymatic method (19). Microfluorometric methods were used to measure blood Ala (20), lactate (21), and β-OHB (22) levels.

Statistical methods

A repeated measures ANOVA was used to identify significant overall and specific (Ala or terbutaline) treatment effects. Curves were also assessed in four time segments: 10–30 (T_1), 45–120 (T_2), 150–240 (T_3), and 270–360 min (T_4). $P < 0.0500$ (overall treatment effect), <0.0100 (specific treatment effect), and <0.01250 (time segment specific treatment effect), respectively, were considered to indicate significant differences. This approach was taken to hold the possibility of type I statistical errors at an acceptable level when multiple comparisons are made. However, it increases the possibility of type II statistical errors. Therefore, all P values are provided for those parameters with overall treatment effect $P < 0.0500$.

RESULTS

Overall treatment effects

In the nondiabetic subjects, the treatment effects were significant on plasma glucose ($P = 0.0046$), insulin ($P = 0.0010$), C-peptide ($P = 0.0001$), glucagon ($P = 0.01840$), NE ($P = 0.0101$), serum NEFA ($P = 0.0001$), and blood Ala ($P = 0.0091$) concentrations. In the IDDM patients, significant treatment effects were observed on plasma glucose ($P = 0.0001$), glucagon ($P = 0.0070$), NE ($P = 0.0006$), serum NEFA ($P = 0.0001$), blood β-OHB ($P = 0.0055$), lactate ($P = 0.0026$), and Ala ($P = 0.0085$) concentrations as well as heart rate ($P = 0.0017$).

Responses to Ala administration

Nondiabetic subjects. Compared with placebos, oral Ala administration to nondiabetic subjects resulted in dose-related increments in plasma glucagon (20 g, $P = 0.0402$; 40 g, $P = 0.0219$), insulin (20 g, $P = 0.0031$; 40 g, $P = 0.0094$) (Fig. 1), and C-peptide (20 g, $P = 0.0006$; 40 g, $P = 0.0014$) (data not shown). Although the glucagon responses did not achieve $P < 0.0100$, they were highly significant ($P =$

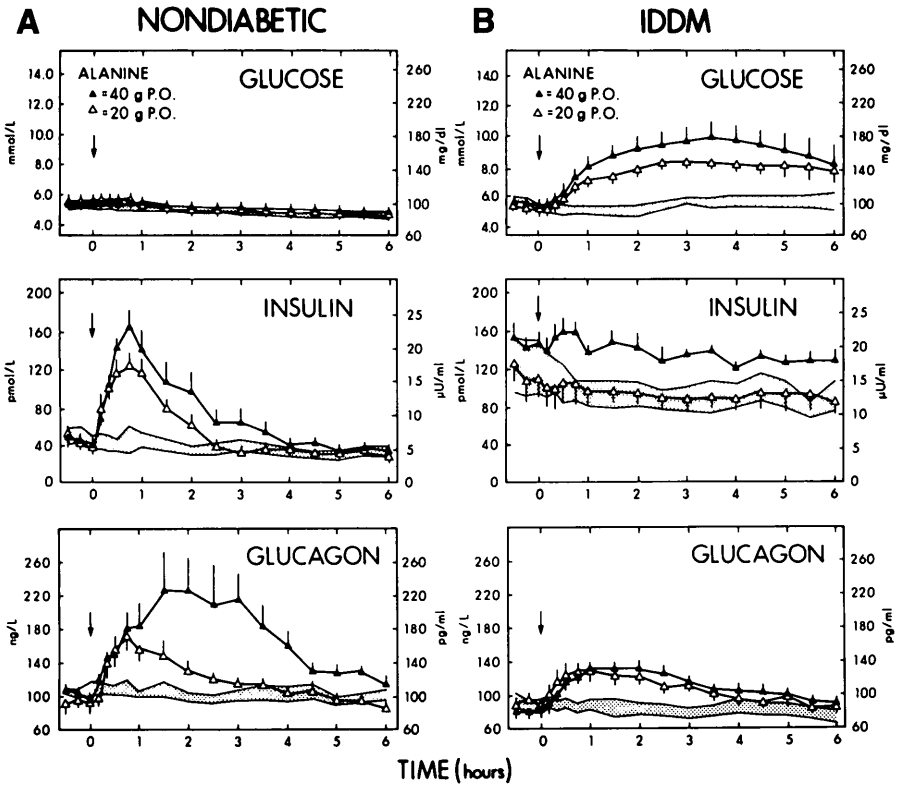

Figure 1—*Mean ± SE plasma glucose, insulin, and glucagon concentrations before and after administration of Ala to nondiabetic subjects (A) and insulin-infused IDDM patients (B). The shaded areas are one SE around the mean for data before and after administration of placebos. (P.O.), per os.*

