Further Defects in Counterregulatory Responses Induced by Recurrent Hypoglycemia in IDDM

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We evaluated the effect of previous experimental hypoglycemia on counterregulatory responses to hypoglycemia in 13 IDDM patients. These patients had defects in counterregulatory responses to hypoglycemia compared with 7 nondiabetic control subjects. Plasma EPI and glucagon responses to hypoglycemia in IDDM patients were ~60% of levels in nondiabetic subjects ($P < 0.02$ and $P < 0.001$, respectively). Hepatic glucose output ([3-^{3}H]glucose) was reduced by ~60% of normal ($P < 0.005$), and the glucose infusion rate required to maintain plasma glucose was correspondingly greater in people with IDDM ($P < 0.001$). With a modified glucose clamp (plasma insulin ~330 pM), the diabetic subjects underwent two sequential 120-min periods of hypoglycemia (~3.0 mM) with an intervening 60-min euglycemic recovery period. In the IDDM patients, there were 30–50% decreases in plasma GH ($P < 0.005$) and cortisol ($P < 0.001$) responses during the second hypoglycemic period compared with the first. In addition, glucose output, already defective compared with that in nondiabetic subjects, was further reduced by 33% ($P = 0.03$) during the second period of experimental hypoglycemia. There was no effect of repeated hypoglycemia on the responses of plasma glucagon, EPI, or NE, though plasma EPI was correlated directly with glucose output ($P < 0.001$) and inversely with glucose uptake ($P < 0.05$). There was no correlation between the rise in glucose output during hypoglycemia and antecedent glycemic control as measured by HbA_1. We conclude that in IDDM patients with preexisting defects in counterregulatory responses to hypoglycemia, recurrent, mild hypoglycemia is associated with additional reductions in pituitary-adrenocortical hormonal secretion and further impairment of hepatic glucose production.
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The treatment of IDDM is characterized by frequent episodes of iatrogenic hypoglycemia, estimated to occur ~3000 times in a patient's lifetime (1). Although most episodes are mild to moderate, the precise frequency of such events is difficult to quantify. Severe hypoglycemia is more readily ascertainable and was estimated in the Diabetes Control and Complications Trial feasibility phase to occur an average of ~17 times per 100 patient-yr in conventionally treated and ~54 times per 100 patient-yr in intensively treated individuals (2). Although the potential clinical sequelae of severe hypoglycemia are obviously very significant, less clear are the possible consequences of episodes of mild to moderate hypoglycemia

Defective glucose counterregulation in association with reduced secretion of glucagon and EPI has been reported in many patients with IDDM (3,4). In IDDM patients treated with intensive therapy, a constellation of more extensive abnormalities exist, including decreased hypoglycemia awareness and defective cortisol, NE, and GH secretion (5). In addition, the level of glucose that stimulates hormone secretion (glycemic threshold) is reduced for EPI, GH, and cortisol in patients under intensive therapy (6). It has been speculated that the more frequent hypoglycemic events that accompany such regimens may be responsible for some of these alterations in glucose counterregulation (7). Evidence to support this possibility comes from studies of nondia-

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IDDM, insulin-dependent diabetes; EPI, epinephrine; NE, norepinephrine; IV, intravenous; GH, growth hormone; HPLC, high-performance liquid chromatography; HYPO 1, period of hypoglycemia between 30 and 150 min; HYPO 2, period of hypoglycemia during last 120 min of study; ANOVA, analysis of variance; NS, not significant; FFA, free fatty acids; MDI, multiple-dose insulin therapy; CSII, continuous s.c. insulin infusion (pump) therapy; CRH, corticotropin-releasing hormone; BMI, body mass index.

