Effect of Plasma Amino Acid Replacement on Glucagon and Substrate Responses to Insulin-Induced Hypoglycemia in Humans

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A defective glucagon response impairs glucose recovery from insulin-induced hypoglycemia in some insulin-dependent (type I) diabetic patients. Our objective was to determine whether the glucagon reponse to insulin-induced hypoglycemia could be stimulated in nondiabetic humans. Because insulin reduces plasma amino acid concentrations, and amino acids are known to stimulate glucagon secretion, we investigated the effect of amino acid replacement during insulin infusion on the glucagon response to hypoglycemia in six healthy nondiabetic subjects. In two separate studies, blood glucose was clamped at the postabsorptive level during a constant infusion of insulin (0.05 U · kg^{-1} · h^{-1}) for 3 h. Blood glucose was then reduced to 45 mg/dl over 20 min. Hormone and substrate concentrations during recovery from hypoglycemia were monitored for 2 h. In the control study, normal saline was infused, and in the other study, an amino acid mixture was infused to prevent the insulin-induced fall in plasma amino acids. Amino acid replacement did not change the basal glucagon levels but resulted in a more robust glucagon response to hypoglycemia (from 184 ± 24 to 292 ± 36 pg/ml) than in the control study (from 176 ± 30 to an average 229 ± 32 pg/ml, $P < 0.01$). Plasma concentrations of epinephrine, norepinephrine, cortisol, and growth hormones increased during hypoglycemia, but the magnitude of the response was not different between the control and amino acid–replacement studies. Amino acid replacement did not affect glucose recovery but inhibited the recovery of plasma concentrations of free fatty acids, glycerol, and β-hydroxybutyrate. We conclude that the glucagon response to insulin-induced hypoglycemia is enhanced by amino acid replacement in humans and that changes in plasma amino acid concentrations modulate the effect of stress hormones on lipid metabolism during hypoglycemia. *Diabetes* 39:376–82, 1990

Cortisol 27.6 nM	= 0.360 pg/ml	Growth hormone 1 μg/L	= 1 ng/ml
Epinephrine 5.46 pM	= 0.183 pg/ml	Insulin 7.18 pM	= 0.139 μU/ml
Glucagon 1 ng/L	= 1 pg/ml	Norepinephrine 0.006 nM	= 169 pg/ml
Glucose 1 mM	= 18 mg/dl		

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Diminished glucagon response to insulin-induced hypoglycemia is reported to delay recovery from hypoglycemia in some insulin-dependent (type I) diabetic patients (1–3). In these patients, the glucagon response to arginine infusion is intact, indicating a functional defect of α-cells of the pancreas during hypoglycemia. The cause of this defective glucagon response remains elusive (1–5). Recurrent hypoglycemic attacks during meticulous diabetic control are frequently observed (2). Our objective was to determine whether we could stimulate α-cell response to insulin-induced hypoglycemia.

In this study, we examined the hypothesis that plasma concentrations of amino acids modulate the α-cell response to hypoglycemia. It is well known that, after a protein meal or amino acid infusion, insulin secretion is stimulated, but hypoglycemia is prevented by glucagon secretion (2,6,7). After a protein meal, plasma amino acid concentrations are high, but unlike after a protein meal, insulin administration alone decreases plasma concentrations of several amino acids by inhibition of proteolysis besides causing hypoglycemia (8,9). Even though many amino acids are known to stimulate glucagon secretion, the effect of amino acids on α-cell response to hypoglycemia is not described. We therefore examined whether preventing the fall in plasma amino acids during insulin infusion before insulin-induced hypoglycemia enhances the glucagon response to hypoglycemia. Because hypoglycemia is associated with increases of epinephrine, norepinephrine, cortisol, and growth hormone, we also determined the effect of replacement of plasma

amino acids on these hormones and on the recovery of glucose and other substrates. These studies were performed in healthy nondiabetic subjects to determine the physiological significance of plasma amino acid concentrations on glucagon secretion and the metabolism of other substrates during hypoglycemia.

