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Resistance to Neuroglycopenia: An Adaptative Response during Intensive Insulin Treatment of Diabetes*

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ABSTRACT

Counterregulation and awareness of hypoglycemia begins at lower plasma glucose levels in insulin-dependent diabetes mellitus (IDDM) subjects given intensive insulin treatment. To determine whether these changes are associated with an alteration in the susceptibility of the brain to mild hypoglycemia, we compared central nervous system responses to hypoglycemia in 8 intensively treated (hemoglobin A_1, 8.3 ± 0.2%; normal, <8%) and 11 conventionally treated IDDM patients (hemoglobin A_1, 14.6 ± 1.3%) with those in 10 healthy subjects. Plasma glucose was lowered from ~4.6 mmol/L in 0.5–0.6 steps using the clamp technique. Glucose levels triggering hormonal responses and perception of hypoglycemic symptoms were significantly lower in intensively treated patients compared to their poorly controlled counterparts ($P < 0.05$), and hormonal responses were suppressed compared to those in healthy controls. Similarly directed changes occurred in the level of circulating glucose required to alter cortical evoked potentials during hypoglycemia. A greater reduction in plasma glucose was required to alter P300 event-related potentials in the intensively treated patients (2.2 mmol/L) compared to those in the conventionally treated and nondiabetic groups (~3.5 and ~3.0 mmol/L, respectively). We conclude that intensively treated IDDM patients are resistant to changes in cortical evoked potentials induced by mild hypoglycemia. This may explain why intensively treated IDDM counterregulate and experience hypoglycemic symptoms at a lower glucose level than conventionally treated patients. (*J Clin Endocrinol Metab* **82:** 1713–1718, 1997)

THE REPORT of the Diabetes Control and Complication Trial Research Group (1) has established the long term benefits of intensive insulin therapy aimed at near normalization of glucose levels in insulin-dependent diabetes mellitus (IDDM). As a result, this therapeutic approach has been recommended for (2) and is being offered to an increasing number of IDDM patients in an effort to prevent or delay microvascular and neuropathic complications. Unfortunately, the frequency and severity of hypoglycemia are markedly increased by such regimens despite close medical supervision (1, 3).

The mechanisms contributing to the greater risk of severe hypoglycemia during intensive insulin therapy in IDDM patients have recently been clarified. Although lowering of target glycemic goals in the face of persisting conventional risk factors would be expected to promote iatrogenic hypoglycemia, impaired counterregulatory defenses against hypoglycemia play an important role as well (4–6). In the early stages of IDDM, the capacity to release glucagon during hypoglycemia is lost (4, 5), and as the duration of the disease increases, epinephrine responses are also diminished in some patients (6). In addition, intensified insulin treatment aimed at restoring glycemia as close to normal as possible leads to a further deterioration of hypoglycemic counterregulation and symptomatic unawareness of hypoglycemia (7–10). Recent studies suggest that this phenomenon is caused by iatrogenic hypoglycemia *per se*. In both healthy subjects and IDDM patients a brief period of moderate hypoglycemia reduces hormonal responses and symptoms during experimentally induced hypoglycemia the following day (11, 12). Conversely, it has been reported that in young, poorly controlled IDDM patients, hypoglycemic symptoms and epinephrine responses can be elicited when glucose is lowered into the normal range (13).

A key issue is whether the divergent changes in glycemic thresholds for counterregulatory responses in well controlled, intensively treated and in poorly controlled, conventionally treated IDDM patients are mirrored by similarly directed changes in brain function as the availability of glucose via the circulation is reduced. This possibility is supported by studies in rats demonstrating that chronic hypoglycemia and hyperglycemia, respectively, increase and decrease the efficiency of glucose extraction by the brain (14–16). Furthermore, in normal human subjects rendered mildly hypoglycemic for several days and IDDM patients who have been well controlled, brain glucose uptake is more effectively preserved during hypoglycemia (17, 18). It is uncertain, however, whether these adaptations in brain glucose uptake during hypoglycemia are accompanied by corresponding alterations in brain function. Studies using tests of neuropsychological performance to evaluate the effect of diabetes treatment on the central nervous system (CNS) response to hypoglycemia have generated contradictory results (19–22). Studies were, therefore, undertaken to assess this question by combining the hypoglycemic clamp with

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measurements of cortical evoked potentials, an end point shown to be sensitive to even modest decrements in plasma glucose (23).

