Intraislet Hyperinsulinemia Prevents the Glucagon Response to Hypoglycemia Despite an Intact Autonomic Response

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Because absence of the glucagon response to falling plasma glucose concentrations plays a key role in the pathogenesis of iatrogenic hypoglycemia in patients with insulin-deficient diabetes and the mechanism of this defect is unknown, and given evidence in experimental animals that a decrease in intraislet insulin is a signal to increased glucagon secretion, we examined the role of endogenous insulin in the physiological glucagon response to hypoglycemia. We tested the hypothesis that intraislet hyperinsulinemia prevents the glucagon response to hypoglycemia despite an intact autonomic—adrenomedullary, sympathetic neural, and parasympathetic neural—response and a low α-cell glucose concentration. Twelve healthy young adults were studied on three separate occasions. Insulin was infused in hourly steps in relatively low doses (1.5, 3.0, 4.5, and 6.0 pmol · kg^{-1} · min^{-1}) from 60 through 300 min on all three occasions. Plasma glucose levels were clamped at euglycemia (~5.0 mmol/l, ~90 mg/dl) on one occasion and at hourly steps of ~4.7, 4.2, 3.6, and 3.0 mmol/l (~85, 75, 65, and 55 mg/dl) from 60 through 300 min on the other two occasions. On one of the latter occasions, the β-cell secretagogue tolbutamide was infused in a dose of 1.0 g/h from 60 through 300 min. Hypoglycemia with tolbutamide infusion, compared with similar hypoglycemia alone, was associated with higher ($P < 0.0001$) C-peptide levels (final values of 1.0 ± 0.2 vs. 0.1 ± 0.0 nmol/l), higher ($P < 0.0001$) rates of insulin secretion (final values of 198 ± 60 vs. 15 ± 4 pmol/min), and higher ($P < 0.0001$) insulin levels (final values of 325 ± 30 vs. 245 ± 20 pmol/l) as expected. The glucagon response to hypoglycemia was prevented during tolbutamide infusion ($P < 0.0001$). Glucagon levels were 17 ± 1 pmol/l at baseline on both occasions, 14 ± 1 vs. 15 ± 1 pmol/l, respectively, during the initial hyperinsulinemic euglycemia, and 15 ± 1 vs. 22 ± 2 pmol/l, respectively, during hypoglycemia with and without tolbutamide infusion. Autonomic—adrenomedullary (plasma epinephrine), sympathetic neural (plasma norepinephrine), and parasympathetic neural (plasma pancreatic polypeptide)—responses to hypoglycemia were not reduced during tolbutamide infusion. We conclude that intraislet hyperinsulinemia prevents the glucagon response to hypoglycemia despite an intact autonomic response and a low α-cell glucose concentration. *Diabetes* 51:958–965, 2002

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Iatrogenic hypoglycemia is the limiting factor in the glycemic management of diabetes mellitus both conceptually and in practice (1,2). It causes recurrent and sometimes permanent physical morbidity, recurrent or persistent psychosocial morbidity, and occasionally death, and it precludes true glycemic control in most patients with type 1 diabetes (3) and many with advanced type 2 diabetes (4). Thus, long-term complications of diabetes can occur despite aggressive attempts to achieve glycemic control (3,4).

Normally, decreased pancreatic β-cell insulin secretion and increased pancreatic α-cell glucagon secretion and, absent the latter, increased adrenomedullary epinephrine secretion prevent or rapidly correct hypoglycemia (2). All three of these defenses against hypoglycemia are compromised in established (i.e., C-peptide negative) type 1 diabetes. As plasma glucose concentrations fall, (exogenous) insulin levels do not decrease, glucagon levels do not increase (5,6), and the epinephrine response to a given level of hypoglycemia is typically attenuated (6,7). The combination of absent glucagon and reduced epinephrine responses causes the syndrome of defective glucose counterregulation, which is associated with a 25-fold or greater increased frequency of severe iatrogenic hypoglycemia during aggressive therapy of type 1 diabetes (8,9). The reduced autonomic (including adrenomedullary epinephrine) and resulting symptomatic responses cause the syndrome of hypoglycemia unawareness, which is also associated with a high frequency of severe hypoglycemia (10). The reduced autonomic and symptomatic responses to a given level of hypoglycemia represent a shift of their glycemic thresholds to lower plasma glucose concentrations and are largely the result of recent antecedent iatrogenic hypoglycemia (2,7).

The mechanism(s) of the loss of the glucagon response to hypoglycemia is unknown. It is a selective defect; glucagon responses to stimuli other than hypoglycemia are largely, if not entirely, intact (2), which suggests an abnormality of signaling to α-cells. Loss of the glucagon response is tightly correlated with the loss of endogenous insulin secretion (11) but not with the presence of classical diabetic autonomic neuropathy (7). Although it is often associated with functional autonomic failure [i.e., hypoglycemia-associated autonomic failure (7,12–14)], the gluca-

gon response is absent in some patients with a normal epinephrine response (6). Clearly, the mechanisms of these two key components of compromised glucose counterregulation in type 1 diabetes are different.