RESEARCH DESIGN AND METHODS

Six healthy subjects (2 women, 4 men: wt 74.3 + 5.4 kg; body mass index [wt/ht^2] 21.8 ± 0.3 kg/m^2; age 22.4 ± 0.9 yr) were studied. The protocol was approved by the Committee on Investigations Involving Human Subjects and the Clinical Research Center Advisory Committee of the University of Rochester. All subjects gave written consent to participate after all procedures and risks were clearly explained.

Two separate studies were performed at 1- to 2-wk intervals. Identical protocols were followed for both studies except that normal saline was infused in one and an amino acid mixture was infused in the other (for composition of the infused amino acid mixture, see Table 1). This special mixture of amino acids was prepared to replace the amino acids that are normally reduced during insulin infusion.

The subjects consumed 2813 ± 116 kcal/day (53:32:15 carbohydrate/fat/protein) for 3 days before each study. Studies were performed after an overnight fast. Blood samples were collected from a heated (70°C) hand vein to get arterialized venous samples. On both occasions, an intravenous infusion of purified regular pork insulin (0.05 U · kg^{-1} · h^{-1}) was started at 0800, and variable doses of 20% dextrose solution were given intravenously to clamp blood glucose at the postabsorptive level for 3 h (10). Dextrose infusion rate was adjusted at 5- to 10-min intervals based on blood glucose measurements (glucose analyzer, YSI). After 3 h, blood glucose was decreased at 2.5- to 5-min intervals by reducing the dextrose infusion rate to achieve a blood glucose level of 45 mg/dl over 20 min. When blood glucose reached 45 mg/dl, dextrose and insulin infusions were discontinued, and blood samples were collected every 15 min until the end of the study to measure glucose, free fatty acids (FFAs), β-*O*-hydroxybutyrate (βOHB), glycerol, amino acids, glucagon, epinephrine, norepinephrine, cortisol, growth hormone, and insulin. The stepwise decrement in dextrose infusion rate ensured an identical blood glucose nadir in the paired experiments. During the entire 5-study period, an infusion of normal saline (control) or amino acids was given. The amino acid mixture was prepared to prevent the fall in plasma amino acids that normally occurs during insulin infusion. In both studies, a continuous infusion of [6,6-$^2H_2$]glucose (3 mg · kg^{-1} · h^{-1}) was given during the entire study period (0 time point until the end of the study) after taking baseline blood samples for measuring the [$^2H_2$]glucose enrichment (11). For calculations of glucose production, blood samples for measurement of [$^2H_2$]glucose enrichment were taken at 120 and 180 min and then at 15-min intervals until the end of the study.

TABLE 1
Amino acid composition (g) in infusate

L-Serine	3
L-Glutamine	15
L-Aspartate	2
L-Histidine	2
L-Threonine	5
L-Glycine	3
L-Arginine hydrochloride	3
L-Valine	8
L-Leucine	8
L-Lysine	3
L-Isoleucine	5
L-Phenylalanine	4
L-Glutamic acid	2

Plasma concentrations of amino acids were measured by high-performance liquid chromatography with postcolumn *o*-phthalaldehyde derivatization (12). Plasma concentrations of βOHB (13), FFA (14), glycerol (15), glucagon (16), insulin (Cambridge Nuclear radioimmunoassay kit, Los Angeles, CA), growth hormone (17), cortisol (Diagnostic Products radioimmunoassay kit, Los Angeles, CA), epinephrine, and norepinephrine (18) were measured as previously described. Plasma [$^2H_2$]glucose enrichment was measured by selective ion monitoring by a gas-chromatograph mass spectrometer as previously described (11).

Approximate values for plasma glucose appearance and disappearance rates were calculated from Steele's equation (19), modified for non-steady-state conditions (20). The modification of this calculation for stable isotopes and its limitations are discussed elsewhere (11). Because we used stable isotopes, isotope enrichment expressed decimally was substituted for specific activity in the appropriate equation. The assumption in the calculations is that the isotopically labeled glucose is handled in a manner identical to the unlabeled glucose. Because stable isotope molecules contribute to the pool size, a slight error in the calculated glucose kinetics is unavoidable. Because these measurements with Steele's equations are approximations, and the same small error is equally applicable to all the groups we studied, the conclusions from these measurements are valid. The endogenous glucose appearance was estimated by deducting the glucose infusion rate from the calculated glucose appearance rate from the tracer.