Subjects and Methods

Subjects

A total of 29 subjects were studied, including 19 IDDM patients and 10 nondiabetic controls. Eight of the patients with IDDM were receiving intensive insulin therapy (multiple daily injections or continuous subcutaneous insulin infusion) for at least 6 months, and 11 were being treated with conventional insulin regimens (twice daily injections of regular and intermediate acting insulin). They were eligible for study if their disease duration was more than 1 yr, they had no symptoms or physical signs of autonomic neuropathy, and they were receiving no medications other than insulin and had no acute illness. The clinical characteristics of all subjects are shown in Table 1. Each gave written informed consent to participate in the study protocol, which was approved by the Yale human investigation committee.

Hypoglycemic clamp procedures

Studies were performed after a 10- to 12-h overnight fast. Subjects with diabetes were admitted to the Yale General Clinical Research Center on the evening before the study. An iv catheter was inserted, and basal insulin was administered as a continuous iv infusion that was adjusted during the night based on plasma glucose measurements obtained every 30–60 min. The plasma glucose concentration did not fall below 4.0 mmol/L in any of the patients during the overnight period. The next morning, a modification of the glucose clamp technique was used to produce a gradual and standardized reduction in plasma glucose. The methods used for this procedure (hypoglycemic clamp) have been previously described (10). Briefly, two venous catheters were employed, one in an antecubital vein for administration of glucose and insulin, and the other in a dorsal hand vein for blood sampling. The hand was placed in a heated (~65 C) box to "arterialize" venous blood (24). After a 60-min basal period during which baseline measurements were obtained, a primed continuous infusion of regular human insulin was given (Novo Nordisk, Princeton, NJ) in a dose of 80 mU/m^2·min. Plasma glucose was measured at the bedside in duplicate at 5-min intervals using a Beckman glucose analyzer (Beckman, Fullerton, CA), and target glucose levels were achieved by varying the rate of an infusion of 20% glucose. In all three groups, plasma glucose was maintained at euglycemic levels (~4.6 mmol/L) for the initial 60 min of the study and then was reduced in 0.5–0.6 mmol/L steps each hour for 240 min. In intensively treated patients, the study was extended for an additional 60-min period to allow for an additional hypoglycemic plateau of 2.2 mmol/L. All subjects were masked to the plasma glucose levels during the study.

Measurements

Blood samples were taken at 10- to 20-min intervals for measurement of insulin and counterregulatory hormones. In the final 20 min of each hypoglycemic plateau (beginning at 4.0 mmol/L), symptoms and electrophysiological data were recorded. Symptoms were assessed using a questionnaire presented on a laptop computer that also recorded subject responses. Subjects rated the following symptoms on a scale of 1 (not at all) to 7 (extreme): headache, difficulty with concentration, weakness, difficulty thinking, sweating, slowed thinking, pounding heart, and shakiness. The sum of these eight items in each subject constituted the total symptom score at the observation point. In addition, four "filler" items (*i.e.* pain in joints, earache, watery eyes, and ringing in ears) were included to control for nonspecific symptoms not referable to hypoglycemia. To obtain cortical evoked potentials, scalp electrodes were placed at Cz and Pz positions, with a reference electrode placed in the opposite ear. A bipolar pair of electrodes was placed above the right eye to monitor for ocular artifacts. P300-evoked potentials were obtained with an auditory categorization ("oddball") task, in which subjects were required to silently count the number of soft clicks. The loud clicks were presented to the ear ipsilateral to the reference electrode, and the soft clicks were delivered to the ear contralateral to the reference electrode. The proportion of soft clicks varied between 10–20% of a total of 200 clicks. Two replications were obtained at each plasma glucose plateau.

