Induction of Hypoglycemia Unawareness by Asymptomatic Nocturnal Hypoglycemia

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Hypoglycemia has been incriminated as a possible factor responsible for development of the hypoglycemia unawareness phenomenon in patients with type I diabetes. Many patients with this condition, however, do not have a history of recent hypoglycemia. Because asymptomatic nocturnal hypoglycemia commonly occurs in type I diabetes, we tested the hypothesis that such episodes might be capable of inducing this phenomenon. Accordingly, autonomic and neuroglycopenic symptoms, counterregulatory hormone responses, and cognitive function were assessed during standardized insulin-induced hypoglycemia in 10 normal volunteer subjects on two occasions—once after induction of asymptomatic nocturnal hypoglycemia and once after control studies in which saline rather than insulin was infused overnight. Compared with control experiments, asymptomatic nocturnal hypoglycemia increased the threshold (required greater hypoglycemia for initiation) and reduced the magnitude of autonomic and neuroglycopenic symptoms, counterregulatory hormone responses, and cognitive dysfunction during subsequent hypoglycemia (all, $P < 0.05$). These results indicate that asymptomatic hypoglycemia may induce hypoglycemia unawareness and, thus, may explain why not every patient with this condition has a history of prior hypoglycemia. Our results therefore support the concept that in type I diabetes this phenomenon may be largely attributable to antecedent hypoglycemia. *Diabetes* 42:1233–37,1993

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Type I diabetes, insulin-dependent diabetes mellitus; BMI, body mass index; EPI, epinephrine; NE, norepinephrine; GH, growth hormone; CI, confidence interval.

Hypoglycemia is common in insulin-treated patients with diabetes; it represents both the principal danger and the major impediment of successful intensive insulin therapy (1). Normally, a characteristic sequence of responses occurs as plasma glucose concentrations decrease (2–4). Initially, an increase in counterregulatory hormone secretion occurs, followed by the appearance of autonomic warning symptoms; and, if plasma glucose concentrations decrease further, the development of neuroglycopenic symptoms and signs of cognitive dysfunction.

This physiological response to hypoglycemia often is disturbed in patients with diabetes (1). Many do not experience appropriate autonomic warning symptoms before development of neuroglycopenia and have reduced counterregulatory hormone responses, a phenomenon referred to as hypoglycemia unawareness (5). It occurs particularly in patients with type I diabetes of long duration (6), in those undergoing intensive insulin therapy, and in those with a history of frequent episodes of hypoglycemia (5). A similar phenomenon has been observed in insulinoma patients and is completely reversed after surgical cure (7). This and other evidence (8–11) suggest that hypoglycemia may induce hypoglycemia unawareness.

However, the extent to which antecedent hypoglycemia accounts for this phenomenon in diabetic patients is unclear because not all diabetic patients have a history of recent hypoglycemia (12). The lack of such a history might be explained by episodes of asymptomatic hypoglycemia, which occur quite commonly in patients with type I diabetes (13). These studies were therefore undertaken to determine whether asymptomatic nocturnal hypoglycemia might induce the hypoglycemia unawareness phenomenon. For this purpose, we assessed counterregulatory hormone responses, autonomic and neuroglycopenic symptoms, and deterioration of cere-

bral function in 10 normal volunteer subjects with and without a prior episode of experimentally induced asymptomatic nocturnal hypoglycemia.

RESEARCH DESIGN AND METHODS

Informed, written consent was obtained from 10 (6 men, 4 women; 9 of European descent, 1 American black) healthy, nonobese (BMI 26 ± 1 kg/m^2) volunteer subjects 31 ± 1 yr of age. The protocol was reviewed and approved by the University of Pittsburgh Institutional Review Board. Data from control studies of 3 normal volunteer subjects have been included in a prior publication (4).