We sought to gain insight into the mechanism of the absent glucagon response in type 1 diabetes by studying the mechanism of the normal glucagon response to hypoglycemia. There is considerable evidence, largely from studies in experimental animals (15,16) but also in humans (17,18), that central nervous system–mediated autonomic nervous system (19)—sympathetic, adrenomedullary, and parasympathetic—activation by hypoglycemia plays an important role in stimulating glucagon secretion. However, we (20) and others (21) found that pharmacological blockade of the actions of the classical autonomic mediators—norepinephrine, epinephrine, and acetylcholine—with adrenergic antagonists, a muscarinic cholinergic antagonist, or both did not reduce the glucagon response to hypoglycemia in humans. Furthermore, the denervated (allografted) human pancreas releases glucagon in response to hypoglycemia (22). These findings suggest that additional mechanisms are involved. It is often assumed that α-cells sense low glucose levels directly, leading to increased glucagon secretion. α-Cells have been reported to express glucokinase but apparently not the glucose transporter GLUT-2 (23). However, demonstrably viable isolated α-cells did not release glucagon in a low glucose (1.4 mmol/l) medium (24). A third possibility is that a decrease in intraislet insulin in response to hypoglycemia stimulates glucagon secretion. Proposed by Samols et al. (25), consistent with the data of Weir et al. (26), supported by the data of Maruyama et al. (27) and Samols et al. (28), and reviewed by Samols and Stagner (29), the intraislet insulin hypothesis posits that a decrease in β-cell insulin secretion and thus a decrease in tonic intraislet α-cell inhibition by insulin is a signal for increased glucagon secretion in response to hypoglycemia. Key findings included evidence that the islet microcirculation flows from β-cells to α-cells in the rat pancreas and that immunoneutralization of insulin stimulates glucagon release from the perfused rat pancreas (27,28). There is substantial evidence that insulin inhibits glucagon secretion in humans: *1*) insulin infusion (during euglycemic clamps) blunts the glucagon response to intravenous arginine (30,31); *2*) tolbutamide infusion (during euglycemic clamps) blunts the glucagon response to intravenous arginine in healthy subjects, who release insulin in response to tolbutamide, but not in patients with type 1 diabetes, who cannot release insulin (32); and *3*) higher, compared with lower, insulin infusion rates and thus higher plasma insulin levels have been reported to blunt the glucagon response to hypoglycemia in healthy humans in two studies (33,34) but not in another (35).

We tested the hypothesis that intraislet hyperinsulinemia prevents the glucagon response to hypoglycemia despite an intact autonomic response and a low α-cell glucose concentration by contrasting the glucagon response to hypoglycemia alone with that to hypoglycemia during infusion of the β-cell secretagogue tolbutamide in healthy subjects. Glucagon responses to tolbutamide-induced, compared with insulin-induced, hypoglycemia have been reported to be similar (36) or only minimally reduced (37,38). Similarly, glucagon responses to insulin-induced hypoglycemia were blunted only in glibenclamide-treated type 2 diabetes (39). The glucagon response was not prevented in these studies. However, C-peptide levels fell, to a greater or lesser degree, during hypoglycemia despite administration of a sulfonylurea. Accordingly, to minimize the possibility of a decrease in insulin secretion during hypoglycemia, our experimental design included infusion of a high dose of tolbutamide (1.0 g/h) throughout.

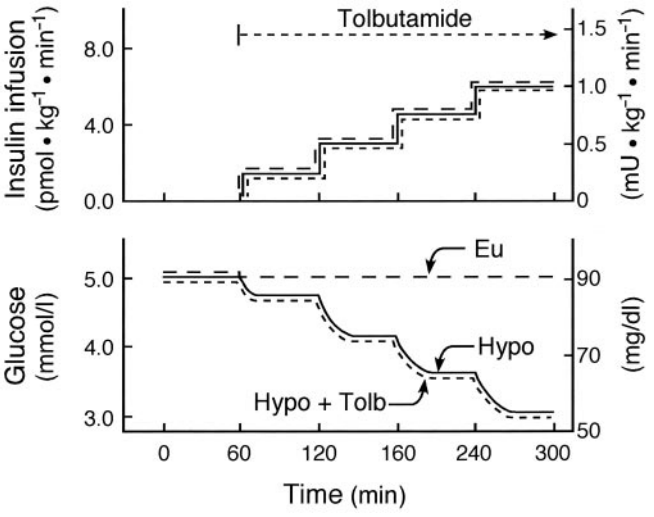

FIG. 1. Experimental design. Insulin was infused intravenously in hourly doses of 1.5, 3.0, 4.5, and 6.0 pmol · kg^{-1} · min^{-1} from 60 through 300 min on all three occasions. Plasma glucose was clamped at ~5.0 mmol/l throughout on one occasion and at hourly steps of 4.7, 4.2, 3.6, and 3.0 mmol/l on two occasions, and tolbutamide was infused in a dose of 1.0 g/h from 60 through 300 min on one of those occasions.

RESEARCH DESIGN AND METHODS

Twelve healthy subjects (five women and seven men, aged 19 to 27 years with BMIs ranging from 18 to 33 kg/m^2) 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. Each subject was studied on three separate occasions. The study protocol is illustrated in Fig. 1. After an overnight fast, the subjects reported to the General Clinical Research Center early in the morning. An intravenous line was inserted in an antecubital vein (for insulin and glucose infusions) and another in a hand vein with that hand kept in an ~60°C plexiglas box (for arterialized venous blood sampling). The subjects rested in the supine position throughout. Observations—blood sampling, blood pressure, and heart rate recordings (Propaq Encore; Protocol Systems, Beverton, OR)—were made at 30-min intervals from 0 through 300 min. The electrocardiogram was monitored through that period. Relatively low-dose intravenous insulin infusions were administered in a stepped manner increased at hourly intervals (1.5, 3.0, 4.5, and 6.0 pmol · kg^{-1} · min^{-1}; 0.25, 0.50, 0.75, and 1.00 mU · kg^{-1} · min^{-1}) from 60 through 300 min on all three occasions. Plasma glucose levels were measured at 5-min intervals, and 20% glucose was infused in doses sufficient to maintain euglycemia (~5.0 mmol/l, 90 mg/dl) on one occasion and to lower plasma glucose levels to hourly clamped steps at ~4.7, 4.2, 3.6, and 3.0 mmol/l (~85, 75, 65, and 55 mg/dl) on the other two occasions. On one of the latter occasions, tolbutamide was infused in a dose of 1.0 g/h also from 60 through 300 min. The hypoglycemia studies were separated by at least 2 weeks.