Determinations and analysis

Catecholamines were measured by radioenzymatic assay (Upjohn, Kalamazoo, MI), and free insulin, GH, cortisol, and glucagon were determined using double antibody RIAs, as described previously (10). Averaged evoked potential waveforms for P300 were calculated on-line and then analyzed off-line by two investigators who were blinded to the subjects experimental group and to the glycemic levels at which the evoked potentials were obtained. Peaks and latencies of the P300 potential were measured with a waveform cursor. P300 evoked potentials were calculated as the difference between potentials evoked by soft clicks and those elicited by loud clicks; this procedure minimized potentials evoked by clicks *per se* and isolated those associated with the counting task.

Demographic data are expressed as the mean ± SD, and all other data are expressed as the mean ± SE. Comparisons of glucose levels, hormone responses, symptoms scores, and P300 latency and amplitude measures between the study groups were made using ANOVA with repeated measures design, followed by Student's *t* test to localize the effects. $P < 0.05$ was considered statistically significant. Hormonal data are presented as the average of measurements obtained during the last 40 min of each glycemic step of the clamp studies.

Results

Glucose and insulin levels

As shown in Fig. 1, the hypoglycemic clamp procedure caused plasma glucose levels to decline in a nearly identical fashion in each of the three groups during the first 240 min of the study, *i.e.* ~0.5 mmol/L each hour to a nadir of ~3 mmol/L. In the intensively treated IDDM subjects, plasma glucose was further reduced to 2.2 mmol/L for an additional 60-min period. Steady state plasma free insulin levels during the clamp studies were not significantly different between the groups (nondiabetic, 847 ± 36 pmol/L; conventionally treated IDDM, 744 ± 60 pmol/L; intensively treated IDDM, 828 ± 72 pmol/L; P = NS).

TABLE 1. Clinical characteristic of the study groups and plasma counterregulatory hormones concentration during baseline

	Conventionally treated IDDM (n = 11)	Intensively treated IDDM (n = 8)	Nondiabetic adults (n = 10)
Age (yr)	23 ± 2 (15–32)	30 ± 3 (20–41)	24 ± 1 (20–28)
Sex (m/f)	6/5	4/4	5/5
Duration of IDDM (yr)	11 ± 1 (5–16)	15 ± 2 (4–27)	
HBA_1 (%)	14.6 ± 1.3 (9.9–22.0)	8.3 ± 0.2 (7.6–8.9)	
Epinephrine (pmol/L)	350 ± 65	308 ± 51	231 ± 35
Cortisol (nmol/L)	342 ± 60	329 ± 73	295 ± 28
GH (μg/L)	6.1 ± 1.3	6.3 ± 2.4	1.9 ± 0.3

Values are expressed as the mean ± SEM, with the range in *parentheses*. HBA_1 values in nondiabetic subjects range from 4–8%.

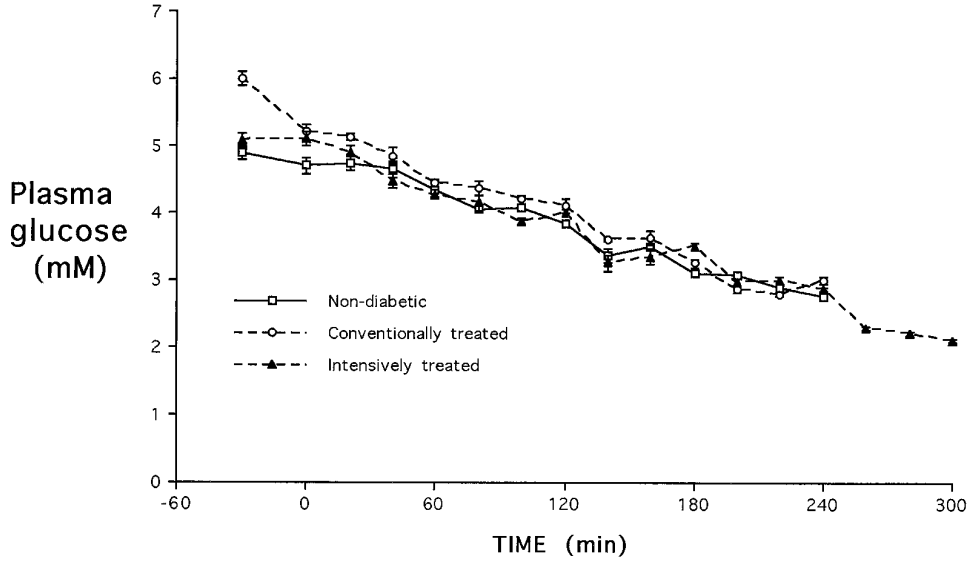

FIG. 1. Plasma glucose levels (mean ± SEM) during the stepped hypoglycemic clamp procedure in intensively treated IDDM (8), conventionally treated IDDM (11), and control (10) subjects.