Each subject was studied twice in random order with at least 2 wk in between. On each occasion, subjects were admitted to the University of Pittsburgh Clinical Research Center the evening before the experiments and were given a standard dinner between 1730 and 1830 (30 kcal/kg, 50% carbohydrate, and 15% protein) and a standard snack (~4 h later) at bedtime (10 kcal/kg, 50% carbohydrate, 35% fat, and 15% protein). At 2200, an antecubital vein for the infusions and a contralateral forearm vein for blood sampling were cannulated. On one occasion (control experiment) at 2300—after collecting baseline samples for plasma glucose, insulin, and counterregulatory hormones—a 4-h saline infusion was started in 7 of 10 subjects. On another occasion, a 4-h intravenous infusion of regular insulin (0.5 mU · kg^{-1} · min^{-1}) was started in all subjects to lower plasma glucose concentrations to 2.2–2.5 mM for ~2 h. All subjects remained asleep during this period. In 3 subjects, glucose was infused to maintain plasma glucose levels >2.2 mM. At 0300, the insulin infusions were stopped and the plasma glucose was allowed to spontaneously return to normal levels. Subsequently, on both occasions, between 0700 and 0730, a hand vein was cannulated retrogradely and maintained in a Plexiglass thermoregulated box (70°C) for sampling of arterialized venous blood. After a 60-min equilibration period, a continuous intravenous infusion of insulin was begun (1 mU · kg^{-1} · min^{-1} for 270 min, followed by 2 mU · kg^{-1} · min^{-1} for an additional 60 min), and plasma glucose was clamped by variable glucose infusions at sequential target 45-min glycemic plateaus of 4.3, 3.7, 3.0, and 2.3 mM, as described previously (4). Arterialized venous blood samples were drawn every 30 min—from 0 to 360 min—for determination of plasma insulin, GH, glucagon, cortisol, EPI, and NE, by methods described previously (4).

A semiquantitative symptom questionnaire was administered every 15 min. Subjects scored from 0 (none) to 5 (severe) each of the following symptoms: dizziness, tingling, blurred vision, difficulty in thinking, faintness, anxiety, palpitations, sweating, irritability, hunger, or tremor. The first 5 symptoms were considered neuroglycopenic, and the other 6 were considered autonomic (14,15). The sum of these symptoms constituted the symptom score.

In addition, during each of the glycemic plateaus the following standard cognitive tests were administered (4): trail making part B, verbal fluency, interference subtest from the Stroop Test, simple and choice visual reaction time, word and color subtests from the Stroop Test, digit vigilance test, trail making part A, verbal memory test, and forward and backward digit span. The evening before a study, subjects were provided with extensive practice on each measure. For the actual study, 6 alternate forms of each test were prepared.

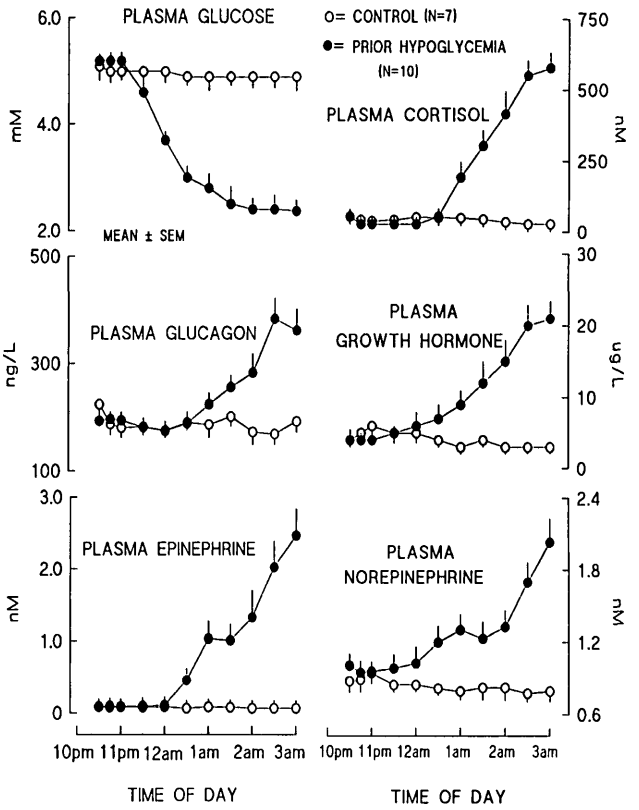

FIG. 1. Plasma glucose, glucagon, EPI, NE, GH, and cortisol during asymptomatic nocturnal hypoglycemia.

Statistical analysis. Glycemic thresholds for various parameters were, as described previously (2,4), the plasma glucose concentration at which changes in the parameters first exceeded the 95% CI observed for those parameters at the corresponding time point in previously published euglycemic-control experiments (4). Results of each cognitive test were transformed to z scores to provide unitless data and changes from baseline were summed (16). Individual peak responses were used to compare magnitudes of various parameters. Results are presented as means ± SE and were evaluated using paired Student's t test (16). $P < 0.05$ was considered statistically significant.