TABLE 1
Clinical characteristics of IDDM patients

IDDM patients	Age (yr)	BMI (kg/m^2)	Sex (M/F)	HbA_1 (%)	Duration of IDDM (yr)	Treatment of IDDM	Total daily insulin dose (U)
1	34	25.1	F	5.9	8	MDI	46
2	25	23.6	F	6.0	5	MDI	37
3	34	29.9	M	9.4	19	CSII	59
4	26	30.2	M	7.5	1	MDI	22
5	38	26.3	M	8.3	24	MDI	60
6	31	24.0	F	9.2	19	Conventional	32
7	40	23.4	F	14.0	14	Conventional	72
8	40	22.8	M	15.6	10	Conventional	30
9	32	24.7	F	10.4	16	Conventional	64
10	37	27.2	M	7.7	3	Conventional	48
11	22	22.0	M	12.5	6	Conventional	48
12	39	23.3	F	10.0	25	Conventional	38
13	34	21.5	M	11.6	14	Conventional	23
Normal range				6.0–8.3			

betic individuals in whom repetitive experimental hypoglycemia produced defects in hormone secretion or resulted in a lowering of the plasma glucose concentration required to stimulate hormone release, resembling the defects in IDDM patients (7–9). In addition, we have reported impairment in nondiabetic humans of hepatic glucose output after recurrent hypoglycemia that was independent of blunted hormonal responses (8). Finally, in patients with recurrent hypoglycemia due to islet-cell tumors, similar defects in counterregulatory hormone secretion have been reported (10,11), which may be reversed after resection of the tumor and return of normal glucose concentrations (10).

The relevance of experimental models of recurrent hypoglycemia has not been examined for IDDM. In particular, IDDM patients may already display a spectrum of preexisting defects in glucose counterregulatory mechanisms, even in the absence of autonomic neuropathy. We therefore studied an unselected group of such patients using a model of repetitive mild hypoglycemia in an attempt to determine whether the defects that we previously reported to be inducible by repeated hypoglycemia in nondiabetic subjects (8) could also occur in people with IDDM.

RESEARCH DESIGN AND METHODS

We recruited 13 patients with IDDM for this study. Their clinical characteristics are shown in Table 1. None of the subjects had symptomatic or clinical evidence of other endocrine diseases (except for treated hypothyroidism in one), and none described a history of repeated episodes of severe hypoglycemia. None of the subjects suffered from symptoms of clinical peripheral or autonomic neuropathy. A group of 7 normal, nondiabetic persons aged 25 ± 1 yr (3 men, 4 women) who had no history of hypoglycemia and were on no medications were included as control subjects to compare the responses to hypoglycemia of IDDM patients. These nondiabetic subjects were studied during a single period of hypoglycemia of equivalent severity, duration, and insulin infusion rates as were used in this study. The data from 4 of these normal subjects was reported previously (8).

On the day before the study, diabetic subjects were admitted to the Clinical Research Center and placed on an IV infusion of short-acting insulin (Humulin, Eli Lilly, Indianapolis, IN). Plasma glucose was measured hourly and normalized with an insulin-infusion algorithm. Subjects were last fed at 2200 the night before the study. The following morning, an IV catheter was placed in retrograde fashion in a distal vein in the wrist for blood withdrawal and in an antecubital vein in the contralateral arm for infusions. Arterialized venous blood was withdrawn at 5- to 10-min intervals from the hand, which was placed in a heated box.

The details of this protocol were described previously (8). Briefly, a primed-continuous infusion of HPLC-purified [3-^{3}H]glucose (Amersham, Arlington Heights, IL) was initiated and continued for 120 min before the experiments for estimation of glucose turnover. After equilibration, the insulin infusion was adjusted at a fixed rate of 17 mU · m^2 body surface area · min^{-1}, and an infusion of 20% dextrose was initiated at a variable rate to clamp the plasma glucose at desired levels (Fig. 1). Plasma glucose was maintained at euglycemic concentrations (~5.0 mM) for 30 min; then the glucose infusion rate was reduced to allow plasma glucose to fall to the hypoglycemic target for study (~3.0 mM). Plasma glu-