Counterregulatory hormones and symptoms

The plasma counterregulatory hormone responses of each group during the hypoglycemic clamp study are summarized in Fig. 2. During the hypoglycemic phase of the study a significant rise in plasma epinephrine above basal values occurred at the 4.0 mmol/L step in the conventionally treated IDDM patients, whereas there was a small, but also significant, increase at the 3.5 mmol/L step in the nondiabetic subjects ($P < 0.05$ for both groups). In the intensively treated patients, a significant rise in plasma epinephrine did not occur until plasma glucose was lowered below 3.0 mmol/L, and plasma epinephrine levels were substantially lower than those in the other groups ($P < 0.05$). Plasma cortisol rose significantly at 3.0 mmol/L glucose in both conventionally treated IDDM and nondiabetic subjects ($P < 0.05$), whereas a glucose level of even 2.2 mmol/L was not sufficient to elicit a significant response in the intensively treated patients. Moreover, significant increments in GH first occurred at a higher glucose level in conventionally treated IDDM and nondiabetic subjects (3.5 mmol/L) compared to the level in the intensively treated patients (3.0 mmol/L; $P < 0.05$).

As shown in Fig. 3, symptomatic awareness of hypoglycemia followed a similar pattern. Hypoglycemic symptom scores increased significantly ($P < 0.05$) above baseline at 4.0 mmol/L in conventionally treated patients, and at 3.0 mmol/L in nondiabetic subjects and the intensively treated group. There were no changes in control symptoms in any of the groups throughout the study (data not shown).

Electrophysiological measures

Figure 4 depicts P300 amplitude in the three groups. At baseline, P300 amplitude was not significantly different among the groups. During the hypoglycemic phase, the conventionally treated IDDM patients showed a significant ($P < 0.05$) reduction in P300 amplitude at the 3.5 mmol/L glucose plateau, a higher glucose level than that producing a significant P300 change in nondiabetic control subjects (3.0 mmol/L). In contrast, the well controlled IDDM patients failed to show a significant change in P300 amplitude until plasma glucose had been reduced to 2.2 mmol/L. Moreover, P300 latency significantly increased by ~9% in the conventionally treated IDDM and in the nondiabetic controls at the 3.0 mmol/L step ($P < 0.05$), whereas a small, but insignificant, increase (~5%) occurred at the 2.2 mmol/L step in the well controlled IDDM patients.

Discussion

In keeping with the earlier reports (7, 8, 10), our intensively treated IDDM patients required a greater hypoglycemic stimulus to trigger counterregulatory hormone responses and symptoms than did poorly controlled, conventionally treated patients with IDDM. A key unresolved issue is whether these changes associated with IDDM therapy are accompanied by a similar shift in the glucose level at which brain function becomes impaired. This is of importance clinically because the consequences of hypoglycemia depend at least in part on the therapeutic window between counterregulatory responses and/or symptoms and onset of CNS dysfunction. The results of the current study suggest that the shift in counterregulatory responses to hypoglycemia in IDDM is, in fact, mirrored by similarly directed changes in neuroglycopenia onset, as assessed by P300 measurements. Thus, intensive therapy may have led to an adaptation in brain glucose metabolism that resulted in greater preservation of cortical evoked potentials in the face of subnormal glucose levels, levels that caused subtle neuroglycopenia in conventionally treated IDDM patients. This conclusion is supported by the experimental data in nondiabetic and diabetic rats (14–16, 25, 26) and in human subjects (17, 18). In rats, chronic hypoglycemia increases and chronic hyperglycemia decreases the efficiency of brain glucose extraction and metabolism (14–16). Furthermore, chronically hyperglycemic BB rats are more susceptible (25) and recurrently hypoglycemic diabetic BB rats are resistant to the adverse effects of hypoglycemia on brain stem function (26). Recent studies suggest that these changes may be due to changes in blood-brain barrier glucose transport (27). In humans, several days of sustained mild hypoglycemia or intensive insulin treat-