A subset of these subjects (n = 5) were available to be restudied using slightly higher insulin infusion rates (4.5, 6.0, 7.5, and 9.0 pmol · kg^{-1} · min^{-1} at hourly intervals from 60 through 300 min) to raise plasma insulin levels to or above those during hypoglycemia with tolbutamide infusion.

Arterialized venous plasma glucose concentrations were measured with a glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin (40), C-peptide (40), glucagon (41), pancreatic polypeptide (42), growth hormone (43), and cortisol (44) were measured with radioimmu-

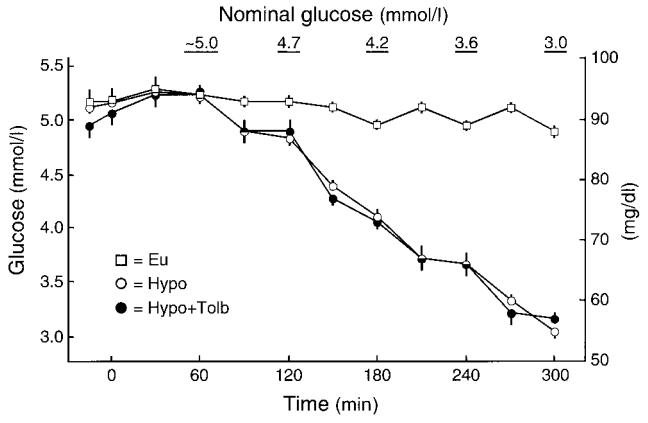

FIG. 2. Mean (±SE) plasma glucose concentrations during low-dose stepped hyperinsulinemic euglycemic clamps (Eu, □), stepped hypoglycemic (4.7, 4.2, 3.6, and 3.0 nmol/l) clamps (Hypo, ○), and stepped hypoglycemic clamps with tolbutamide 1.0 g/h (Hypo + Tolb, ●) from 60 through 300 min.

noassays. Plasma epinephrine and norepinephrine were measured with a single isotope derivative (radioenzymatic) method (45). Serum nonesterified fatty acids (46) and blood β-hydroxybutyrate (47), lactate (48), and alanine (49) were measured with enzymatic methods.

Insulin secretion rates were derived by stochastic deconvolution of plasma C-peptide levels (50) using the program WINSTODEC (STOchastic DEConvolution) (51). The C-peptide impulse response was described by the two-compartment model first proposed by Eaton et al. (52). Values of the impulse response parameters were calculated in each subject using a population approach (53) based on individual anthropometric parameters, i.e., age, sex, body surface area, and health condition (here nondiabetic, nonobese).

Data in this manuscript are expressed as means ± SE. Data were analyzed by general linear model repeated measures ANOVA. $P < 0.05$ was considered to indicate statistically significant differences.

RESULTS

Plasma glucose concentrations on the three study occasions are shown in Fig. 2. Target plasma glucose levels were achieved.

Plasma C-peptide concentrations are shown in Fig. 3. C-peptide levels declined from 0.6 ± 0.1 to 0.2 ± 0.0 nmol/l (1.7 ± 0.2 to 0.7 ± 0.1 ng/ml) during hyperinsulinemic euglycemia and to a greater extent, from 0.6 ± 0.1 to 0.1 ± 0.0 nmol/l (1.7 ± 0.2 to 0.3 ± 0.1 ng/ml), during hyperinsulinemic hypoglycemia in the absence of tolbutamide. During tolbutamide infusion and hyperinsulinemic hypoglycemia, C-peptide levels rose from 0.7 ± 0.1 nmol/l (2.0 ± 0.3 ng/ml) to a peak of 1.7 ± 0.2 nmol/l (5.1 ± 0.5 ng/ml) and a final value of 1.0 ± 0.2 nmol/l (3.1 ± 0.6 ng/ml; $P < 0.0001$ vs. hypoglycemia alone).

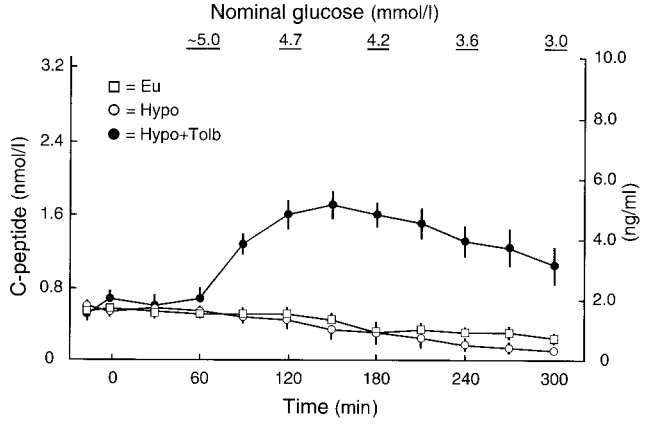

FIG. 3. Mean (±SE) plasma C-peptide concentrations during low-dose stepped hyperinsulinemic euglycemic clamps (Eu, □), stepped hypoglycemic (4.7, 4.2, 3.6 and 3.0 mmol/l) clamps (Hypo, ○), and stepped hypoglycemic clamps with tolbutamide 1.0 g/h (Hypo + Tolb, ●) from 60 through 300 min. C-peptide levels were significantly higher ($P < 0.0001$) with tolbutamide.