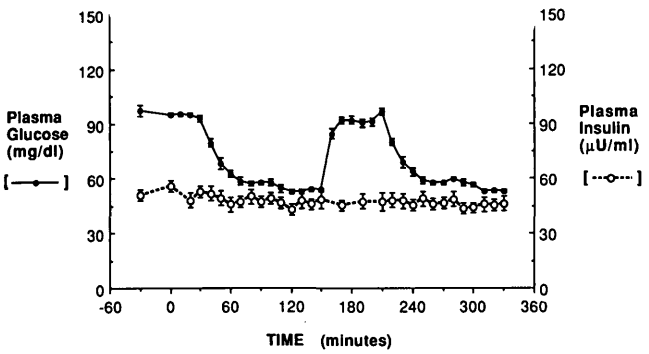

FIG. 1. Plasma glucose and insulin concentrations during clamps in subjects with IDDM (see text for details). To convert glucose in mg/dl to mM, multiply by 0.05551. To convert insulin in μU/ml to pM, multiply by 7.175.

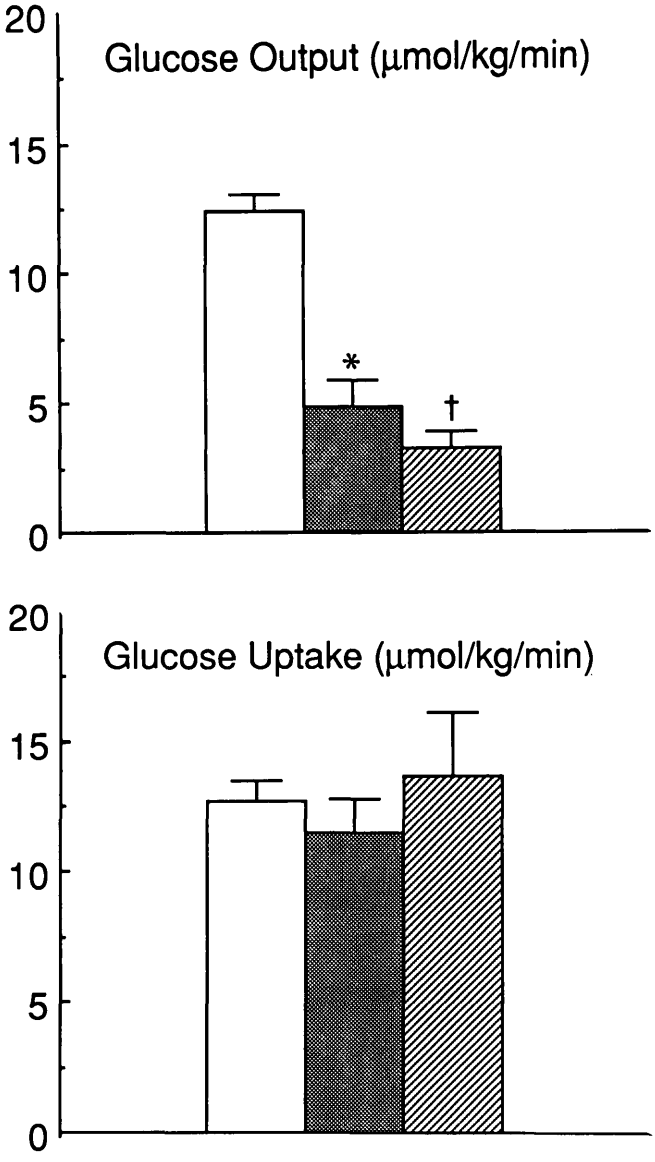

FIG. 2. Average hepatic glucose output and uptake rates at baseline before hypoglycemia (open bars) and during the final 60 min of HYPO 1 (stippled bars) and HYPO 2 (hatched bars). *Glucose output remained significantly below baseline during HYPO 1 ($P < 0.001$). †Further impairment in glucose output in HYPO 2 vs. HYPO 1 was significant ($P = 0.03$).