0.0067) after the 40-g dose in the T_1 time segment. No significant changes were observed in plasma EPI, NE, pancreatic polypeptide, growth hormone, or cortisol (data not shown). Serum NEFA (20 g, $P = 0.0821$; 40 g, $P = 0.0086$) fell and then rose to control levels (Fig. 2). The decrements were significant in the T_2 time segment ($P = 0.0010$) after the 20-g dose and in the T_2 ($P = 0.0120$) and T_3 ($P = 0.0122$) time segments after the 40-g dose. No significant changes in blood β-OHB were observed (data not shown). Blood lactate appeared to rise slightly, but this was not statistically significant. Blood Ala rose substantially (20 g, $P = 0.0031$; 40 g $P = 0.0158$) as expected (Fig. 2). There were no changes in heart rate or blood pressure (data not shown).

IDDM patients. Compared with placebos, oral Ala administration to insulin-infused, initially euglycemic IDDM patients resulted in dose-related increments in plasma glucagon (20 g, $P = 0.0070$; 40 g, $P = 0.0001$) (Fig. 2), but, of course, no increments in plasma insulin (Fig. 1) or C-peptide (data not shown), with substantial increments in plasma glucose (20 g, $P = 0.0006$; 40 g, $P = 0.0094$) (Fig. 1). Mean ± SE plasma glucose concentrations increased from 5.1 ± 0.3 mM to a peak of 8.3 ± 0.3 mM at 180 min after 20 g of Ala and from 5.4 ± 0.2 mM to a peak of 10.0 ± 1.0 mM at 210 min after 40 g of Ala. Increments were apparent as early as 20 min after Ala ingestion (Fig. 1). As in the nondiabetic subjects, no significant changes in plasma EPI, NE, pancreatic polypeptide, growth hormone, or cortisol were observed (data not shown). Apparent decrements in serum NEFA levels (Fig. 2) were not significant statistically. No significant changes in blood β-OHB were observed (data not shown). Blood lactate appeared to rise (Fig. 2), but this was not significant. Blood Ala rose substantially (20 g, $P = 0.0041$; 40 g, $P = 0.0072$) (Fig. 2). There were no changes in heart rate or blood pressure (data not shown).

Responses to terbutaline administration

Nondiabetic subjects. Compared with placebos, oral terbutaline administration to nondiabetic subjects resulted in small, dose-related increments in plasma glucose (Fig. 3). Mean plasma glucose concentrations appeared to increase from 5.4 ± 0.2 mM to a peak of 5.8 ± 0.2 mM at 120 min after ingestion of 2.5 mg of terbutaline and from 5.6 ± 0.3 mM to a peak of 6.3 ± 0.3 mM at 60 min after ingestion of 5.0 mg of terbutaline ($P = 0.0134$). Similar, but more rapid, small apparent increments (from 5.3 ± 0.2 to 6.0 ± 0.3 mM at 30 min, $P = 0.0133$) followed subcutaneous administration of 0.25 mg of terbutaline. None of these achieved $P < 0.0100$. Thus none was significant by the conservative criteria used. Significant increments in plasma insulin after oral terbutaline (2.5 mg, $P = 0.0067$; 5.0 mg, $P = 0.0021$) (Fig. 3), and in C-peptide (data not shown) after subcutaneous terbutaline ($P = 0.0004$) were observed. Insulin levels were increased in the T_2 time segment ($P = 0.0024$) after 2.5 mg and in the T_1 ($P = 0.0120$) and T_2 ($P = 0.0073$) time segments after 5.0 mg orally. Apparent increments in the T_1 time segment ($P = 0.0163$) followed 0.25 mg subcutaneously. The latter also raised C-peptide in the T_1 ($P = 0.002$), T_2 ($P = 0.0029$), and T_3 ($P = 0.0003$) time segments (data not shown).

