

# Loss of the Decrement in Intraislet Insulin Plausibly Explains Loss of the Glucagon Response to Hypoglycemia in Insulin-Deficient Diabetes

## Documentation of the Intraislet Insulin Hypothesis in Humans

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The intraislet insulin hypothesis for the signaling of the glucagon secretory response to hypoglycemia states that a decrease in arterial glucose → a decrease in  $\beta$ -cell insulin secretion → a decrease in tonic  $\alpha$ -cell inhibition by insulin → an increase in  $\alpha$ -cell glucagon secretion. To test this hypothesis in humans, a hyperinsulinemic-euglycemic ( $\sim 5.0$  mmol/l [90 mg/dl]  $\times 2$  h) and then a hypoglycemic ( $\sim 3.0$  mmol/l [55 mg/dl]  $\times 2$  h) clamp was performed in 14 healthy young adults on two occasions, once with oral administration of the ATP-sensitive potassium channel agonist diazoxide to selectively suppress baseline insulin secretion and once with the administration of a placebo. The decrement in plasma C-peptide during the induction of hypoglycemia was reduced by  $\sim 50\%$  in the diazoxide clamps (from  $0.3 \pm 0.0$  to  $0.1 \pm 0.0$  nmol/l [ $0.8 \pm 0.1$  to  $0.4 \pm 0.1$  ng/ml]) compared with the placebo clamps (from  $0.4 \pm 0.0$  to  $0.1 \pm 0.0$  nmol/l [ $1.2 \pm 0.1$  to  $0.4 \pm 0.1$  ng/ml]) ( $P = 0.0015$ ). This reduction of the decrement in intraislet insulin during induction of hypoglycemia caused an  $\sim 50\%$  reduction ( $P = 0.0010$ ) of the increase in plasma glucagon in the diazoxide clamps (from  $29 \pm 3$  to  $35 \pm 2$  pmol/l [ $102 \pm 9$  to  $123 \pm 8$  pg/ml]) compared with the placebo clamps (from  $28 \pm 2$  to  $43 \pm 5$  pmol/l [ $98 \pm 7$  to  $151 \pm 16$  pg/ml]). Baseline glucagon levels, the glucagon response to intravenous arginine, and the autonomic (adrenomedullary, sympathetic neural, and parasympathetic neural) responses to hypoglycemia were not altered by diazoxide. These data indicate that a decrease in intraislet insulin is a signal for the glucagon secretory response to hypoglycemia in healthy humans. The absence of that signal plausibly explains the loss of the glucagon response to falling plasma glucose concentrations, a key feature of the pathogenesis of iatrogenic hypoglycemia, in insulin-deficient (type 1 and advanced type 2) diabetes. *Diabetes* 54:757–764, 2005

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ECG, electrocardiogram.

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**H**ypoglycemia, the result of the interplay of relative or absolute insulin excess and compromised glucose counterregulation, is the limiting factor in glycemic management in diabetes (1). It causes morbidity in most people with type 1 diabetes and in many with advanced type 2 diabetes and is sometimes fatal. It also precludes the maintenance of euglycemia over a lifetime of diabetes and thus a full realization of the benefits of glycemic control. As plasma glucose concentrations decline, decrements in pancreatic islet  $\beta$ -cell insulin secretion, increments in pancreatic islet  $\alpha$ -cell glucagon secretion, and, absent the latter, increments in adrenomedullary epinephrine secretion normally prevent or rapidly correct hypoglycemia (2). These defenses against hypoglycemia are compromised in insulin-deficient type 1 diabetes and advanced type 2 diabetes; insulin levels do not decrease, glucagon levels do not increase, and the increase in epinephrine levels is typically attenuated (1).

Loss of the glucagon secretory response to falling plasma glucose concentrations is a key feature of the pathophysiology of glucose counterregulation; specifically, this includes the clinical syndrome of defective glucose counterregulation (1) in type 1 diabetes (3,4) and advanced (i.e., absolutely insulin-deficient) type 2 diabetes (5). The mechanism of this glucose counterregulatory defect is unknown. It is known to be a selective defect, as the glucagon secretory response to other stimuli, such as amino acid administration (6,7), remains intact. Therefore, it must be a signaling, rather than a structural,  $\alpha$ -cell abnormality. It is closely linked with the loss of endogenous insulin secretion (5,8), but not with classical diabetic autonomic neuropathy (9). Although it is often associated with functional sympathoadrenal failure (i.e., hypoglycemia-associated autonomic failure) (1), the glucagon response is absent in some patients with a normal epinephrine response (4). Therefore, the mechanisms of the loss of the glucagon response and the attenuated sympathoadrenal response are almost assuredly different.