Data are given as means ± SE. Paired *t* tests were performed to test the significance of difference in baseline values between the two paired studies. Analysis of variance for measurements repeated over time (Scheffe's multiple comparisons) was performed to assess the effect of hypoglycemia, with or without amino acid replacement, on glucagon and other hormones and substrates. $P < 0.05$ was considered statistically significant.

RESULTS

Plasma amino acids. When insulin and glucose alone were infused, there was a significant decrease in the plasma concentrations of arginine, cystine, isoleucine, leucine, valine, methionine, phenylalanine, serine, threonine, tyrosine, glutamine, glutamic acid, and lysine ($P < 0.05$; Table 2). The amino acid–mixture infusion prevented the decrease of all of these amino acids with the exception of tyrosine. Methionine and phenylalanine were slightly overreplaced ($P < 0.05$). At 300 min, leucine, isoleucine, and valine were also significantly above the baseline ($P < 0.05$) when the amino acid mixture was infused.

Glucose. Infusion rates of dextrose between the control

TABLE 2
Plasma concentrations of amino acids during glucose clamp without and with amino acid replacement (AA)

	Time (min)									
	0		60		180		240		300	
Amino acids	Control	AA	Control	AA	Control	AA	Control	AA	Control	AA
Alanine	259 ± 34	253 ± 29	248 ± 23	256 ± 21	244 ± 25	253 ± 19	248 ± 19	259 ± 22	230 ± 3*	239 ± 21
Arginine	66 ± 9	84 ± 5	59 ± 7	76 ± 8	50 ± 4*	72 ± 5	48 ± 6*	66 ± 7	48 ± 11*	81 ± 12
Cystine	21 ± 4	19 ± 4	15 ± 3	19 ± 3	13 ± 2*	20 ± 6	16 ± 2*	17 ± 2	15 ± 2*	21 ± 1
Glycine	144 ± 9	143 ± 13	142 ± 15	154 ± 13	137 ± 13	153 ± 9	125 ± 8*	133 ± 8	131 ± 10*	130 ± 7
Glutamine	438 ± 9	410 ± 20	403 ± 16	410 ± 18	360 ± 14*	390 ± 19	339 ± 14*	344 ± 21*	379 ± 29	368 ± 25
Glutamic acid	57 ± 5	59 ± 4	45 ± 4*	57 ± 3	46 ± 5*	43 ± 2*	53 ± 4	53 ± 4	50 ± 1	62 ± 7
Isoleucine	48 ± 1	56 ± 3	33 ± 1*	74 ± 2	17 ± 1*	62 ± 4	22 ± 3	63 ± 3	31 ± 3	73 ± 5*
Leucine	98 ± 4	104 ± 5	73 ± 3*	133 ± 3	44 ± 2*	113 ± 6	52 ± 5*	113 ± 4	67 ± 5*	140 ± 4*
Methionine	17 ± 1	17 ± 2	14 ± 1*	33 ± 1*	8 ± 1*	32 ± 2*	8 ± 2*	32 ± 1*	7 ± 1*	35 ± 1*
Phenylalanine	42 ± 2	43 ± 2	38 ± 3*	66 ± 2	28 ± 2*	74 ± 3	28 ± 2*	68 ± 2	31 ± 2*	70 ± 3
Serine	76 ± 3	76 ± 4	70 ± 6	76 ± 5	54 ± 3*	67 ± 6	53 ± 5*	62 ± 5*	60 ± 4*	68 ± 4
Threonine	83 ± 4	84 ± 4	71 ± 4	103 ± 5	61 ± 3*	98 ± 5	59 ± 5*	99 ± 5	60 ± 4*	107 ± 4
Tyrosine	43 ± 4	44 ± 5	34 ± 2*	34 ± 4	20 ± 2*	23 ± 4*	25 ± 1*	24 ± 3	30 ± 3	31 ± 2
Valine	183 ± 8	187 ± 9	167 ± 10*	250 ± 6	127 ± 7*	248 ± 8	126 ± 5*	251 ± 5	139 ± 8*	292 ± 16*

Values are means ± SE in μmol.
*$P < 0.05$ vs. baseline.

studies and amino acid replacements were not different (Fig. 1). Plasma concentrations of glucose before, during, and at the time of recovery from hypoglycemia were similar in both studies (Fig. 1). Endogenous glucose appearance rate increased significantly during hypoglycemia and then decreased ($P < 0.05$). There were no significant differences between the studies. There was no difference between control studies and amino acid studies in the pattern of glucose disappearance rate (Fig. 2).