Terbutaline administration, most notably the 0.25 mg subcutaneous dose, to nondiabetic subjects also increased plasma NE ($P = 0.0151$) (Fig. 3). No significant changes in plasma glucagon, EPI, pancreatic polypeptide, growth hormone, or cortisol were noted (data not

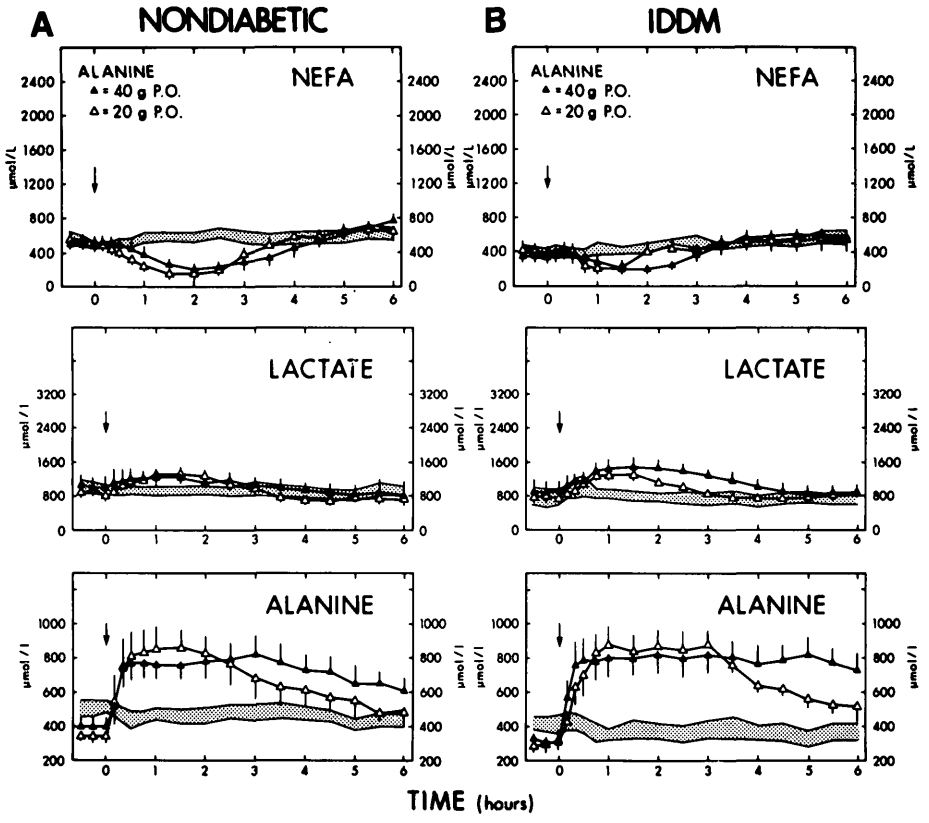

Figure 2—*Mean ± SE serum NEFA and blood lactate and Ala concentrations before and after administration of Ala to nondiabetic subjects (A) and insulin-infused IDDM patients (B). The shaded areas are one SE around the mean for data before and after administration of placebos. (P.O.), per os.*

shown). Serum NEFA and blood β-OHB appeared to increase, at least after 5.0 mg orally and 0.25 mg subcutaneously (Fig. 4), but these were not significant. However, NEFA increased significantly in the T_1 time segment ($P = 0.0104$) after subcutaneous terbutaline. Blood lactate showed an apparent but insignificant dose-related increase (Fig. 4); lactate was increased significantly in the T_3 time segment ($P = 0.0006$) after subcutaneous terbutaline. Blood Ala showed no change (data not shown). Heart rate appeared to increase slightly, at least after subcutaneous terbutaline, but this was not significant; there were no changes in blood pressure (data not shown).