It is our premise that insight into the mechanism supporting the normal glucagon secretory response to falling plasma glucose concentrations in nondiabetic individuals

will shed light on the mechanism behind the loss of the glucagon response in those with insulin-deficient diabetes. There is considerable evidence, largely from animal studies (10,11), but also some from human studies (12,13), that activation of the central nervous system—mediated autonomic nervous system (sympathetic, parasympathetic, and adrenomedullary) by hypoglycemia plays a role in stimulating glucagon secretion. However, we (14) and others (15) have found that pharmacological blockade of the actions of the classical autonomic mediators norepinephrine, acetylcholine, and epinephrine with  $\alpha$ - and  $\beta$ -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 (16).  $\alpha$ -Cells are also thought to sense low glucose concentrations directly, leading to increased glucagon secretion (17).

Our focus is on a third mechanism, the inraislet insulin hypothesis. First proposed by Samols et al. (18), the inraislet insulin hypothesis posits that a decrease in  $\beta$ -cell insulin secretion, and thus a decrease in inraislet insulin and tonic inraislet  $\alpha$ -cell inhibition by insulin, is a signal for increased glucagon secretion in response to hypoglycemia. It is supported by three findings from studies of the perfused rat pancreas: 1) the islet microcirculation flows from  $\beta$ -cells to  $\alpha$ -cells (19), 2) perfusion with an antibody to insulin increases glucagon release (20), and 3) suppression of insulin release at baseline and throughout prevents glucagon release in response to perfusion with a low-glucose medium (21). It is also supported by *in vivo* studies in rats with streptozotocin-induced diabetes (22). Similar to people with insulin-deficient diabetes, these insulin-deficient animals have no glucagon response to hypoglycemia. However, when insulin is infused into the superior pancreaticoduodenal artery before the induction of hypoglycemia and is then switched off when hypoglycemia is induced, circulating glucagon concentrations increase (22). In addition, it has been found that both normal islets and islets from streptozotocin-administered rats can respond to glucose deprivation by releasing glucagon if they are first provided with increased endogenous or exogenous insulin (23). Furthermore, there is considerable evidence, including that from human studies (24–29), that insulin suppresses glucagon secretion (17).

The inraislet insulin hypothesis is attractive not only because of the rodent data that support it (18–23), but also because if it were documented in healthy humans that a decrease in inraislet insulin is normally a signal for the glucagon secretory response to hypoglycemia, then the absence of that signal would plausibly explain the loss of the glucagon response to hypoglycemia in insulin-deficient diabetes (3–9). In our initial study in healthy humans, we found that inraislet hyperinsulinemia, produced by an infusion of the  $\beta$ -cell secretagogue tolbutamide, prevented the glucagon response to hypoglycemia despite an intact autonomic response and a low  $\alpha$ -cell glucose concentration (30). Although that finding is consistent with the inraislet insulin hypothesis, it is conceivable that the prevention of the glucagon response was the result of inraislet hyperinsulinemia *per se* rather than the absence of a decrease in inraislet insulin. Gosmanov et al. (31)

reported that suppression of insulin (and glucagon and growth hormone) secretion with somatostatin for 1 h before and during the 1st h of a 2-h hyperinsulinemic-hypoglycemic clamp partially reduces the increment in plasma glucagon during the 2nd h of hypoglycemia in healthy humans. Although those study results might have been confounded by ongoing suppression of glucagon secretion, the offset of the somatostatin effect was rapid. Indeed, given a rebound, postsomatostatin increase in the growth hormone response, a similar rebound increase in the glucagon response might have been expected. If so, the data may have underestimated an effect of the absence of a decrement in inraislet insulin to reduce the glucagon response to hypoglycemia. Accordingly, we tested the hypothesis that selective suppression of insulin secretion with the ATP-sensitive potassium channel agonist diazoxide (32) before the induction of hypoglycemia and the resulting reduction of the decrement in inraislet insulin during the induction of hypoglycemia, reduces the glucagon secretory response to hypoglycemia in healthy humans.

## RESEARCH DESIGN AND METHODS

For this study, 14 healthy young adults (7 women and 7 men) gave their written informed consent to participate. The study was approved by the Washington University Medical Center Human Studies Committee and conducted at the Washington University General Clinical Research Center. Subjects' mean age ( $\pm$ SD) was  $26 \pm 4$  years and their mean BMI was  $23 \pm 4$  kg/m<sup>2</sup>. All subjects had normal fasting plasma glucose concentrations, serum creatinine concentrations, and hematocrits and normal electrocardiograms (ECG).