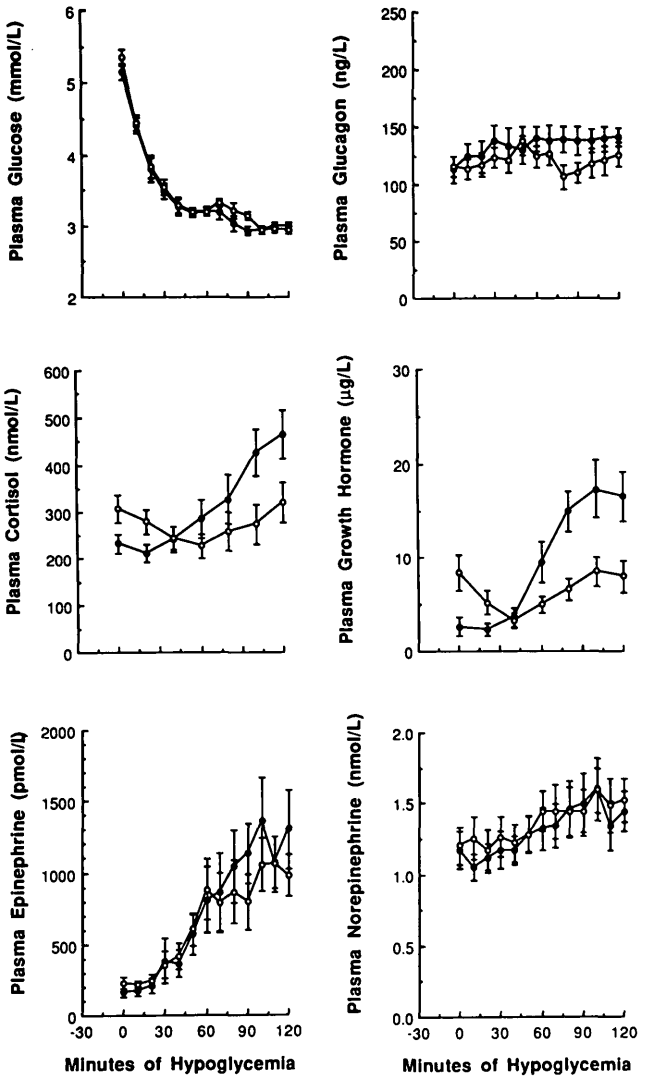

FIG. 3. Plasma glucose, glucagon, cortisol, growth hormone, EPI, and NE responses during 120 min of hypoglycemia. Response during HYPO 1 (solid circles) was compared with HYPO 2 (open circles). There was evidence for reduction in plasma cortisol and GH responses during HYPO 2 compared with HYPO 1 ($P < 0.005$).

cose was clamped at this level for a 120-min experimental period (t = 30–150 min, HYPO 1). Interval euglycemia (~5.0 mM) was established between 150 and 210 min by increasing the glucose infusion rate (recovery period). Finally, a second period of hypoglycemia (~3.0 mM) was induced for the final 120 min of the study (HYPO 2).

The methods for estimation of glucose turnover, plasma free insulin, glucagon, EPI, NE, GH, cortisol, and FFAs were described previously (12–15). Statistical analyses were performed with ANOVA with repeated measures for effects over time and paired Student's *t* test analyses for grouped data (16). Results in the figures are means ± SE.

RESULTS

Basal plasma glucose was clamped in diabetic subjects at normal concentrations (5.3 ± 0.2 mM) with exogenous insulin and then maintained at 3.1 ± 0.1 mM during HYPO 1 (Fig. 1). During the euglycemic recovery period, plasma glucose averaged 5.3 ± 0.2 mM and then was reduced for a second interval during HYPO 2 to 3.0 ± 0.1 mM. Hypoglycemia during the two hypoglycemic periods was thus nearly identical (NS), and the rate of decline also was similar. Plasma free insulin averaged 352 ± 36 pM during HYPO 1 and 330 ± 29 pM during HYPO 2 (Fig. 1). Plasma FFA concentrations (not shown) averaged 172 ± 40 μM at baseline and 342 ± 56 and 425 ± 70 μM during HYPO 1 and HYPO 2, respectively (NS).