FFAs, glycerol, and βOHB. In both studies, plasma concentrations of FFA and βOHB decreased from the baseline level to very low levels during the combined infusions of insulin and dextrose (Table 3; Fig. 3). Before hypoglycemia, plasma concentrations of FFA, glycerol, and βOHB were similar in both control and amino acid studies. During hypoglycemia and the recovery from hypoglycemia, plasma concentrations of FFA increased ($P < 0.001$), but the magnitude of increase in FFA was lower during amino acid replacement than during the control study ($P < 0.001$). Plasma concentrations of glycerol were measured in five of the six subjects. Plasma glycerol concentration was in a steady state before hypoglycemia. There was no difference between the two studies. In both studies, there was a significant elevation of plasma glycerol during the recovery phase of hypoglycemia ($P < 0.02$). The increase in plasma glycerol during hypoglycemia was significantly higher during the control study than the amino acid study ($P < 0.001$). In the control study, there was an increase of βOHB during the hypoglycemia and recovery phase ($P < 0.01$; Fig. 2). The increment of βOHB during amino acid replacement was lower than during the control study ($P < 0.01$).

Hormonal concentrations. Plasma concentrations of insulin remained steady during the first 3 h of the study and then decreased significantly in a similar fashion during both the amino acid and saline infusions (Fig. 4). There was no significant difference in plasma concentrations of insulin between saline and amino acid experiments during the entire study.

Plasma concentrations of cortisol and growth hormone were steady during the first 3 h of the study in both experiments. Both hormones increased during the period of hypoglycemia ($P < 0.05$), but there were no differences in the response to hypoglycemia between the two experiments.

During the first 3 h of each study, plasma concentrations of glucagon did not change, and there was no significant difference in the plasma glucagon values between the two experiments. In both experiments, there was a significant increase in plasma concentrations of glucagon during hypoglycemia ($P < 0.01$), and then there was a decline compared with the peak value ($P < 0.01$; Fig. 5). During amino acid infusion, the increment in plasma concentrations of glucagon was higher than during saline infusion ($P < 0.003$).

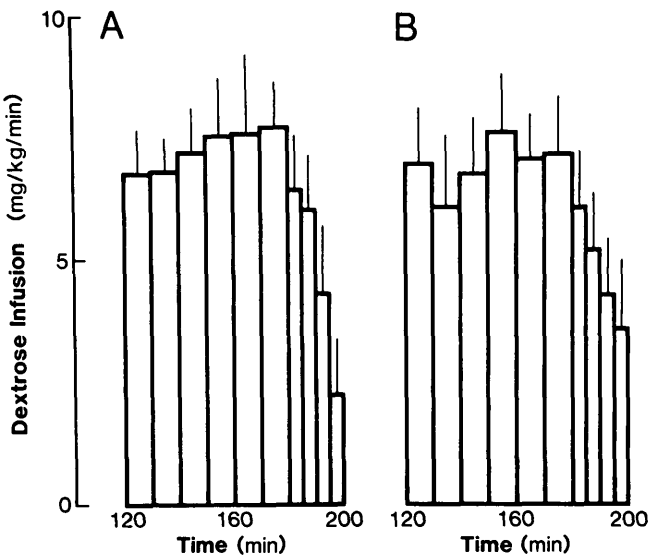

FIG. 1. Dextrose infusion rate during control (*A*) and amino acid–infusion (*B*) studies. There was no significant difference between studies.

TABLE 3
Baseline plasma concentrations of hormones and substrates in postabsorptive state

	Control study	Amino acid study
Insulin (μU/ml)	14 ± 3	14 ± 3
Glucagon (pg/ml)	202 ± 51	196 ± 46
Cortisol (μg/ml)	15 ± 3	11 ± 46
Growth hormone (ng/ml)	2.2 ± 0.8	1.5 ± 0.6
Epinephrine (pg/ml)	76 ± 19	60 ± 13
Norepinephrine (pg/ml)	133 ± 16	132 ± 11
Glucose (mg/dl)	86 ± 2	84 ± 2
Free fatty acids (μeq/L)	490 ± 35	486 ± 47
Glycerol (μM)	171 ± 20	151 ± 20
β-Hydroxybutyrate (μM)	80 ± 10	66 ± 10

Plasma concentrations of epinephrine and norepinephrine did not change during the first 180 min, and then there was a significant increase ($P < 0.001$), which reached a peak at 225 min. After 225 min, epinephrine and norepinephrine levels decreased from the peak value ($P < 0.01$). There was no significant difference between the two experiments during the entire study period.