Subjects reported to the research center early in the morning after an overnight fast on two occasions separated by at least 2 weeks. After normal blood pressures and heart rates in the supine and standing positions were documented, subjects assumed the supine position and remained in that position until the study was completed. Lines were inserted into an antecubital vein (for insulin and glucose infusions) and a dorsal hand vein, with that hand being kept in an  $\sim 55^\circ\text{C}$  Plexiglas box (for arterialized venous sampling) at  $t = \sim -30$  min. A hyperinsulinemic ( $2.0 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ )-euglycemic ( $\sim 5.0 \text{ mmol/l}$  [90 mg/dl], 0–120 min) clamp followed by a hypoglycemic ( $\sim 3.0 \text{ mmol/l}$  [55 mg/dl], 120–240 min) clamp was performed on both occasions. In random sequence, placebo or diazoxide (Proglycem; Schering-Plough, Kenilworth, NJ), 6.0 mg/kg, *p.o.* was administered at  $t = 0$  min. Samples were obtained and blood pressures and heart rates (Propaq Encore; Protocol Systems, Beaverton, OR) were recorded at  $t = -15, 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225,$  and  $240$  min. The ECG was monitored throughout. Neurogenic (autonomic) and neuroglycopenic symptoms of hypoglycemia were assessed at  $t = -15, 0, 30, 60, 90, 120, 150, 180, 210,$  and  $240$  min. Symptoms were quantitated by asking the subjects to score (from 0 [none] to 6 [severe]) 12 symptoms chosen based on our published data (14): 6 neurogenic (adrenergic: pounding heart, feeling shaky/tremulous, or feeling nervous/anxious; and cholinergic: feeling sweaty, hungry, or tingling) and 6 neuroglycopenic (difficulty thinking/feeling confused, and feeling tired, drowsy, weak, warm, faint, or dizzy) symptoms were assessed. Arginine hydrochloride (5.0 g) was injected intravenously after the 240-min sample; additional samples were obtained at  $t = 243, 245,$  and  $247$  min.

**Analytical methods.** Plasma glucose concentrations were measured by the glucose oxidase method (Yellow Springs Analyzer 2; Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin (33), C-peptide (33), glucagon (34), pancreatic polypeptide (35), growth hormone (36), and cortisol (37) levels were measured with radioimmunoassays. Plasma epinephrine and norepinephrine levels were measured with a single isotope derivative (radioenzymatic) method (38). Serum nonesterified fatty acids (39) and blood lactate (40) were measured with enzymatic techniques.

**Statistical methods.** Data are given as mean  $\pm$  SE, except where the SD is specified. Baseline adjusted data were analyzed by mixed-procedure, repeated-measures ANOVA. *P* values  $<0.0500$  were considered to indicate statistically significant differences. Condition (diazoxide or placebo) or condition  $\times$  time interaction ANOVA *P* values are reported. Increments in plasma glucagon under the two conditions were contrasted with a *t* test for paired data.

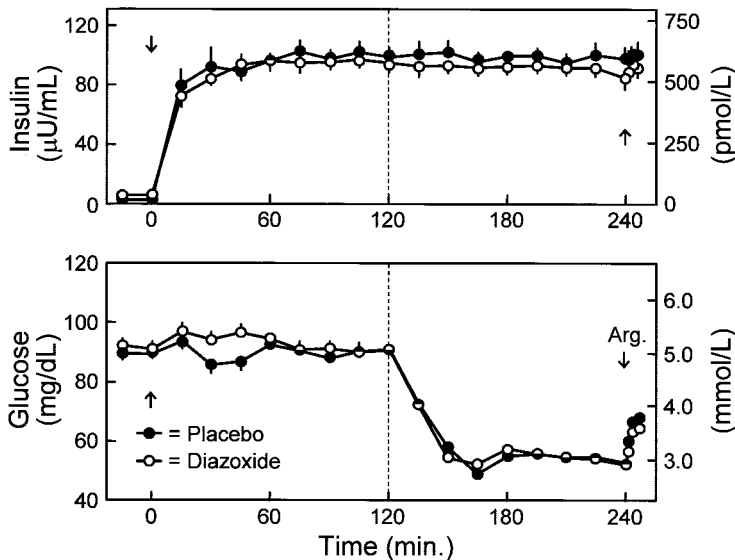


FIG. 1. Plasma insulin and glucose concentrations during hyperinsulinemic-euglycemic and then hypoglycemic clamps, with diazoxide (6.0 mg/kg) or placebo given orally after the 0-min sample and arginine hydrochloride (Arg.; 5.0 g) injected intravenously after the 240-min sample. Data are means  $\pm$  SE.

Pearson correlation coefficients were determined for the relation between decrements in plasma C-peptide and increments in plasma glucagon.

## RESULTS

Plasma insulin concentrations were similar and target plasma glucose concentrations were achieved during both clamp studies (Fig. 1). Plasma C-peptide concentrations were lower during the euglycemic phase of the diazoxide compared with the placebo clamp ( $P = 0.0015$ ) (Fig. 2). Plasma C-peptide concentrations declined from  $0.6 \pm 0.1$  nmol/l ( $1.7 \pm 0.2$  ng/ml) at  $t = 0$  min to  $0.4 \pm 0.0$  nmol/l ( $1.2 \pm 0.1$  ng/ml) at  $t = 120$  min after placebo and from  $0.6 \pm 0.1$  nmol/l ( $1.7 \pm 0.2$  ng/ml) at  $t = 0$  min to  $0.3 \pm 0.0$  nmol/l ( $0.8 \pm 0.1$  ng/ml) at  $t = 120$  min after diazoxide administration. During the hypoglycemic phase, plasma C-peptide concentrations declined further in both clamps, to  $0.1 \pm 0.0$  nmol/l ( $0.4 \pm 0.1$  ng/ml) with placebo and  $0.1 \pm 0.0$  nmol/l ( $0.4 \pm 0.1$  ng/ml) with diazoxide (Fig. 2). Thus, during the induction of hypoglycemia, the decrement in mean C-peptide in the diazoxide clamps (0.4 ng/ml) was 50% of that in the placebo clamps (0.8 ng/ml) ( $P = 0.0015$ ). It was notable that intravenous arginine did not raise C-peptide levels during hypoglycemia in either study (Fig. 2).