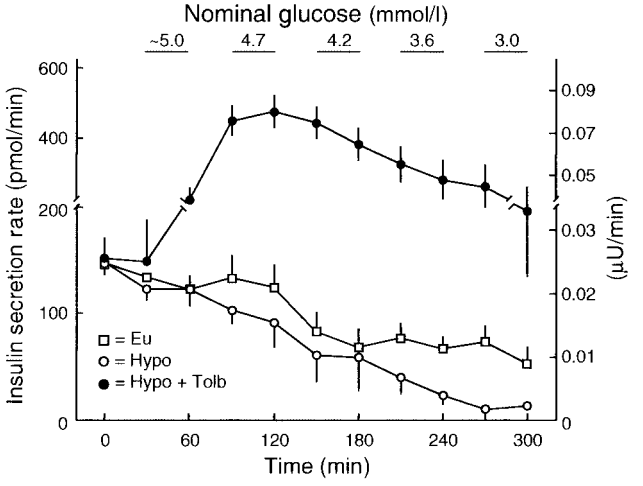

FIG. 4. Mean (±SE) insulin secretion rates during low-dose stepped hyperinsulinemic euglycemic clamps (Eu, □), stepped hypoglycemic (4.7, 4.2, 3.6, and 3.0 mmol/l) clamps (Hypo, ○), and stepped hypoglycemic clamps with tolbutamide 1.0 g/h (Hypo + Tolb, ●) from 60 through 300 min. Insulin secretion rates were higher ($P < 0.0001$) with tolbutamide.

Insulin secretion rates are shown in Fig. 4. Insulin secretion declined from 147 ± 16 to 54 ± 13 pmol/min (24 ± 3 to 9 ± 3 nU/min) during hyperinsulinemic euglycemia and to a greater extent, from 148 ± 12 to 15 ± 4 pmol/min (25 ± 2 to 2 ± 1 nU/min), during hypoglycemia in the absence of tolbutamide. During tolbutamide infusion and initial hyperinsulinemic euglycemia, insulin secretion rose from 154 ± 18 to a peak of 479 ± 41 pmol/min (26 ± 3 to 80 ± 7 nU/min). It then declined to a final value of 198 ± 60 pmol/min (33 ± 10 nU/min) during hypoglycemia ($P < 0.0001$ vs. hypoglycemia alone).

Plasma insulin concentrations are shown in Fig. 5. Insulin levels were virtually the same during hyperinsu-

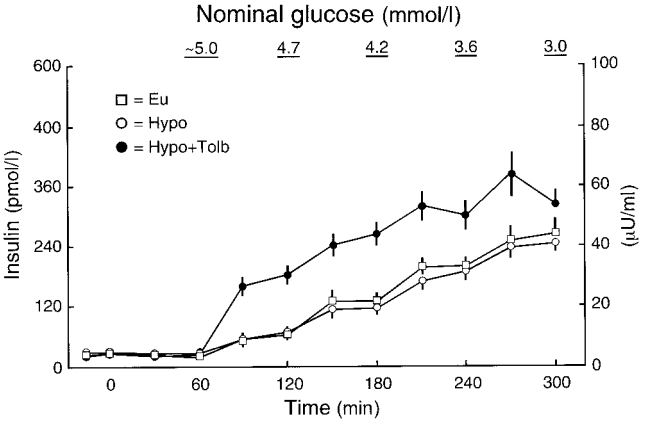

FIG. 5. Mean (±SE) plasma insulin concentrations during low-dose stepped hyperinsulinemic euglycemic clamps (Eu, □), stepped hypoglycemic (4.7, 4.2, 3.6, and 3.0 mmol/l) clamps (Hypo, ○), and stepped hypoglycemic clamps with tolbutamide 1.0 g/h (Hypo + Tolb, ●) from 60 through 300 min. Insulin levels were significantly higher ($P = 0.0143$) with tolbutamide.

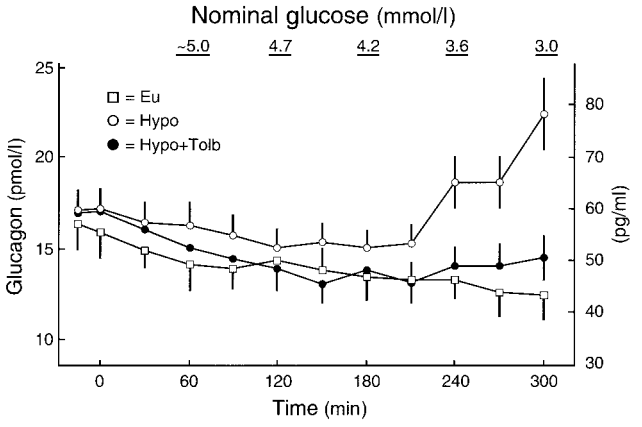

FIG. 6. Mean (±SE) plasma glucagon concentrations during low-dose stepped hyperinsulinemic euglycemic clamps (Eu, □), stepped hypoglycemic (4.7, 4.2, 3.6, and 3.0 mmol/l) clamps (Hypo, ○), and stepped hypoglycemic clamps with tolbutamide 1.0 g/h (Hypo + Tolb, ●) from 60 through 300 min. Glucagon levels were significantly lower ($P < 0.0001$) during hypoglycemia with tolbutamide than without tolbutamide.

linemic euglycemia and during hyperinsulinemic hypoglycemia without tolbutamide. Insulin levels were ~120 pmol/l (20 μU/ml) higher ($P < 0.0001$) during hyperinsulinemic hypoglycemia with tolbutamide infusion.