DISCUSSION

In this study, we demonstrated that, in healthy human subjects, the glucagon response to hypoglycemia is enhanced when the fall in plasma amino acids during insulin infusion is prevented. Although many amino acids stimulate glucagon secretion, the amount of amino acids we infused in this study was not enough to stimulate glucagon secretion in the basal state (21). However, this small increase in plasma amino acid concentrations seems to have modulated the α-cell response to insulin-induced hypoglycemia. It is well known that many amino acids stimulate α-cell secretion in the basal state, and our findings demonstrate that amino acids also enhance α-cell response to insulin-induced hypoglycemia.

The enhanced glucagon response, however, failed to accelerate glucose recovery. The reason for this is not entirely clear. Persistent insulin action may have inhibited the effect of glucagon on hepatic glucose production, although a significant increase in glucose production in both studies argues against this possibility. It has been demonstrated that glucagon is not essential for recovery from hypoglycemia when epinephrine and norepinephrine responses to hypoglycemia are intact (5). In the same study, Rizza et al. also infused somatostatin and glucagon during hypoglycemia, increasing both peripheral (>2.2 times) and hepatic levels of glucagon (theoretically by 22%) higher than the control subjects. Careful review of their results shows that increased glucagon level did not accelerate glucose production. Increase in plasma glucagon during amino acid infusion in our subjects was comparable to that of Rizza et al. (5). Note that in the control study, increased levels of epinephrine, norepinephrine, stimulated glucagon (>30% from baseline), and hypoglycemia in conjunction stimulated glucose recovery. Even though no data are available on the maximal hepatic glucose output possible during hypoglycemia, in our control study, the hepatic glucose output was similar or higher than has been reported in the literature (4,5). A possibility is that hepatic glucose production is maximally stimulated without amino acid infusion, and a further modest increase in glucagon response may not augment glucose production any further. It remains to be tested whether the glucagon dose-response curve is any different during hypoglycemia than during the euglycemic state. Glucose production is probably modulated by the availability of substrates for gluconeogenesis. Thus, reduced lipolysis may have decreased substrate availability for glucose production, causing the lack of increase in glucose production due to enhanced glucagon levels. Recent studies by Caprio et al. (22) indicate that FFA concentrations in blood had an effect on carbohydrate metabolism.

Another important finding of our study was the demonstration of diminished FFA, glycerol, and βOHB recoveries during hypoglycemia when the amino acid mixture was infused. A 3-h insulin infusion and glucose clamp reduced