Plasma glucagon concentrations declined similarly during the euglycemic phase of both clamp studies (Fig. 3). Plasma glucagon concentrations declined from  $34 \pm 3$

pmol/l ( $117 \pm 9$  pg/ml) at  $t = 0$  min to  $28 \pm 2$  pmol/l ( $98 \pm 7$  pg/ml) at  $t = 120$  min with placebo and from  $36 \pm 3$  pmol/l ( $124 \pm 9$  pg/ml) at  $t = 0$  min to  $29 \pm 3$  pmol/l ( $102 \pm 9$  pg/ml) at  $t = 120$  min with diazoxide. The glucagon response to hypoglycemia was reduced ( $P = 0.0010$ ) with the diazoxide compared with the placebo clamps (Fig. 3). Plasma glucagon concentrations rose to  $43 \pm 5$  pmol/l ( $151 \pm 16$  pg/ml) at  $t = 240$  min in the placebo clamps and to  $35 \pm 2$  pmol/l ( $123 \pm 8$  pg/ml) at  $t = 240$  min in the diazoxide clamps. Thus, during hypoglycemia, the increment in plasma glucagon in the diazoxide clamps ( $21 \pm 5$  pg/ml) was 45% of that in the placebo clamps ( $47 \pm 11$  pg/ml) ( $P = 0.027$ ). It was notable that the glucagon response to intravenous arginine was not reduced by diazoxide (Fig. 3). The increments in plasma glucagon were related to the decrements in plasma C-peptide ( $r = -0.593$ ,  $P = 0.015$ ) during hypoglycemia; the correlation coefficient was  $-0.965$  ( $P < 0.001$ ) in the placebo clamps.

Plasma epinephrine ( $P = 0.1583$ ) and norepinephrine ( $P = 0.2599$ ) concentrations (Fig. 4), neurogenic symptom scores ( $P = 0.6859$ ), plasma pancreatic polypeptide concentrations ( $P = 0.1456$ ) (Fig. 5), and growth hormone and cortisol responses (Table 1) to hypoglycemia were not reduced in the diazoxide clamps. Indeed, the growth hormone response was enhanced ( $P = 0.0134$ ). Cortisol levels were slightly lower during the euglycemic phase of

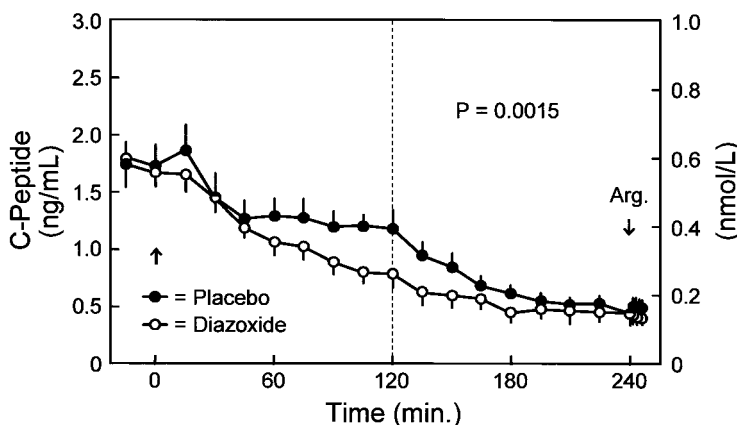


FIG. 2. Plasma C-peptide concentrations during hyperinsulinemic-euglycemic and then hypoglycemic clamps, with diazoxide (6.0 mg/kg) or placebo given orally after the 0-min sample and arginine hydrochloride (Arg.; 5.0 g) injected intravenously after the 240-min sample. Data are means  $\pm$  SE.

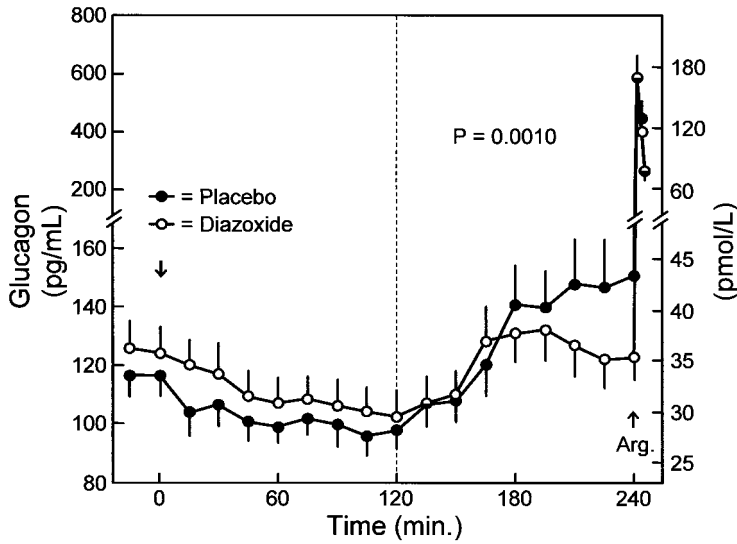


FIG. 3. Plasma glucagon concentrations during hyperinsulinemic-euglycemic and then hypoglycemic clamps, with diazoxide (6.0 mg/kg) or placebo given orally after the 0-min sample and arginine hydrochloride (Arg.; 5.0 g) injected intravenously after the 240-min sample. Data are means  $\pm$  SE.