RESULTS

Overnight period: plasma glucose and counterregulatory hormones. After starting the infusion of insulin at 2300, plasma glucose decreased from 5.2 ± 0.2 to 2.7 ± 0.1 mM after 2 h and averaged 2.4 ± 0.1 mM during the next 2 h (Fig. 1). Although stages of sleep were not assessed, one investigator directly observed all subjects to document that they remained asleep during this period. After stopping the insulin infusion at 0300,

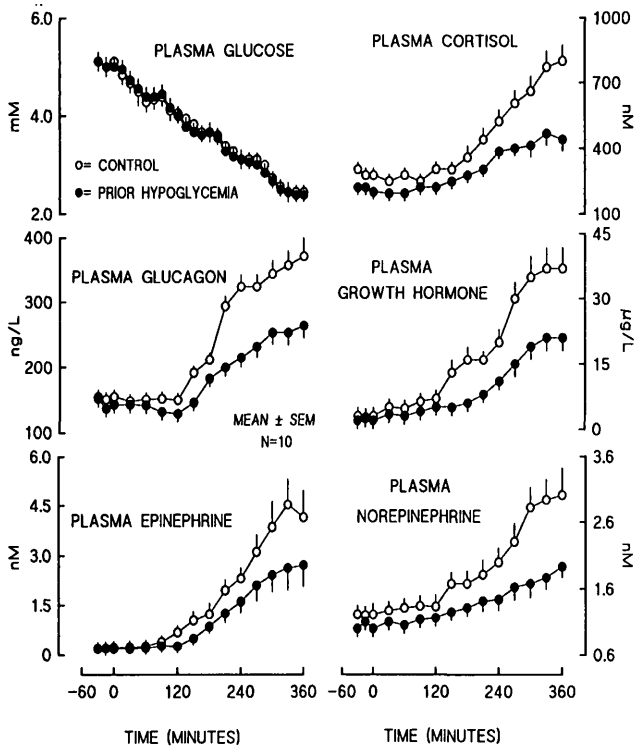

FIG. 2. Plasma glucose, glucagon, EPI, NE, GH, and cortisol during stepwise hypoglycemia.

plasma glucose increased to values observed in control (saline infusion) experiments by 0430 (5.0 ± 0.1 vs. 4.9 ± 0.1 mM, data not shown). All (glucagon, EPI, NE, GH, and cortisol) counterregulatory hormones increased significantly during the asymptomatic hypoglycemia and returned to values observed in control experiments by 0700.

Subsequent stepwise hypoglycemic clamp experiments: plasma glucose, insulin, and counterregulatory hormones. Plasma insulin concentrations were not significantly different on the two experimental days (data not shown). Plasma glucose decreased in similar steps to a final plateau of ~2.4 mM on both experimental days (Fig. 2). Despite the fact that basal plasma counterregulatory hormone concentrations were comparable on both experimental days, significantly reduced responses were observed for all counterregulatory hormones in experiments preceded by asymptomatic nocturnal hypoglycemia. Moreover, initial increases for all counterregulatory hormones began at significantly lower plasma glucose concentrations on the experimental day after the asymptomatic nocturnal hypoglycemia.

Symptom scores. Both autonomic and neuroglycopenic symptom scores were significantly lower than those in control experiments after asymptomatic nocturnal hypoglycemia (Fig. 3, Tables 1 and 2). Moreover, the plasma glucose concentrations at which both these symptoms began were significantly lower than those in the control experiments. However, the plasma EPI and NE concentrations (1.4 ± 0.3 and 1.7 ± 0.2 nM, respectively) at which autonomic symptoms began after asymptomatic hypoglycemia were not significantly different from those (1.1 ± 0.2 and 1.4 ± 0.2 nM) in control experiments.