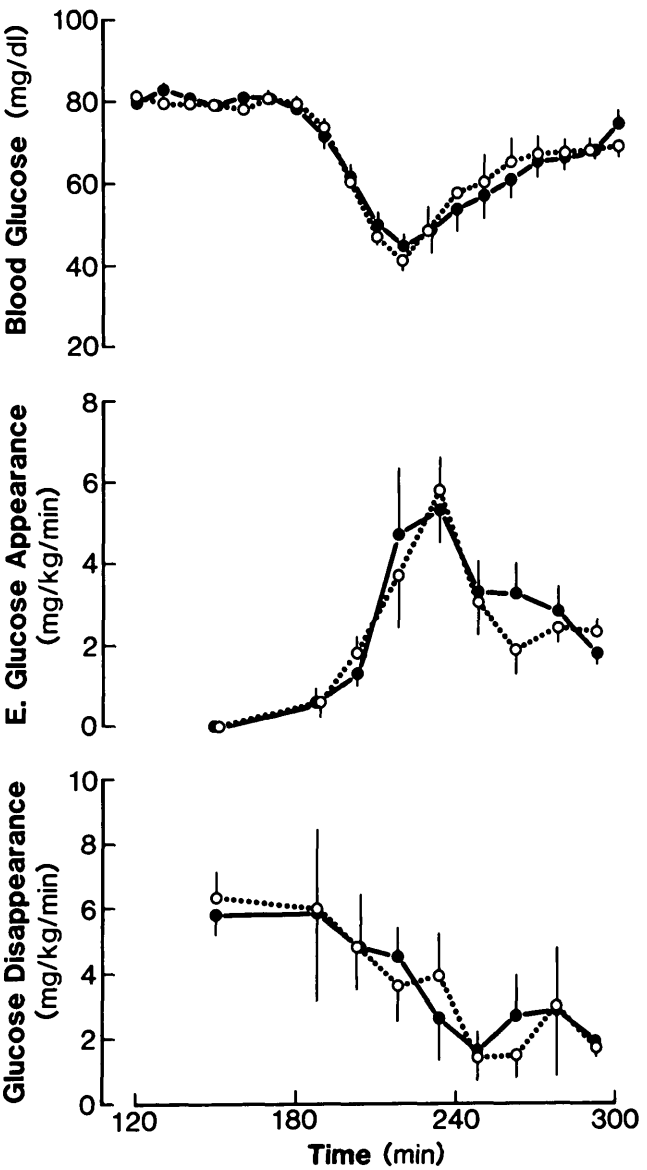

FIG. 2. Blood glucose and glucose kinetics in control (○) and amino acid–infusion (●) studies. There was no difference in blood glucose and endogenous (E.) glucose appearance and disappearance rates between groups.

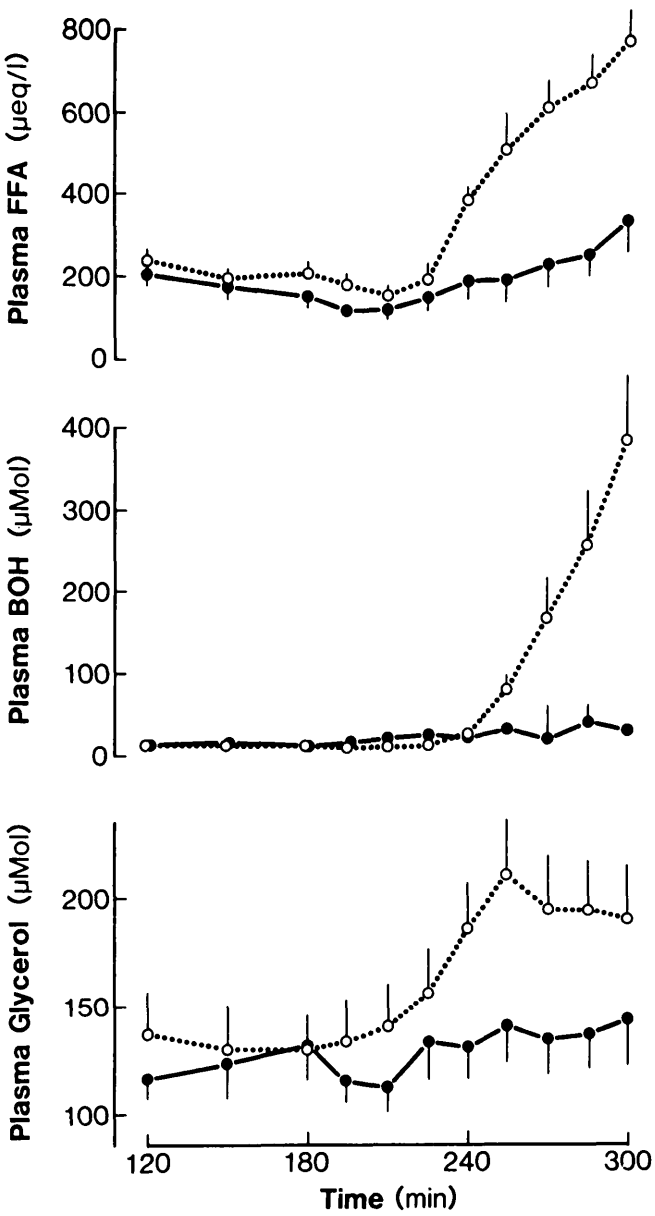

FIG. 3. Plasma concentrations of free fatty acids (FFA), β-O-hydroxybutyrate (BOH), and glycerol in control (○) and amino acid–infusion (●) studies. Rebound increment in FFA, BOH, and glycerol were lower during amino acid infusion than during control study ($P < 0.01$).