the diazoxide clamps, but rose to levels comparable with those in the placebo clamps during hypoglycemia ( $P = 0.0255$ ). Neuroglycopenic symptom scores were not altered (data not shown).

Heart rates and blood pressures were similar in both clamps (Table 2), although the mean heart rate tended to be higher ( $P = 0.1835$ ) and the mean diastolic blood pressure tended to be lower ( $P = 0.0545$ ) during the hypoglycemic phase of the diazoxide clamps. Blood lactate and serum nonesterified fatty acid levels ( $P = 0.6683$ ) were similar under both conditions, although the increment in blood lactate during hypoglycemia was reduced slightly ( $P = 0.0154$ ) in the diazoxide clamps (Table 3).

**DISCUSSION**

These data demonstrate, in healthy humans, that an  $\sim 50\%$  reduction in the decrement in insulin secretion, and thus in the decrement in inraislelet insulin, during induction of hypoglycemia causes a  $>50\%$  reduction in the glucagon response to hypoglycemia. Thus, they document the inraislelet insulin hypothesis (18–23,30,31) for the signaling of the glucagon response to hypoglycemia in humans: a decrease in arterial glucose  $\rightarrow$  a decrease in  $\beta$ -cell insulin secretion  $\rightarrow$  a decrease in inraislelet insulin  $\rightarrow$  a decrease

in tonic  $\alpha$ -cell inhibition by insulin  $\rightarrow$  an increase in glucagon secretion. Compared with placebo, diazoxide selectively, but only partially, suppressed plasma C-peptide concentrations, an index of insulin secretion, during the hyperinsulinemic-euglycemic phase of the clamps. C-peptide concentrations then decreased to comparable levels during hypoglycemia. Thus, there was a decrement in inraislelet insulin during the induction of hypoglycemia and a subsequent increment in glucagon secretion during hypoglycemia in both the diazoxide and the placebo clamp studies. However, both the decrement in inraislelet insulin during the induction of hypoglycemia and the subsequent increment in glucagon secretion during hypoglycemia were smaller in the diazoxide compared with the placebo clamps. Stated differently, a reduced signal (decrement in inraislelet insulin) during induction of hypoglycemia led to a reduced response (increment in plasma glucagon) during hypoglycemia. The timing of the glucagon response was similar under both conditions, but the magnitude of the increment in plasma glucagon was related to the magnitude of the decrement in inraislelet insulin.

The effect of diazoxide cannot be attributed to a direct inhibitory action of the drug on  $\alpha$ -cell glucagon secretion. Baseline plasma glucagon concentrations and the brisk

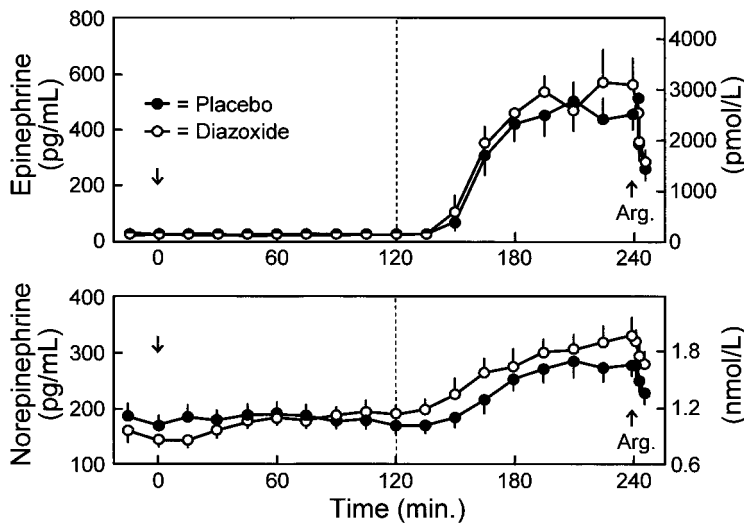


FIG. 4. Plasma epinephrine and norepinephrine concentrations during hyperinsulinemic-euglycemic and then hypoglycemic clamps, with diazoxide (6.0 mg/kg) or placebo given orally after the 0-min sample and arginine hydrochloride (Arg.; 5.0 g) injected intravenously after the 240-min sample. Data are means  $\pm$  SE.