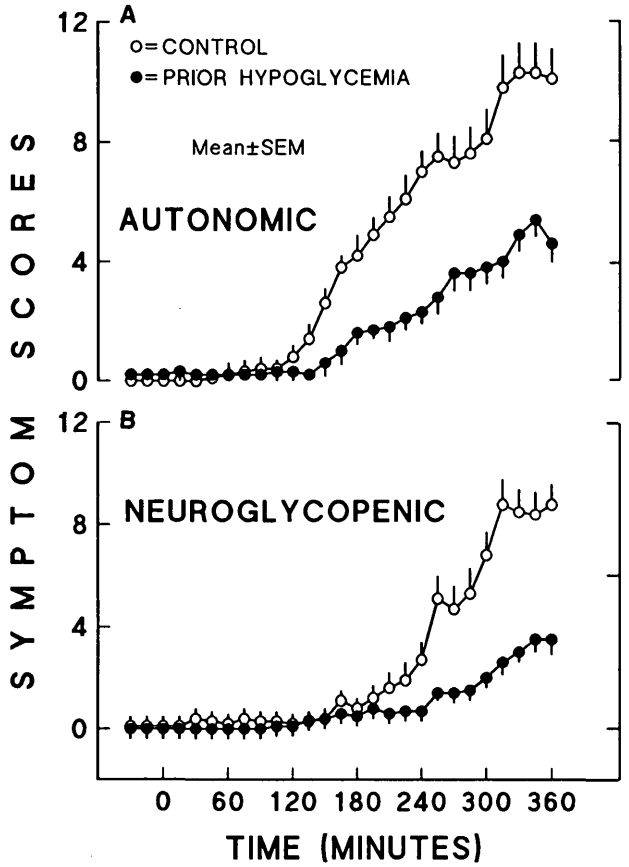

FIG. 3. Scores of autonomic (*A*) and neuroglycopenic (*B*) symptoms.

Cognitive tests. The deterioration of cognitive function during hypoglycemia was significantly less on the experimental day after asymptomatic nocturnal hypoglycemia, compared with control experiments (Fig. 4, Tables 1 and 2). Moreover, the plasma glucose concentration at which deterioration began also was significantly lower on the experimental day after asymptomatic nocturnal hypoglycemia.

DISCUSSION

This study demonstrates that a single episode of moderate asymptomatic nocturnal hypoglycemia (2.4 mM for 2

TABLE 1
Magnitudes of counterregulatory hormone responses, symptoms, and cognitive dysfunction

	Control studies	Prior hypoglycemia
EPI (nM)	5.0 ± 0.8	2.8 ± 0.7*
NE (nM)	3.4 ± 0.4	1.9 ± 0.2*
Glucagon (ng/L)	426 ± 78	301 ± 35*
GH (μg/L)	39 ± 4	23 ± 2*
Cortisol (nM)	803 ± 58	497 ± 52*
Autonomic symptom score	10 ± 2	5 ± 1*
Neuroglycopenic symptom score	9 ± 2	4 ± 1*
Cognitive dysfunction†	16 ± 4	7 ± 1*

Data are means ± SE.
*$P < 0.05$ vs. control study.
†Sum of z scores.

TABLE 2
Glycemic thresholds for counterregulatory hormone responses, symptoms, and cognitive dysfunction*

	Control studies	Prior hypoglycemia
EPI	3.9 ± 0.1	3.4 ± 0.1†
NE	3.9 ± 0.1	3.4 ± 0.2†
Glucagon	3.6 ± 0.2	3.1 ± 0.1†
GH	4.0 ± 0.1	3.6 ± 0.2†
Cortisol	3.3 ± 0.1	2.9 ± 0.1†
Autonomic symptoms	3.7 ± 0.1	3.3 ± 0.1†
Neuroglycopenic symptoms	3.1 ± 0.1	2.7 ± 0.1†
Cognitive functions	2.8 ± 0.1	2.3 ± 0.1†

Data are means ± SE.
*Measured in mM.
†$P < 0.05$ vs. control study.

h) reduces autonomic and neuroglycopenic symptoms, impairs counterregulatory hormone responses, and diminishes cognitive dysfunction during subsequent hypoglycemia—changes similar to those observed in patients with the hypoglycemia unawareness phenomenon (5,7). The fact that asymptomatic hypoglycemia can induce these changes may thus explain why many patients with this condition do not give a history of recent symptomatic hypoglycemia. Our results in healthy volunteer subjects support the proposal that hypoglycemia may be the principal cause for hypoglycemia unawareness phenomenon in patients with diabetes, and indicate that neither autonomic neuropathy nor a long duration of diabetes is a prerequisite for its development.

Previous studies (8,9,11) have consistently shown that symptomatic hypoglycemia reduces counterregulatory hormone responses and autonomic warning symptoms during a subsequent episode of hypoglycemia. However, these studies did not assess alterations in cognitive function and have been inconsistent regarding an effect on neuroglycopenic symptoms. Widom and Simonson (11) recently reported that repetitive episodes of moderate hypoglycemia reduced autonomic symptoms without apparently affecting neuroglycopenic symptoms. In contrast, Heller and Cryer (8) and Dagogo-Jack et al. (10) found that an episode of prior hypoglycemia reduced both neuroglycopenic and autonomic symptoms. Our findings are thus in agreement with the latter two reports (8,10). Moreover, our findings of reduced neuroglycopenic symptoms and reduced deterioration of cognitive function are consistent with observations in insulinoma patients who have had repetitive episodes of hypoglycemia (7,17).