Plasma glucagon concentrations are shown in Fig. 6. Glucagon levels were similar at baseline in all three studies, 16 ± 1, 17 ± 1, and 17 ± 1 pmol/l (55 ± 5, 60 ± 4, and 60 ± 4 pg/ml), and declined to similar levels during the euglycemic phases (through 120 min) in all three studies, 14 ± 1, 15 ± 1, and 14 ± 1 pmol/l (50 ± 5, 52 ± 4, and 48 ± 4 pg/ml), in the studies with subsequent euglycemia, hypoglycemia alone, and hypoglycemia with tolbutamide infusion, respectively. At the end of the studies (300 min), glucagon levels were 12 ± 1 pmol/l (43 ± 5 pg/ml) during euglycemia, 22 ± 2 pmol/l (78 ± 7 pg/ml) during hypoglycemia alone, and 15 ± 1 pmol/l (51 ± 4 pg/ml) during hypoglycemia with tolbutamide infusion ($P < 0.0001$ vs. hypoglycemia alone).

Plasma epinephrine and norepinephrine responses to hypoglycemia were virtually identical ($P = 0.8956$ and 0.9537, respectively) with and without tolbutamide infusion (Table 1). Plasma pancreatic polypeptide responses to hypoglycemia were also not reduced; indeed, they were increased ($P = 0.0037$) during tolbutamide infusion (Table 1). Plasma growth hormone responses to hypoglycemia were unaltered ($P = 0.1324$), but cortisol responses were reduced slightly ($P = 0.0386$; Table 1).

Serum nonesterified fatty acid ($P = 0.3795$) and blood β-hydroxybutyrate ($P = 0.3618$) and alanine ($P = 0.1792$) levels were similar under all study conditions (Table 2). Blood lactate levels were lower ($P = 0.0024$) initially during hypoglycemia with tolbutamide, but the final values were similar (1,516 ± 117 and 1,280 ± 129 μmol/l during hypoglycemia with and without tolbutamide, respectively; Table 2). Heart rates, systolic blood pressures, and diastolic blood pressures were unaltered by tolbutamide infusion (data not shown).

In the subset of patients ($n = 5$) who were restudied with slightly higher insulin infusion rates, peripheral plasma insulin concentrations (Table 3) were raised ($P = 0.0389$) above those observed during hyperinsulinemic hypoglycemia with tolbutamide infusion (Fig. 4). Despite the higher insulin levels, plasma glucagon concentrations increased during hypoglycemia (Table 3), were no different from those during hypoglycemia alone produced with the lower insulin doses ($P = 0.5961$), and were higher than those during hypoglycemia with tolbutamide infusion ($P =$

TABLE 1
Mean ± SE plasma epinephrine, norepinephrine, pancreatic polypeptide, growth hormone, and cortisol concentrations during low-dose stepped hyperinsulinemic euglycemic clamps (Eu), stepped hypoglycemic (4.7, 4.2, 3.6, and 3.0 mmol/l) clamps (Hypo), and stepped hypoglycemic clamps with tolbutamide 1.0 g/h (Hypo + Tolb) from 60 to 300 min*

	Time (min)							
	−15	0	60	120	180	240	300	P†
Epinephrine (pmol/l)								
Eu	130 ± 40	120 ± 35	95 ± 10	115 ± 15	130 ± 20	130 ± 15	145 ± 20	
Hypo	85 ± 10	110 ± 20	85 ± 10	100 ± 15	145 ± 20	670 ± 190	1,500 ± 315	
Hypo + Tolb	85 ± 10	75 ± 10	75 ± 10	80 ± 10	135 ± 10	790 ± 265	1,560 ± 250	0.8956
Norepinephrine (nmol/l)								
Eu	0.85 ± 0.09	0.77 ± 0.09	0.77 ± 0.12	0.84 ± 0.12	0.90 ± 0.15	0.93 ± 0.10	0.87 ± 0.08	
Hypo	0.87 ± 0.12	0.74 ± 0.25	0.72 ± 0.09	0.82 ± 0.12	0.88 ± 0.12	0.96 ± 0.11	1.16 ± 0.15	
Hypo + Tolb	0.87 ± 0.08	0.85 ± 0.10	0.95 ± 0.05	0.87 ± 0.09	0.93 ± 0.12	0.97 ± 0.11	1.16 ± 0.12	0.9537
Pancreatic polypeptide (pmol/l)								
Eu	16 ± 4	16 ± 4	24 ± 13	15 ± 4	13 ± 3	12 ± 2	14 ± 4	
Hypo	25 ± 5	19 ± 4	19 ± 6	18 ± 4	16 ± 3	37 ± 10	78 ± 18	
Hypo + Tolb	30 ± 8	27 ± 5	28 ± 8	19 ± 3	24 ± 5	50 ± 12	139 ± 16	0.0037
Growth hormone (pmol/l)								
Eu	53 ± 18	62 ± 22	35 ± 19	44 ± 18	194 ± 66	106 ± 44	75 ± 26	
Hypo	62 ± 31	49 ± 18	35 ± 13	26 ± 4	49 ± 18	362 ± 115	340 ± 88	
Hypo + Tolb	84 ± 35	71 ± 26	26 ± 0	35 ± 9	53 ± 31	128 ± 44	402 ± 119	0.1324
Cortisol (nmol/l)								
Eu	510 ± 45	495 ± 60	380 ± 55	355 ± 45	330 ± 40	260 ± 25	235 ± 25	
Hypo	435 ± 60	410 ± 60	315 ± 50	305 ± 40	255 ± 35	355 ± 50	420 ± 50	
Hypo + Tolb	430 ± 55	390 ± 55	335 ± 50	330 ± 45	275 ± 35	255 ± 35	410 ± 35	0.0386