plasma concentrations to a very low level. During hypoglycemia, an enhanced sympathetic response, growth hormone and cortisol secretions increased the plasma concentrations of these substrates as expected. Although it enhanced glucagon secretion, amino acid infusion inhibited the recovery of these substrates. There is no evidence that changes in these substrates are related to the increased glucagon level. In fact, glucagon is known to enhance ketogenesis in the liver and stimulates lipolysis in vitro (23). Reduced plasma concentrations of FFA suggest reduced lipolysis, increased clearance of FFA, or enhanced reesterification. Decreased plasma glycerol concentrations indicate that decreased lipolysis is the most likely explanation for the low FFA levels observed during amino acid infusion.

Even though diminished lipolysis may have caused the reduced FFA and βOHB recovery, the mechanism of this is unclear from this study. Plasma concentrations of norepinephrine, cortisol, and growth hormone are not different between the studies. Although plasma concentrations of norepinephrine are not different between the studies, the possibility of reduced sympathetic activity in adipose tissue during amino acid infusions cannot be excluded. The increased plasma concentrations of amino acids may have modulated the effect of these lipolytic hormones. Studies by Cersosimo et al. (24) indicate that a physiological increment in glutamine inhibits lipolysis in dogs. In our subjects, plasma concentrations of glutamine were not different from the baseline when amino acids were infused but were lower than baseline when no amino acids were infused. The observed effect of amino acid infusion on the recovery of FFA, glycerol,

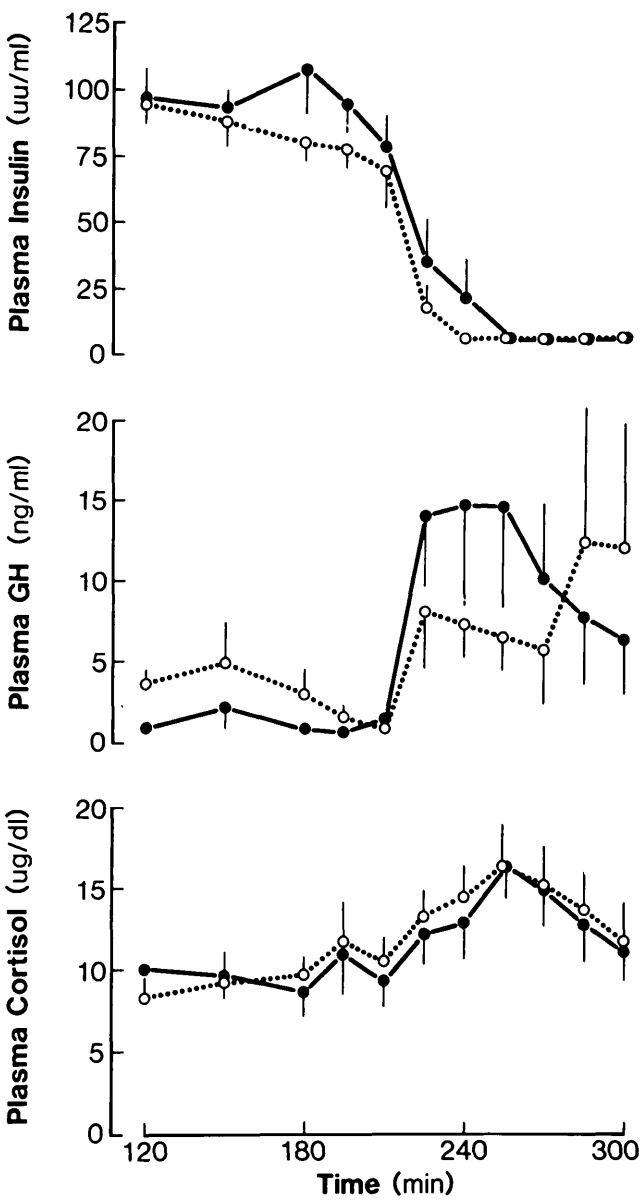

FIG. 4. Plasma concentrations of insulin, growth hormone (GH), and cortisol in control (○) and amino acid–infusion (●) studies. There was no significant difference between studies.