Before the start of the glucose clamp, glucose output had been normalized with the overnight infusion of insulin and averaged 12.38 ± 0.56 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (Fig. 2).

TABLE 2
Counterregulatory hormone concentrations and glucose kinetics during hypoglycemia in nondiabetic and IDDM subjects

	Nondiabetic subjects	IDDM patients
Plasma EPI (pM)	2160 ± 450	1300 ± 270*
Plasma NE (nM)	1.51 ± 0.18	1.44 ± 0.22
Plasma glucagon (ng/L)	229 ± 24	140 ± 8†
Plasma GH (μg/L)	17.7 ± 5.2	16.5 ± 2.6
Plasma cortisol (nM)	550 ± 100	460 ± 50
Plasma insulin (pM)	330 ± 22	352 ± 34
Plasma glucose (mM)	3.1 ± 0.1	3.1 ± 0.1
Glucose output ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	12.82 ± 1.22	4.88 ± 1.17‡
Glucose uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	16.93 ± 2.00	11.44 ± 1.33
Glucose infusion rate ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	2.78 ± 1.00	8.88 ± 1.94†

Values are means ± SE.
*$P < 0.02$, †$P < 0.001$, ‡$P < 0.005$, IDDM patients vs. nondiabetic subjects.

After the euglycemic clamp was initiated, endogenous glucose output was initially suppressed (data not shown), but during the final 60 min of each hypoglycemic interval, glucose counterregulation resulted in an increase in glucose output above the suppressed levels. However, glucose output remained significantly below basal during HYPO 1 ($P < 0.001$) and was 33% lower during HYPO 2 than during HYPO 1 ($P = 0.03$; Fig. 2). Glucose uptake was comparable during the final 60 min of HYPO 1 and HYPO 2 (Fig. 2).

Plasma glucagon was unaltered by the mild, recurrent hypoglycemia (Fig. 3). In contrast, the plasma concentrations of the other major counterregulatory hormones all increased by 30–500% over baseline during induction of the first hypoglycemia (Fig. 3). For plasma GH and cortisol, however, the rise induced by hypoglycemia during HYPO 1 was significantly greater than that seen during HYPO 2 ($P < 0.005$ for GH and $P < 0.001$ for cortisol; Fig. 3). Plasma EPI and NE increased comparably during the two hypoglycemic periods (Fig. 3), though plasma EPI tended to be lower during HYPO 2 ($0.05 < P < 0.10$).

The responses of IDDM patients during HYPO 1 differed substantially from normal control subjects during equivalent hypoglycemia (Table 2). Table 2 compares the average values over the final 60 min of hypoglycemia in IDDM patients and an identical period of hypoglycemia in nondiabetic control subjects. The IDDM subjects had decreased secretion of glucagon ($P < 0.001$), EPI ($P < 0.02$), and glucose output ($P < 0.005$). Concomitant with impaired endogenous glucose output, the glucose infusion rate required to maintain identical hypoglycemia was threefold higher in IDDM patients than control subjects (8.88 ± 1.94 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ vs. 2.78 ± 1.00 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.001$). The increases in plasma cortisol, GH, and NE during HYPO 1, however, were equivalent to values in nondiabetic control subjects during hypoglycemia. Thus, as a group, these IDDM patients had impaired counterregulation of hypoglycemia (though the plasma glucose level had been clamped to prevent further decrements in plasma glucose).

A multiple regression analysis was used to evaluate the influence of antecedent glycemic control on the hormonal and kinetic variables of glucose counterregulation. The relationship between HbA_1 during the study and the plasma EPI responses during HYPO 1 and 2 were positively correlated ($r = 0.58$, $P < 0.04$). In the initial hypoglycemic period, the EPI response was highly correlated with glucose output ($r = 0.75$, $P < 0.001$) and inversely correlated with glucose uptake ($r = -0.46$, $P < 0.05$). There was no significant correlation between the rise in glucose output during this hypoglycemic period and HbA_1.