IDDM patients. Compared with placebos, oral terbutaline administration to insulin-infused, initially euglycemic IDDM patients resulted in substantial dose-related increments in plasma glucose (Fig. 3). Mean plasma glucose concentrations increased from 5.4 ± 0.1 to 10.2 ± 1.0 mM at 330 min after ingestion of 2.5 mg of terbutaline ($P = 0.0078$) and from 5.1 ± 0.3 mM to a peak of 14.0 ± 0.6 mM at 300 min after ingestion of 5.0 mg of terbutaline ($P = 0.0001$). Increments were apparent as early as 20 min after terbutaline ingestion. Similar increments in plasma glucose (from 5.6 ± 0.3 to 10.3 ± 0.7 mM) followed subcutaneous administration of 0.25 mg of terbutaline ($P = 0.0017$). Increments were apparent at the first sampling point, 10 min after injection (Fig. 3). Notably, inhalation of 0.4 mg of terbutaline resulted in plasma glucose increments (from 5.1 ± 0.3 to 10.0 ± 1.5 mM at 300 min) over a time course similar to that following 2.5 mg orally. There were, of course, no changes in plasma insulin (Fig. 3) or C-peptide (data not shown).

As in the nondiabetic subjects, terbutaline administration, at least the 0.25 mg subcutaneous dose ($P = 0.0159$ in the T_1 time segment) raised plasma NE (Fig. 3). No significant changes in plasma glucagon, EPI, pancreatic polypeptide, growth hormone, or cortisol were noted (data not shown). Serum NEFA and blood β-OHB appeared to increase (2.5 mg, $P = 0.0113$; 5.0 mg, $P = 0.0015$), more prominently than in nondiabetic subjects, after terbutaline administration (Fig. 4). Serum NEFA increased significantly in the T_2 ($P = 0.0001$) and T_3 ($P = 0.0045$) time segments after 5.0 mg orally and in the T_1 ($P = 0.0038$) time segment after 0.25 mg subcutaneously. Similarly, blood β-OHB was increased in the T_2 time segment ($P = 0.0078$) after 5.0 mg orally and in the T_1 ($P = 0.0118$) and T_2 ($P = 0.0026$) time segments after 0.25 mg subcutaneously. There was a dose-related increase in blood lactate (2.5 mg, NS; 5.0 mg, $P = 0.0091$) (Fig. 4), but no change in blood Ala (data not shown). Heart rate appeared to increase slightly, at least after subcutaneous terbutaline ($P = 0.0155$); there were no changes in blood pressure (data not shown).

No symptoms were attributable to either Ala or terbutaline administration. However, several subjects found the taste of the Ala solution unpleasant.

CONCLUSIONS— These data document minimal glycemic effects of Ala and terbutaline in nondiabetic subjects but substantial glycemic effects of Ala and terbutaline in insulin-infused, initially euglycemic IDDM patients. This difference is undoubtedly the result of the inability of the latter patients to secrete insulin in response to the agents administered, to rising plasma glucose concentrations, or both.