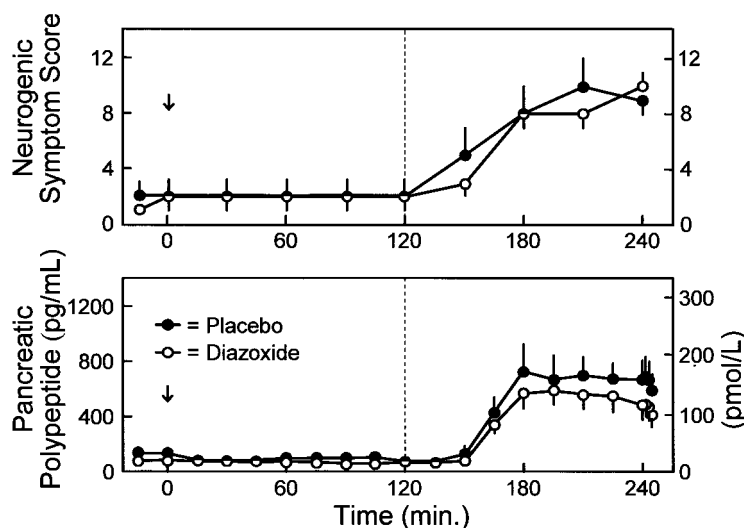


FIG. 5. Neurogenic symptom scores and plasma pancreatic polypeptide concentrations during hyperinsulinemic-euglycemic and then hypoglycemic clamps, with diazoxide (6.0 mg/kg) or placebo given orally after the 0-min sample and arginine hydrochloride (Arg.; 5.0 g) injected intravenously after the 240-min sample. Data are means  $\pm$  SE.

glucagon response to intravenous arginine were not reduced in the diazoxide, compared with the placebo, clamps. Furthermore, neither intravenous nor oral diazoxide suppressed plasma glucagon concentrations in healthy humans under nonclamped conditions (32). In addition, although by suppressing baseline insulin release diazoxide reduced the glucagon release response to low glucose from the perfused rat pancreas, consistent with the intraislet insulin hypothesis, it did not suppress basal glucagon

TABLE 1

Plasma growth hormone and cortisol concentrations during hyperinsulinemic, euglycemic (0–120 min) and then hypoglycemic (120–240 min) clamps before and after administration of diazoxide or placebo and later arginine hydrochloride

Time (min)	Growth hormone (ng/ml)		Cortisol ( $\mu$ g/dl)	
	Placebo	Diazoxide	Placebo	Diazoxide
-15	4.7 $\pm$ 1.5	5.3 $\pm$ 1.2	16.0 $\pm$ 1.6	16.4 $\pm$ 1.6
0	4.7 $\pm$ 1.6	6.1 $\pm$ 1.4	15.3 $\pm$ 1.6	15.4 $\pm$ 1.6
15	4.3 $\pm$ 1.3	5.7 $\pm$ 1.5	13.7 $\pm$ 1.2	13.4 $\pm$ 1.2
30	5.0 $\pm$ 1.7	4.4 $\pm$ 1.2	13.1 $\pm$ 1.3	12.8 $\pm$ 1.0
45	5.0 $\pm$ 2.1	3.8 $\pm$ 1.2	13.0 $\pm$ 1.1	11.7 $\pm$ 1.0
60	3.5 $\pm$ 1.4	2.8 $\pm$ 1.0	12.6 $\pm$ 1.1	11.4 $\pm$ 1.0
75	1.6 $\pm$ 0.5	2.2 $\pm$ 0.9	11.2 $\pm$ 1.1	11.4 $\pm$ 1.2
90	1.4 $\pm$ 0.5	1.8 $\pm$ 0.8	11.3 $\pm$ 0.9	9.8 $\pm$ 1.0
105	0.9 $\pm$ 0.2	1.4 $\pm$ 0.6	10.9 $\pm$ 0.8	9.1 $\pm$ 0.9
120	1.0 $\pm$ 0.2	1.4 $\pm$ 0.5	10.6 $\pm$ 0.9	8.4 $\pm$ 0.9
135	2.5 $\pm$ 1.2	1.2 $\pm$ 0.4	9.8 $\pm$ 0.8	8.1 $\pm$ 0.8
150	2.9 $\pm$ 1.4	1.6 $\pm$ 0.4	9.9 $\pm$ 0.8	8.0 $\pm$ 0.9
165	3.7 $\pm$ 1.4	5.7 $\pm$ 1.5	10.8 $\pm$ 0.8	9.9 $\pm$ 1.2
180	10.7 $\pm$ 2.5	16.4 $\pm$ 2.9	14.2 $\pm$ 1.0	12.9 $\pm$ 1.2
195	17.6 $\pm$ 3.6	23.3 $\pm$ 3.9	16.6 $\pm$ 1.1	16.9 $\pm$ 1.4
210	17.0 $\pm$ 2.5	28.5 $\pm$ 5.4	18.4 $\pm$ 1.1	18.8 $\pm$ 1.8
225	19.4 $\pm$ 3.0	28.1 $\pm$ 4.6	19.5 $\pm$ 1.5	19.6 $\pm$ 1.9
240	20.9 $\pm$ 2.9	25.3 $\pm$ 4.2	19.4 $\pm$ 1.7	18.9 $\pm$ 1.8
243	21.4 $\pm$ 3.0	22.9 $\pm$ 3.1	17.9 $\pm$ 1.5	18.3 $\pm$ 1.6
245	21.2 $\pm$ 3.3	24.4 $\pm$ 3.2	17.7 $\pm$ 1.6	17.2 $\pm$ 1.6
247	22.4 $\pm$ 3.3	25.8 $\pm$ 3.5	18.5 $\pm$ 1.5	17.8 $\pm$ 1.7