DISCUSSION

Other laboratories (7,9) as well as ours (8) have reported effects of antecedent experimental hypoglycemia on variables of the glucose counterregulatory response in healthy, nondiabetic subjects. This study examined a group of IDDM patients in whom previous glycemic control was variable. However, none of the subjects had symptomatic severe hyperglycemia or hypoglycemia, and most were treated with conventional insulin therapy. As a group, these patients had evidence of defective glucose counterregulation compared with nondiabetic subjects studied during an equivalent single period of hypoglycemia. Consistent with previous reports (3,4), this defect in counterregulation consisted of a marked impairment in glucagon and EPI secretory responses to hypoglycemia and a blunted rise in hepatic glucose output during hypoglycemia. The responses of plasma cortisol, NE, and GH, however, were similar to those in nondiabetic subjects. During experiments in which hypoglycemia was repeated in the IDDM patients, the plasma levels of cortisol and GH were lower compared with the initial period of hypoglycemia. EPI and glucagon secretion, already quite impaired, did not decline further, but hepatic glucose production was further impaired in the recurrent hypoglycemia.

A recent report by Heller and Cryer found evidence that previous experimental hypoglycemia could induce changes in hypoglycemic symptoms and counterregulatory hormone secretion in nondiabetic subjects when recurrent 2-h periods of experimental hypoglycemia were induced over a 24-h period (7). In those studies, the secretion of EPI, NE, and glucagon were all reduced when an interval of hypoglycemia was induced ~18 h before an episode of hypoglycemia (7). In contrast, we reported that two recurrent episodes of hypoglycemia induced in the same fashion as in our present study did not produce decreased responses of catecholamines or glucagon, but did reduce the responses of GH, cortisol, and glucose output (8). In that sense, our previous findings in nondiabetic subjects are consistent with the present data in IDDM patients in spite of the background of impaired counterregulation of these IDDM subjects. Widom and Simonson (9) reported that the plasma glucose thresholds for counterregulatory hormone secretion were higher in nondiabetic subjects after repeated hypo-

glycemia, though this was not statistically significant for the EPI response. As a whole, this evidence provides support for the notion that a wide range of counterregulatory hormone defects can be induced experimentally, but particular models of antecedent hypoglycemia may have different specific effects.

The degree of glucose counterregulation in the IDDM patients was closely related to the rise in circulating arterial plasma EPI. These findings are compatible with previous work that demonstrated the codependence of hepatic glucose output on increments in glucagon and EPI secretion in the acute posthypoglycemic period (3,4). We recently reported that after hypoglycemia, the reduction in glucose disposal in IDDM subjects also is correlated with the rise in EPI and NE (17). This study suggests that antecedent glycemic control may indeed be a determinant of the extent of the EPI response to hypoglycemia. A recent report found that the glucose thresholds for EPI release and hypoglycemic symptoms were reduced (that is, a lower glucose could stimulate EPI secretion) in a cohort of IDDM subjects with HbA_1 values <11% compared with the group with HbA_1 levels >11% (18).

The mechanisms that underlie reduced hormonal responses to recurrent hypoglycemia have not been clearly defined. Negative feedback of GH secretion by GH itself (19) has been reported, although antecedent elevations of GH per se (20,21) do not attenuate the GH response to hypoglycemia. In short-term studies, two sequential stimuli appeared to potentiate GH responses to hypoglycemia (21,22), and two brief, closely spaced episodes of hypoglycemia resulted in sustained GH elevations (20). Kerr et al. (20) also reported that the cortisol response during the second of two periods of hypoglycemia was lower compared with one prolonged hypoglycemic period. It is possible that cortisol secretion during HYPO 1 might modulate cortisol release during later hypoglycemia. In fact, the responses of ACTH and cortisol to CRH are regulated by basal cortisol concentrations (23), although in our study, plasma cortisol levels before hypoglycemia were equal. Our data suggest that hypoglycemia may have generalized effects on pituitary hormone secretion that result in diminution of the GH and cortisol responses to repetitive hypoglycemia in IDDM. Previous reports of the counterregulatory defects in intensively treated IDDM patients include reduction in the responses of GH and cortisol and lowering of the thresholds for secretion of these hormones (5,6). Plasma GH and cortisol may indeed have important actions in glycemic recovery, occurring less rapidly, but having more prolonged effects (24,25).