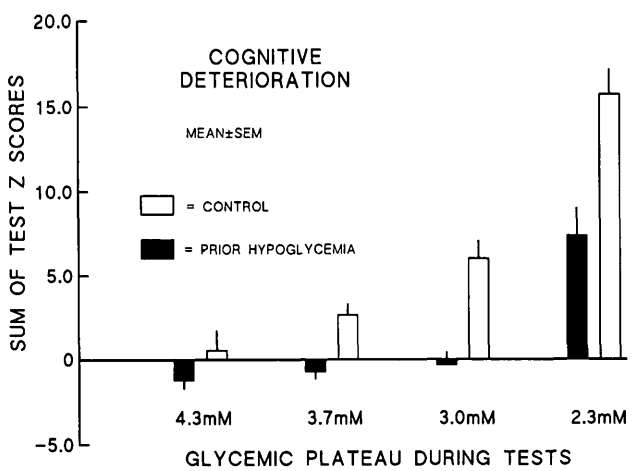

FIG. 4. Changes in sum of *z* scores of cognitive function tests.

The reduction in neuroglycopenic symptoms and cognitive deterioration found in these studies—and in studies of insulinoma patients (7,17)—support the proposal that the mechanism responsible for the hypoglycemia unawareness phenomenon may be an adaptive increase in blood brain glucose transport (18). Such a theory predicts that counterregulatory hormone responses, autonomic warning symptoms, neuroglycopenic symptoms, and cognitive dysfunction all should be affected in the same direction, although not necessarily to the same degree.

Early reports by Amiel et al. (19) and Widom and Simonson (3), which indicate that the thresholds for counterregulatory hormone responses and autonomic symptoms—but not neuroglycopenic symptoms nor cognitive dysfunction—were increased in diabetic patients undergoing intensive therapy appear to argue against this theory. However, it is possible that intensive insulin therapy may affect counterregulatory hormone responses and the resultant autonomic warning symptoms in ways other than causing hypoglycemia. Moreover, the failure of Amiel et al. (19) and Widom and Simonson (3) to detect a significant alteration in thresholds for neuroglycopenic symptoms and cognitive dysfunction could have been attributable to factors such as a lack of statistical power (a result of the small number of subjects studied), absence of a control (nonhypoglycemic) experiment, and arbitrary definitions for thresholds.

In this study, dizziness, tingling, blurred vision, difficulty in thinking, and faintness were classified as neuroglycopenic symptoms; whereas, anxiety, palpitations, sweating, irritability, hunger, and tremor were considered autonomic symptoms. The latter were considered the result of activation of the sympathetic and parasympathetic nervous system, whereas the former were considered secondary to reduced brain glucose utilization. The appropriateness of this classification is supported by the modification of symptoms observed in studies of β-adrenergic blockade (15) and in patients with cervical cord transaction (20) in which hunger, sweating, tremor, anxiety, and palpitations were reduced or absent, while difficulty in thinking, blurred vision, dizziness, faintness, and tiredness were not affected. Moreover Hepburn et al. (14), applying factor analysis to symptoms reported by diabetic patients during experimental hypoglycemia, found that hunger, sweating, trembling, anxiety, and palpitations occurred separately from other symptoms, such as confusion, drowsiness, inability to concentrate, blurred vision, and tingling.

Our results are consistent with a generalized CNS adaptation to repetitive hypoglycemia, which could be explained by increases in blood brain glucose or other substrate transport. It also has been suggested that

reduced β-adrenergic sensitivity may be responsible for hypoglycemia unawareness in patients with type I diabetes (21). The observations of this study—that antecedent asymptomatic hypoglycemia did not affect the plasma catecholamine concentrations at which autonomic symptoms began—argues against this proposal. Furthermore, although it is possible repetitive hypoglycemia might somehow alter β-adrenergic sensitivity, this would not explain the alterations in counterregulatory hormone and cognitive responses observed in patients with the hypoglycemia unawareness phenomenon.

Finally, these studies have implications regarding the management of hypoglycemia unawareness. Our demonstration that asymptomatic nocturnal hypoglycemia induces changes similar to those observed in diabetic and insulinoma patients with hypoglycemia unawareness supports the concept that hypoglycemia might be the main cause of the phenomenon in patients with diabetes, by providing an explanation for why not every patient with this condition has a history of prior hypoglycemia. The reversal of the phenomenon after surgical cure in insulinoma patients (7) suggests that prevention of hypoglycemia—or at least a reduction in its frequency—also may reverse the phenomenon in patients with diabetes. Indeed, preliminary studies (22) suggest that this may be the case.

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