Data are means  $\pm$  SE. Diazoxide (6.0 mg/kg) or placebo was given orally after the 0-min sample and arginine hydrochloride (5.0 g) was injected intravenously after the 240-min sample. (Convert ng/ml to pmol/l by multiplying by 44.15, and convert  $\mu$ g/dl to nmol/l by multiplying by 27.59.)  $P = 0.0134$  for placebo vs. diazoxide in growth hormone measurements;  $P = 0.0255$  for placebo vs. diazoxide in cortisol measurements.

release and enhanced, rather than inhibited, the glucagon release response to perfusion with high glucose (20). Finally, diazoxide did not decrease basal glucagon release from  $\alpha$ -TC glucagonoma cells (41).

An important finding of our study, in the context of other signals for the glucagon response to hypoglycemia, was that the reduction of the intraislet insulin signal in the diazoxide clamps reduced the glucagon response to hypoglycemia, despite a low  $\alpha$ -cell glucose concentration and an intact autonomic nervous system response. Activation of all three components of the autonomic nervous system—the adrenomedullary (plasma epinephrine and norepinephrine) (42), sympathetic neural (neurogenic symptoms) (42), and parasympathetic neural (plasma pancreatic polypeptide)—during hypoglycemia was similar in the diazoxide and placebo clamps. These data do not negate a role for autonomic activation in stimulating glucagon secretion during hypoglycemia (10–16). However, they do demonstrate a role for an intraislet insulin signal independent of the autonomic responses. The growth hormone and cortisol responses to hypoglycemia were also not reduced after diazoxide administration.

Several findings of this study, although not novel (2), are of interest. First, intravenous arginine did not stimulate insulin secretion during hypoglycemia under either clamp condition. This further illustrates that hypoglycemia suppresses insulin secretion, and does so potentially. Second, the increase in plasma glucagon stimulated by intravenous arginine was followed by prompt increments in plasma glucose. This further illustrates that glucagon stimulates glucose production potently, despite substantial hyperinsulinemia. Third, as plasma glucose levels approached the physiological postabsorptive range, plasma epinephrine and norepinephrine levels fell. This further illustrates that the glycemic thresholds for catecholamine release, like those for other glucose counterregulatory hormones, lie below the physiological range.

Bingham et al. (43) found no significant effect of diazoxide on the glucagon response to hypoglycemia in healthy men, although the mean peak glucagon concentration tended to be lower after diazoxide than after placebo administration. However, they used a lower dosage of diazoxide and did not document an effect of the drug on insulin secretion by measuring plasma C-peptide concen-

TABLE 2

Heart rates and systolic and diastolic blood pressures during hyperinsulinemic, euglycemic (0–120 min) and then hypoglycemic (120–240 min) clamps before and after administration of diazoxide or placebo and later arginine hydrochloride

Time (min)	Heart rate (bpm)		Systolic blood pressure (mmHg)		Diastolic blood pressure (mmHg)	
	Placebo	Diazoxide	Placebo	Diazoxide	Placebo	Diazoxide
-15	64 ± 3	67 ± 3	116 ± 2	113 ± 3	63 ± 2	65 ± 2
0	67 ± 3	68 ± 3	115 ± 3	115 ± 3	62 ± 3	63 ± 2
15	64 ± 2	70 ± 3	117 ± 3	118 ± 3	64 ± 2	64 ± 2
30	67 ± 3	74 ± 3	117 ± 3	119 ± 3	63 ± 3	62 ± 2
45	67 ± 3	74 ± 4	116 ± 3	116 ± 3	62 ± 2	61 ± 2
60	68 ± 3	71 ± 3	119 ± 2	118 ± 3	63 ± 2	62 ± 3
75	69 ± 3	79 ± 3	116 ± 3	115 ± 3	60 ± 2	60 ± 2
90	74 ± 4	76 ± 4	121 ± 3	118 ± 3	63 ± 2	59 ± 2
105	70 ± 3	78 ± 3	116 ± 3	117 ± 4	60 ± 2	56 ± 2
120	73 ± 3	81 ± 3	119 ± 3	115 ± 3	61 ± 2	57 ± 2
135	71 ± 3	80 ± 4	117 ± 3	115 ± 4	59 ± 2	55 ± 2
150	73 ± 3	81 ± 4	117 ± 3	117 ± 3	59 ± 3	55 ± 2
165	80 ± 4	83 ± 4	116 ± 3	112 ± 4	58 ± 3	52 ± 2
180	77 ± 4	84 ± 5	119 ± 4	113 ± 5	55 ± 3	49 ± 3
195	74 ± 4	81 ± 4	116 ± 4	112 ± 4	54 ± 3	47 ± 2
210	76 ± 3	81 ± 4	117 ± 4	115 ± 4	55 ± 3	48 ± 2
225	72 ± 5	83 ± 4	116 ± 4	110 ± 4	54 ± 2	46 ± 2
240	72 ± 4	78 ± 4	115 ± 3	114 ± 4	53 ± 2	47 ± 2
243	70 ± 4	79 ± 4	113 ± 4	107 ± 4	52 ± 2	44 ± 2
245	70 ± 4	79 ± 4	112 ± 4	109 ± 4	52 ± 2	45 ± 2
247	74 ± 4	81 ± 4	114 ± 3	110 ± 3	54 ± 3	47 ± 2