Although glucose kinetics were

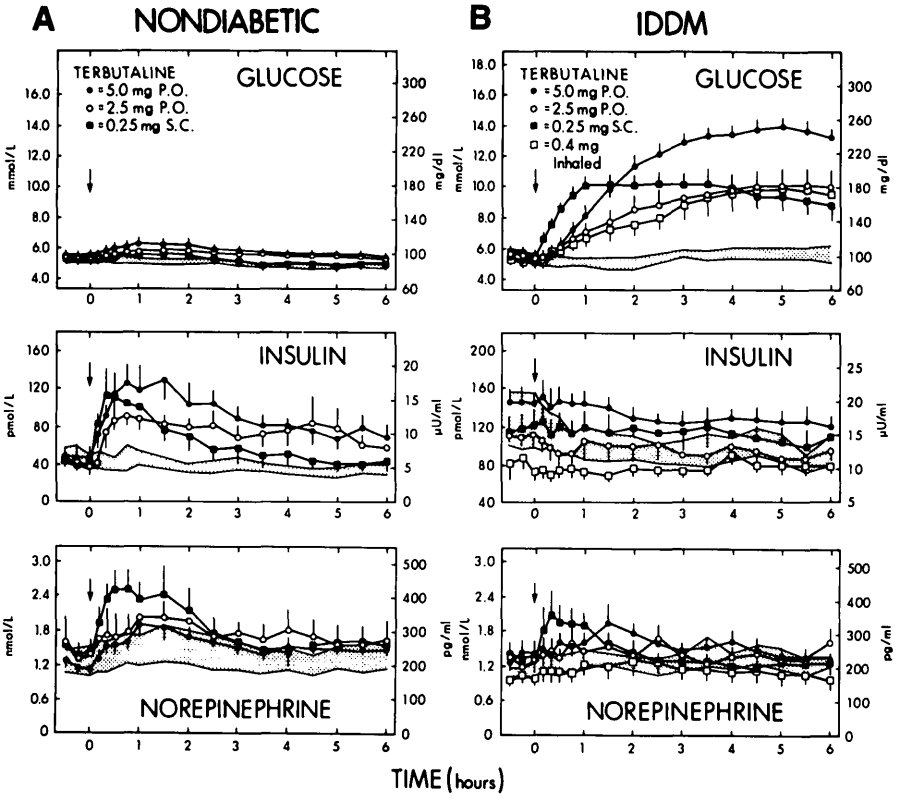

Figure 3—*Mean ± SE plasma glucose, insulin, and NE concentrations before and after administration of terbutaline to nondiabetic subjects (A) and insulin-infused IDDM patients (B). The shaded areas are one SE around the mean for data before and after administration of placebos. (P.O.), per os; (S.C.), subcutaneously.*

not measured in this study, the mechanisms of the glycemic actions of Ala and terbutaline in IDDM are almost assuredly different. Ala stimulated glucagon secretion. Glucagon would tend to increase hepatic glucose production and plasma glucose (5,6), an effect that would be countered by increased insulin secretion in nondiabetic individuals but not those with IDDM. By analogy with the β_2-adrenergic actions of EPI (7), terbutaline would be expected to both stimulate hepatic glucose production and limit glucose utilization directly and thus increase plasma glucose; again, effects that would be countered by increased insulin secretion in nondiabetic individuals but not those with IDDM. Among the indirect glycemic actions, these data indicate that terbutaline increases plasma NE concentrations. This must reflect stimulation of sympathetic neural NE release because plasma EPI levels, a sensitive marker of adrenomedullary secretion (23), did not increase. This effect could be the result of stimulation of prejunctional β_2-adrenergic receptors on sympathetic axon terminals (24), a CNS effect, or both. Finally, terbutaline raised serum NEFA levels, which might have contributed to the glycemic response.

In the nondiabetic subjects, transient decrements in NEFA levels followed Ala ingestion. This is best attributed to the potent antilipolytic effect of increased insulin levels. Ala ingestion had no clear effect on ketogenesis, as evidenced by unaltered β-OHB levels in both groups. It did increase blood lactate levels, most prominently in the IDDM patients. Because lactate utilization as an hepatic gluconeogenic precursor was probably increased, at least in the latter group, this finding suggests increased peripheral lactate production from Ala per se, increased glycolysis, or both. Finally, blood Ala levels rose, as expected, after Ala ingestion. Although peak concentrations did not differ, Ala elevations were more sustained after ingestion of 40 compared with 20 g.

Terbutaline administration increased NEFA and blood β-OHB levels prominently in IDDM patients with small (and statistically nonsignificant) changes in these in nondiabetic subjects. This difference almost assuredly reflects increased insulin secretion in the latter, but not the former, group. The increments in NEFA and β-OHB imply that the β2-adrenergic agonist stimulated lipolysis and ketogenesis, respectively. Because human fat cells contain β2-adrenergic as well as β1-adrenergic receptors (25,26), this effect could have been the result of the β2-adrenergic action of the drug, the β1-adrenergic (greater than β2-adrenergic) action of endogenous NE, or both. Mediation through a variant β-adrenergic receptor subtype (27) cannot be excluded. Terbutaline also raised blood lactate levels. If lactate utilization was increased under these conditions, this suggests increased lactate mobilization in response to the agonist. Blood Ala levels were unchanged after terbutaline administration.