*To convert epinephrine to pg/ml, divide by 5.458; norepinephrine to pg/ml, divide by 0.005911; pancreatic polypeptide to pg/ml, divide by 0.239; growth hormone to ng/ml, divide by 44.15; cortisol to μg/l, divide by 27.59. †Hypo versus Hypo + Tolb.

TABLE 2
Mean ± SE serum nonesterified fatty acids and blood β-hydroxybutyrate, lactate, and alanine concentrations during low-dose stepped hyperinsulinemic euglycemic clamps (Eu), stepped hypoglycemic (4.7, 4.2, 3.6, and 3.0 mmol/l) clamps (Hypo), and stepped hypoglycemic clamps with tolbutamide 1.0 g/h (Hypo + Tolb) from 60 to 300 min

	Time (min)							
	−15	0	60	120	180	240	300	*P**
Nonesterified fatty acids (μmol/l)								
Eu	592 ± 50	586 ± 43	522 ± 55	274 ± 29	136 ± 15	101 ± 11	101 ± 12	
Hypo	487 ± 47	512 ± 69	445 ± 46	260 ± 40	157 ± 32	118 ± 15	110 ± 15	
Hypo + Tolb	601 ± 56	602 ± 52	592 ± 55	206 ± 20	157 ± 18	130 ± 16	113 ± 13	0.3795
β-Hydroxybutyrate (μmol/l)								
Eu	141 ± 35	174 ± 43	133 ± 23	68 ± 8	56 ± 13	38 ± 7	36 ± 6	
Hypo	77 ± 14	106 ± 26	94 ± 20	49 ± 8	52 ± 16	38 ± 11	31 ± 6	
Hypo + Tolb	179 ± 60	142 ± 48	162 ± 41	73 ± 9	62 ± 9	49 ± 10	50 ± 9	0.3618
Lactate (μmol/l)								
Eu	775 ± 85	872 ± 103	805 ± 131	878 ± 78	966 ± 78	1,078 ± 129	1,130 ± 125	
Hypo	1,074 ± 128	1,049 ± 132	1,186 ± 156	1,169 ± 138	1,214 ± 175	1,079 ± 89	1,280 ± 129	
Hypo + Tolb	889 ± 145	749 ± 127	839 ± 123	933 ± 103	816 ± 74	1,051 ± 71	1,516 ± 117	0.0024
Alanine (μmol/l)								
Eu	330 ± 66	394 ± 75	361 ± 78	344 ± 77	413 ± 92	384 ± 77	315 ± 48	
Hypo	349 ± 39	468 ± 56	420 ± 54	465 ± 67	440 ± 59	363 ± 51	297 ± 44	
Hypo + Tolb	518 ± 98	414 ± 67	485 ± 107	410 ± 66	374 ± 55	376 ± 62	334 ± 49	0.1792

*Hypo versus Hypo + Tolb.

0.0002) even after adjustment for apparent differences at baseline. Plasma epinephrine, norepinephrine, pancreatic polypeptide, growth hormone, and cortisol concentrations also increased (Table 3). Again, the plasma cortisol response was greater ($P = 0.0010$) than that during hypoglycemia with tolbutamide infusion.

Glucose infusion rates on all three study occasions in all of the subjects ($n = 12$) and on all three of those occasions plus the occasion when slightly higher doses of insulin were infused (with identical stepped hypoglycemia) in a subset of the subjects ($n = 5$) are shown in Table 4. The glucose infusion rates required to maintain the various glucose clamps, an index of glucose utilization under these conditions, were highest during hyperinsulinemic euglycemia, lowest during hyperinsulinemic hypoglycemia alone (when the glucagon response occurred), and intermediate at the lowest glucose step during hypoglycemia with tolbutamide (when the glucagon response was prevented; all $P < 0.0001$). This pattern was also apparent in the data from the five subjects who were infused with slightly higher does of insulin. Early in the latter study, the glucose infusion rates required were higher during infusion of the higher insulin doses than during infusion of the lower insulin doses alone ($P = 0.0045$) but were similar to those during infusion of the lower insulin doses plus tolbutamide ($P = 0.1345$). Notably, however, at the lowest glucose step, the glucose infusion rates were virtually identical during hypoglycemia produced by infusion of the lower and higher doses of insulin (when the glucagon response occurred on both occasions) and lower than those required during similar hypoglycemia when tolbutamide was also infused (when the glucagon response did not occur).