Data are means ± SE. Diazoxide (6.0 mg/kg) or placebo was given orally after the 0-min sample and arginine hydrochloride (5.0 g) was injected intravenously after the 240-min sample.

TABLE 3

Blood lactate and serum nonesterified fatty acid concentrations during hyperinsulinemic, euglycemic (0–120 min) and then hypoglycemic (120–240 min) clamps before and after administration of diazoxide or placebo and later arginine hydrochloride

Time (min)	Lactate (μmol/l)		Nonesterified fatty acid (μmol/l)	
	Placebo	Diazoxide	Placebo	Diazoxide
-15	762 ± 93	772 ± 95	518 ± 44	505 ± 49
0	674 ± 54	822 ± 84	485 ± 33	518 ± 54
15	686 ± 58	760 ± 75	386 ± 24	334 ± 35
30	985 ± 78	909 ± 89	203 ± 31	232 ± 36
45	1,118 ± 58	1,174 ± 96	146 ± 24	203 ± 33
60	1,202 ± 79	1,283 ± 99	124 ± 22	156 ± 31
75	1,061 ± 75	1,262 ± 127	131 ± 26	138 ± 23
90	1,151 ± 66	1,224 ± 82	96 ± 13	127 ± 16
105	1,154 ± 80	1,158 ± 74	107 ± 14	106 ± 20
120	1,089 ± 69	1,285 ± 81	115 ± 20	114 ± 20
135	1,037 ± 107	1,185 ± 79	100 ± 13	125 ± 19
150	1,084 ± 107	1,223 ± 107	109 ± 27	122 ± 28
165	1,134 ± 82	1,174 ± 75	116 ± 19	148 ± 26
180	1,462 ± 115	1,458 ± 104	121 ± 20	168 ± 26
195	1,601 ± 116	1,509 ± 133	131 ± 20	118 ± 19
210	1,638 ± 134	1,523 ± 136	124 ± 19	155 ± 31
225	1,559 ± 186	1,460 ± 136	102 ± 18	134 ± 21
240	1,664 ± 161	1,628 ± 109	128 ± 17	146 ± 28
243	1,661 ± 141	1,619 ± 128	95 ± 13	166 ± 36
245	1,571 ± 118	1,707 ± 153	92 ± 11	156 ± 30
247	1,892 ± 205	1,618 ± 94	114 ± 18	143 ± 28

Data are means ± SE. Diazoxide (6.0 mg/kg) or placebo was given orally after the 0-min sample and arginine hydrochloride (5.0 g) was injected intravenously after the 240-min sample. *P* = 0.0154 for placebo vs. diazoxide for lactate measurements.

trations. Furthermore, the precise temporal relation between diazoxide administration and induction of hypoglycemia is unclear in their report, and the frankly hypoglycemic glucose level (43 mg/dl) was maintained for only 40 min. In the present study, the effect of a reduced in-trasilet insulin signal to reduce the glucagon response became apparent during the 2nd h of hypoglycemia.

McCrimmon et al. (44) have reported that diazoxide injected bilaterally into the rat ventromedial hypothalamus increases the plasma epinephrine and glucagon responses to hypoglycemia. In the present study, systemic diazoxide administration did not alter the plasma epinephrine response and reduced the glucagon response.

Although the present data indicate that a decrease in in-trasilet insulin is a signal for glucagon secretion in response to hypoglycemia, a decrement in insulin secretion alone does not stimulate glucagon secretion. Plasma C-peptide concentrations declined during the hyperinsulinemic-euglycemic clamp, but plasma glucagon concentrations did not increase. In streptozotocin-induced diabetic rats, the switch off of superior pancreaticoduodenal artery insulin infusion elicited a glucagon response only when that was done at the time hypoglycemia was induced (22). Thus, there appears to be an interaction between a decrease in in-trasilet insulin and a decrease in α-cell glucose that triggers increased glucagon secretion (22,30).

In conclusion, the present data indicate that a decrease in in-trasilet insulin is a signal for the normal glucagon secretory response to hypoglycemia in healthy humans. The absence of that signal plausibly explains the loss of the glucagon response to falling plasma glucose concentrations, a key feature of the pathogenesis of iatrogenic

hypoglycemia, in insulin-deficient (type 1 and advanced type 2) diabetes (3–9).

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