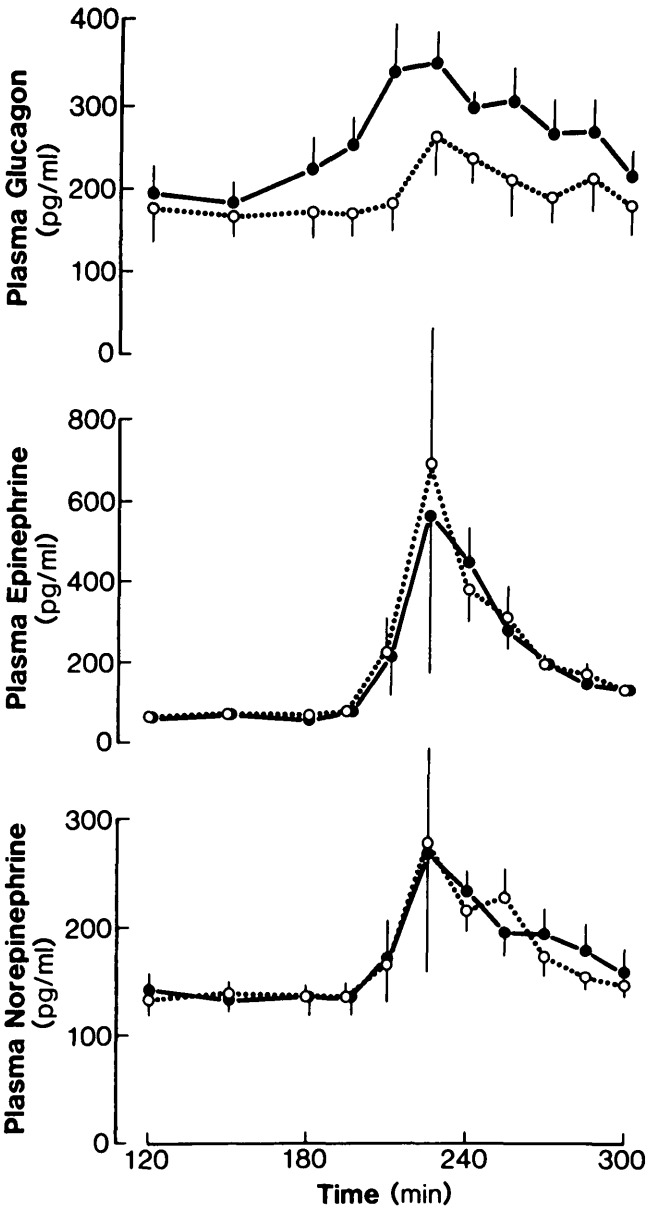

FIG. 5. Plasma concentrations of glucagon, epinephrine, and norepinephrine in control (○) and amino acid–infusion (●) studies. Glucagon levels were higher during hypoglycemia on amino acid–infusion study than during control study ($P < 0.01$).

and βOHB during hypoglycemia may be due to glutamine alone or the combined effect of all the amino acids. It is not known whether a similar effect is exhibited by other amino acids. Alanine has been shown to be antiketogenic, but alanine levels were not different between our paired experiments (25). Similar effects have not been demonstrated for any other amino acids. A decreased βOHB recovery is presumably due to the reduced availability of FFA (26).

It has been reported that secretion of epinephrine and norepinephrine is diminished in diabetic patients after intensive insulin treatment (27). Adrenergic sensitivity is also reported to be diminished in type I diabetic patients of all ages (28). We postulate that in these patients, enhanced glucagon responses to hypoglycemia are critical for recovery from hypoglycemia.

We conclude from our study that amino acid replacement during insulin infusion enhances the glucagon response to hypoglycemia. Although the enhanced glucagon secretion did not improve glucose recovery in nondiabetic subjects during hypoglycemia, we propose that such an enhanced glucagon secretion is critical for glucose recovery from hypoglycemia in type I diabetic patients with autonomic neuropathy. The inhibition of diminished FFA and βOHB recovery during amino acid infusion suggests a modulation of catabolic hormonal action by amino acids.

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