DISCUSSION

These data document that the glucagon response to hypoglycemia is prevented by intraislet hyperinsulinemia despite an intact autonomic—adrenomedullary (plasma

TABLE 3
Mean ± SE plasma glucose, insulin, C-peptide, glucagon epinephrine, norepinephrine, and pancreatic polypeptide concentrations during slightly higher insulin infusion rate (4.5, 6.0, 7.5, and 9.0 pmol · kg^{-1} · min^{-1}) stepped hyperinsulinemic hypoglycemic clamps from 60 to 300 min in a subset of subjects ($n = 5$)*

	Time (min)							
	−15	0	60	120	180	240	300	*P*†
Glucose (mmol/l)	5.3 ± 0.3	5.3 ± 0.2	5.3 ± 0.2	4.7 ± 0.1	4.2 ± 0.1	3.6 ± 0.2	3.0 ± 0.0	0.8175
Insulin (pmol/l)	38 ± 4	40 ± 8	42 ± 11	265 ± 11	366 ± 62	421 ± 40	515 ± 38	0.0389
C-peptide (nmol/l)	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0010
Glucagon (pmol/l)	23 ± 2	21 ± 1	21 ± 2	21 ± 2	22 ± 3	25 ± 3	29 ± 1	0.0002
Epinephrine (pmol/l)	110 ± 15	125 ± 50	80 ± 10	125 ± 40	225 ± 95	1,090 ± 255	2,300 ± 515	0.1035
Norepinephrine (nmol/l)	0.89 ± 0.05	0.87 ± 0.09	0.82 ± 0.08	0.96 ± 0.14	0.95 ± 0.07	1.11 ± 0.12	1.40 ± 0.27	0.1897
Pancreatic polypeptide (pmol/l)	30 ± 3	30 ± 3	28 ± 3	29 ± 3	39 ± 2	56 ± 11	117 ± 32	0.2158
Growth hormone (pmol/l)	76 ± 55	71 ± 49	72 ± 50	49 ± 27	72 ± 50	107 ± 28	365 ± 105	0.1074
Cortisol (nmol/l)	430 ± 95	365 ± 75	285 ± 65	290 ± 40	295 ± 65	385 ± 70	590 ± 75	0.0010

*To convert glucose to mg/dl, divide by 0.0551; C-peptide to ng/ml, divide by 0.331; insulin to μU/ml, divide by 6.0; glucagon to pg/ml, divide by 0.2871; epinephrine to pg/ml, divide by 5.458; norepinephrine to pg/ml, divide by 0.005911; pancreatic polypeptide to pg/ml, divide by 0.239; growth hormone to ng/ml, divide by 44.15; cortisol to μg/dl, divide by 27.59. †Compared with responses to lower doses of insulin with tolbutamide.

TABLE 4
Mean ± SE glucose infusion rates ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)* during low-dose stepped hyperinsulinemic euglycemic clamps (Eu), stepped hypoglycemic (4.7, 4.2, 3.6, and 3.0 mmol/l) clamps (Hypo), and stepped hypoglycemic clamps with tolbutamide 1.0 g/h (Hypo + Tolb) from 60 to 300 min ($n = 12$) and in a subset of subjects ($n = 5$) during higher dose insulin and otherwise identical stepped hypoglycemic clamps (Higher Ins, Hypo)

	Time (min)							
	−15	0	60	120	180	240	300	*P*
All subjects ($n = 12$)								
Eu	0	0	1.4 ± 0.6	5.1 ± 1.4	18.5 ± 1.8	36.5 ± 2.6	47.2 ± 2.7	
Hypo	0	0	1.5 ± 0.6	4.0 ± 1.7	12.1 ± 2.6	18.9 ± 2.8	18.3 ± 3.7	
Hypo + Tolb	0	0	2.5 ± 0.9	14.8 ± 2.4	32.5 ± 2.7	39.6 ± 2.6	30.6 ± 4.3	<0.0001†
Subset of subjects ($n = 5$)								
Eu	0	0	1.2 ± 0.8	2.3 ± 1.3	20.4 ± 3.8	39.9 ± 4.4	48.1 ± 4.4	
Hypo	0	0	1.3 ± 0.8	5.4 ± 4.1	16.7 ± 4.8	19.5 ± 5.5	19.2 ± 8.5	
Hypo + Tolb	0	0	1.4 ± 0.9	17.4 ± 2.9	31.6 ± 5.1	37.2 ± 4.8	32.1 ± 8.3	0.0331†
Higher Ins, Hypo	0	0	1.6 ± 1.2	18.3 ± 4.7	33.5 ± 3.5	34.5 ± 3.0	20.0 ± 5.6	0.0045‡

*To convert $\mu mol \cdot kg^{-1} \cdot min^{-1}$ to $mg \cdot kg^{-1} \cdot min^{-1}$, divide by 5.551; †Hypo versus Hypo + Tolb; ‡Hypo versus Higher Ins, Hypo.

epinephrine), sympathetic neural (plasma norepinephrine), and parasympathetic neural (plasma pancreatic polypeptide)—response and a low α-cell glucose concentration in humans.

Prevention of the glucagon response to hypoglycemia by tolbutamide was reflected biologically in the glucose infusion rates required to maintain the various glucose clamps. Reflecting the effect of tolbutamide to stimulate insulin secretion, as documented by higher plasma C-peptide and insulin concentrations and insulin secretion rates, the required glucose infusion rates were higher during the initial euglycemic steps (4.7 and 4.2 mmol/l). Albeit lower than during hyperinsulinemic euglycemia (likely reflecting the glycemic effects of intact epinephrine secretion), when the glucagon response was prevented during hypoglycemia, the required glucose infusion rates were higher than during hypoglycemia alone (when the glucagon response occurred). Notably, at the lowest glucose step, the required glucose infusion rates were identical during hypoglycemia alone with both the lower and the higher insulin infusion doses when the glucagon response was intact. Thus, these data provide additional support for the importance of glucagon as a glucose counterregulatory hormone (2).