In the IDDM patients, the glycemic effect of Ala ingestion was apparent at 20 min, but not 10 min after ingestion, peaked at 3–4 h, and persisted throughout the 6-h study period. Note that the latter occurred despite the ongoing infusion of insulin in doses that were sufficient to maintain euglycemia at baseline and after placebo administration. The glycemic response was dose-related with peak plasma glucose concentrations of 8.3 ± 0.3 and 10 ± 1.0 mM after ingestion of 20 and 40 g of Ala, respectively. In the patients, the glycemic effect of terbutaline ingestion was also apparent at

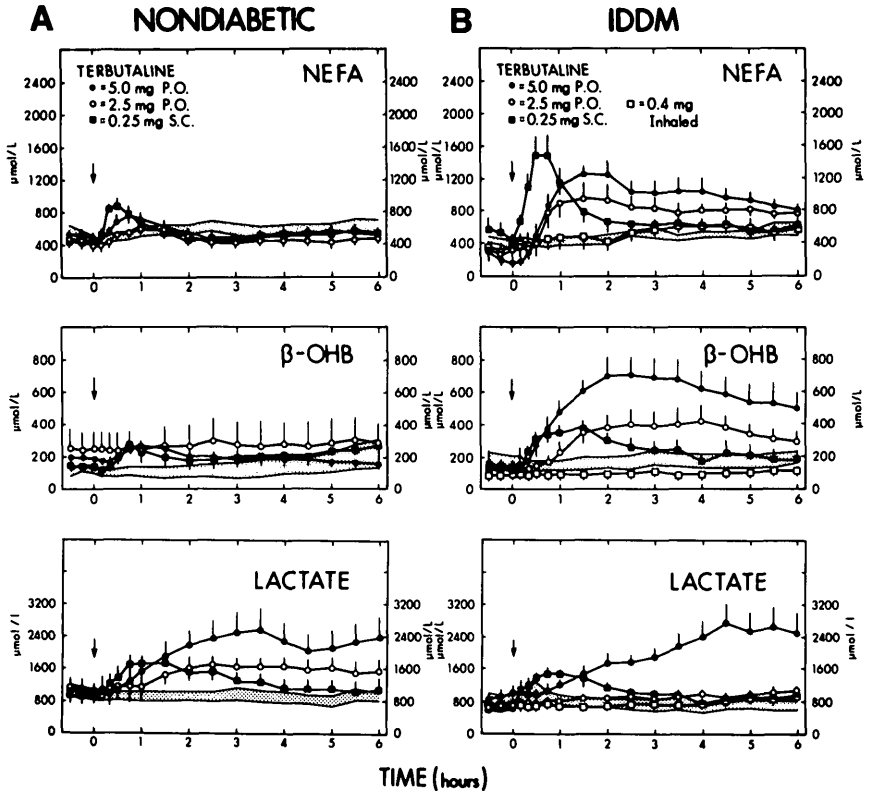

Figure 4—*Mean ± SE serum NEFA and blood β-OHB and lactate concentrations before and after administration of terbutaline to nondiabetic subjects (A) and insulin-infused IDDM patients (B). The shaded areas are one SE around the mean for data before and after administration of placebos. (P.O.), per os; (S.C.), subcutaneously.*

20 min, but not 10 min after ingestion. It peaked at 4–5 h and persisted throughout the 6-h study period. The glycemic response was dose-related with peak plasma glucose concentrations of 10.2 ± 1.0 and 14.0 ± 0.6 mM after ingestion of 2.5 and 5.0 mg of terbutaline, respectively. As expected, the glycemic response to subcutaneous terbutaline was more rapid. Plasma glucose levels were clearly increased at the first sampling point, 10 min after injection. Peak glucose levels (10.3 ± 0.7 mM) were achieved at 60 min. However, plasma glucose levels remained elevated throughout the 6-h study period. In contrast, the glycemic response to inhaled terbutaline (0.4 mg) was delayed (and quite comparable to an ingested dose of 2.5 mg).

In summary, these data demonstrate substantially greater glycemic responses to Ala and terbutaline administration in IDDM patients than in nondiabetic subjects and suggest different mechanisms of the glycemic actions of these two agents. Thus, Ala and terbutaline represent potential new approaches to the treatment, and perhaps the prevention, of iatrogenic hypoglycemia in IDDM patients. It would be premature, however, to advocate their use for that purpose, because they first need to be tested in a clinically appropriate model of insulin-induced hypoglycemia in IDDM patients. This has been done (this issue, B.V. Wiethop and P.E. Cryer, p. 1131–36).

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