Note that these experiments do not show an effect of acute hypoglycemia on the impaired secretion of glucagon and EPI preexisting in these patients with IDDM. It is possible that to detect an effect of repeated hypoglycemia on these hormones, one would need more frequent, severe, or prolonged events (7), or that the mechanism of the reduction of these secretory responses is attributable to other factors (3,18). For example, recent studies from our laboratory suggest that the defective secretion of glucagon induced in normal subjects by repeated hypoglycemia may be explained by the duration of hyperinsulinemia (8,26). Nevertheless, our data indicate that brief, antecedent hypoglycemia cannot account for the observed defects in EPI and glucagon secretion characteristic of some patients with IDDM.

Finally, these studies confirm our previous observations in nondiabetic subjects that suggested that hepatic glucose production may be impaired after recurrent hypoglycemia (8). In those studies, glucose output was reduced by ~30% in the second of two hypoglycemic periods, unexplained by changes in counterregulatory hormones. The mechanism for the further reduction of glucose output by repeated hypoglycemia in the previous study in nondiabetic subjects and these experiments in IDDM patients remains unclear. Although there were significant decreases in GH and cortisol responses in IDDM during the second period of hypoglycemia, and although GH and cortisol play a role in counterregulation (24,25), the time course for these effects (>2.5 h after secretion) suggest that differences in GH and cortisol secretion in the second hypoglycemic period are unlikely to be responsible for the lower rate of glucose production. It is also possible that the modest differences in plasma EPI and glucagon observed during the two periods of hypoglycemia, although not statistically significant, may have resulted in a biologically significant effect on glucose output. Despite the absence of a significant further impairment in EPI response, note that glucose output during the first hypoglycemic period was correlated with the mean plasma EPI concentration. This suggests that even though EPI secretion may be reduced in patients with IDDM, the hormone still plays a major counterregulatory role (3,4,17). Finally, we cannot exclude the possibility that reduction in the liver's sensitivity to counterregulatory hormones may occur over the time course of these studies, as has been suggested in the case of the response to glucagon infusion in IDDM patients (27).

It is also possible that this impairment in the liver's response might be attributable to limitation of neurogenic or substrate-driven hepatic glucose release. Though we observed a decrease in the NE response to repeated hypoglycemia in nondiabetic subjects (8), we failed to detect such an effect in IDDM patients. Our findings are compatible with a possible effect of hypoglycemia on the central nervous system or autoregulation of hepatic glucose output (28–31). Although it has been suggested that these mechanisms come into play only during severe hypoglycemia, it is conceivable that they may play a role in recurrent hypoglycemia as well.

Although repeated reductions in plasma glucose may alter some hormonal and glucose kinetic parameters of counterregulation and may relate to episodes of previous iatrogenic hypoglycemia in IDDM patients, our current results indicate that the phenomenon of impaired counterregulation spans a broad range of antecedent glycemic control, including patients with prior poor control. Thus, such defective counterregulatory responses to hypoglycemia were not limited to subjects with lower HbA_1 values. Although retrospective, our estimate of the frequency of previous episodes of severe hypoglycemia

suggests that our subjects did not suffer from a recent excess of such events. Because it is possible to worsen the impairment in counterregulatory responses in IDDM patients who already demonstrate such defects by the induction of experimental hypoglycemia, steps to reduce the frequency of even mild hypoglycemic episodes may be an important clinical goal.

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