In contrast to the markedly reduced glucagon response, most of the other responses to hypoglycemia were not reduced during tolbutamide infusion. Notably, from the perspective of our hypothesis, plasma epinephrine, norepinephrine, and pancreatic polypeptide responses, indexes of adrenomedullary, sympathetic neural, and parasympathetic neural responses, respectively (2), were not reduced during hypoglycemia with tolbutamide compared with during hypoglycemia without tolbutamide. It is interesting that although unaltered by tolbutamide during euglycemia, the plasma pancreatic polypeptide response to hypoglycemia was enhanced. Although the final plasma cortisol values were similar, the cortisol response seemed to be shifted to a lower plasma glucose concentration during hypoglycemia with tolbutamide infusion. The growth hormone response, as well as nonesterified fatty acid, β-hydroxybutyrate, and alanine levels, was unaltered. Blood lactate levels were inexplicably lower initially in the tolbutamide study, but the final values, during hypoglycemia, were similar to those during hypoglycemia without tolbutamide.

Might the observed prevention of the glucagon response to hypoglycemia during tolbutamide infusion have been the result of direct suppression of α-cell glucagon secretion by tolbutamide or indirect suppression of α-cell glucagon secretion by neural mechanisms activated by the higher portal and systemic insulin levels that resulted from tolbutamide infusion rather than prevention of a decrease in intraislet insulin? With respect to the first consideration, tolbutamide has been reported to stimulate, not suppress, exocytosis of glucagon from isolated rat α-cells (54). With respect to the second consideration, hyperinsulinemia per se increases sympathetic neural but not adrenomedullary or parasympathetic neural activity (55). However, if anything, increased sympathetic neural activity would be expected to increase, rather than decrease, glucagon secretion (16,19). Nonetheless, elevation of peripheral plasma insulin levels by as little as ~120 pmol/l (~20 μU/ml) has been reported to blunt the glucagon response to a decrease in plasma glucose induced by administration of a phosphorylase inhibitor in dogs (56). Accordingly, we restudied a subset of subjects with higher insulin infusion rates (without tolbutamide) that produced insulin levels ~120 pmol/l (~20 μU/ml) higher than those during the initial hypoglycemia with tolbutamide study and ~240 pmol/l (~40 μU/ml) higher than those during the initial hypoglycemia without tolbutamide study. These higher insulin levels did not reduce the glucagon response to hypoglycemia.

These data are consistent with but not definitive evidence of the intraislet insulin hypothesis (25–29)—a decrease in β-cell insulin secretion and thus a decrease in tonic intraislet α-cell inhibition by insulin is a signal for increased glucagon secretion in response to hypoglycemia—in humans. This hypothesis is attractive because if a decrease in intraislet insulin were an important signal to increased glucagon secretion during hypoglycemia, then the absence of this signal would plausibly explain the absence of the glucagon response in insulin-deficient diabetes (2,5,6). However, it is conceivable that prevention of the glucagon response to hypoglycemia in the present study was the result of tolbutamide-induced intraislet hyperinsulinemia per se rather than of the absence of a decrease in intraislet insulin. It is notable that glucagon levels were not suppressed during the initial euglycemic (4.7 mmol/l, 85 mg/dl) glucose step (120 min) in the

tolbutamide study compared with the other two studies. The latter finding is consistent with data from the perfused rat pancreas in which very high perfusate insulin concentrations (up to 20 μmol/l) did not suppress glucagon secretion at a normal perfusate glucose concentration (5.6 mmol/l) (53). Indeed, remarkably high insulin concentrations (600 but not 60 nmol/l) were required to blunt the glucagon response to a low perfusate glucose concentration (1.4 mmol/l) (57). It is also consistent with the finding that micromolar concentrations of insulin did not suppress amino acid–stimulated glucagon release from isolated α-cells (24). The interpretation that a decrease in intraislet insulin per se is a signal to increased glucagon secretion during hypoglycemia could be challenged by the finding that insulin secretion decreased but glucagon levels did not increase during hyperinsulinemic euglycemia in the present study. However, insulin secretion decreased to a greater extent during hypoglycemia than during euglycemia. Insulin secretion rates fell by 92% during hypoglycemia alone, and nadir rates were only 22% of those during euglycemia. Clearly, additional data are needed to confirm or refute the intraislet insulin hypothesis in humans.

Might there normally be an interaction of autonomic activation [albeit nonadrenergic and nonmuscarinic cholinergic (20,21)], low α-cell glucose, and decreased intraislet insulin signals that stimulate glucagon secretion during hypoglycemia? Autonomic activation per se does not stimulate glucagon secretion, despite low α-cell glucose, in the setting of intraislet hyperinsulinemia as documented in the present data. Furthermore, the denervated (allografted) human pancreas releases glucagon in the setting of low α-cell glucose and decreased intraislet insulin (22). High insulin per se does not suppress glucagon secretion from the perfused rat pancreas at a normal (5.6 mmol/l) glucose concentration (53), nor did it suppress glucagon levels during the euglycemic phases of the present experiments. Low glucose per se does not stimulate glucagon secretion from isolated α-cells (24) or in the absence of a decrement in intraislet insulin in type 1 diabetes (2,5–7,11–14) and in the present data. However, immunoneutralization of insulin increases glucagon release from the perfused pancreas (27,28).

We conclude that the glucagon response to hypoglycemia is prevented by intraislet hyperinsulinemia despite an intact autonomic response and a low α-cell glucose concentration in humans.

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