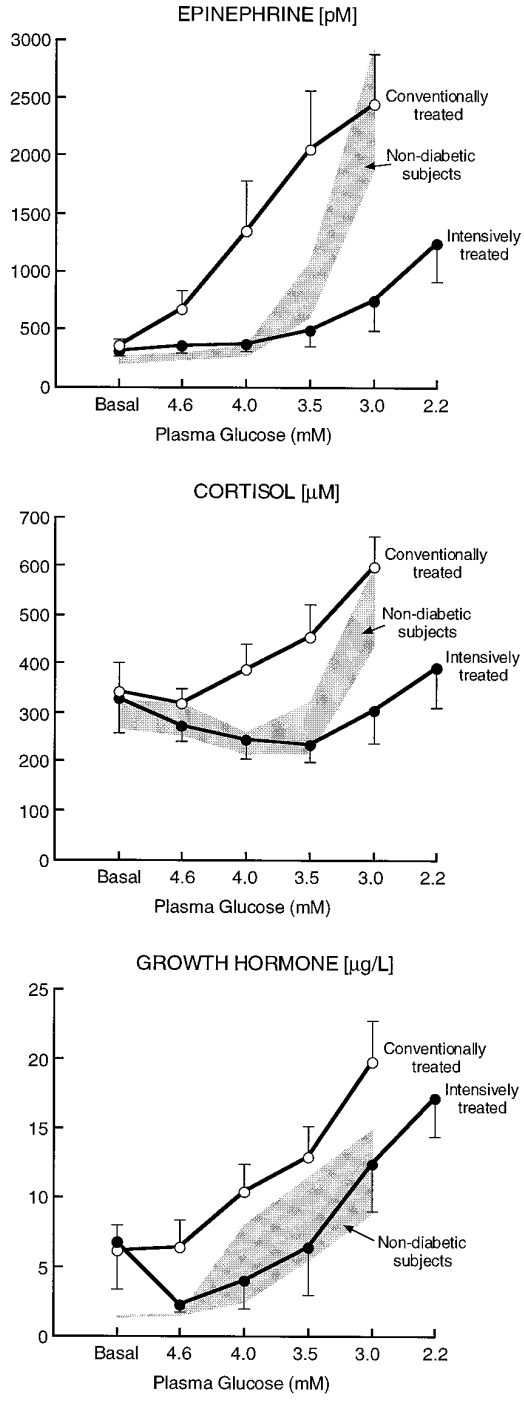

FIG. 2. Plasma counterregulatory hormones responses of each subject group to stepped reduction in plasma glucose concentrations during hypoglycemic clamp studies.

ment leading to near-normal glycated hemoglobin levels in IDDM patients has been reported to prevent the decline in whole brain glucose uptake (measured by internal jugular venous sampling) produced by experimental hypoglycemia (~3.0 mmol/L) using the stepped hypoglycemic clamp technique (17, 18).

Previous studies examining the effect of glycemic control of IDDM on CNS function during hypoglycemia, however,

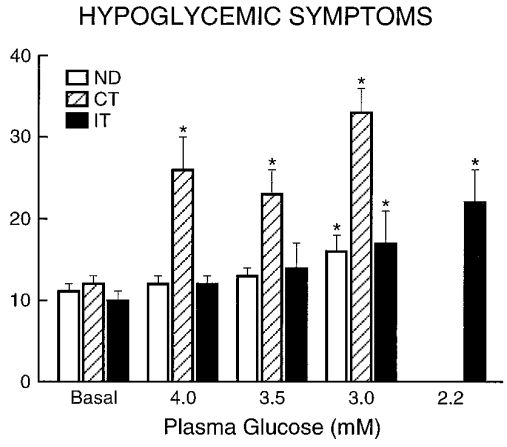

FIG. 3. Total hypoglycemic symptoms score (mean ± SEM) of each subject group during hypoglycemic clamp studies. The total symptom score represents the sum of eight hypoglycemic symptoms. *Asterisks* represents significant difference *vs.* baseline ($P < 0.05$).

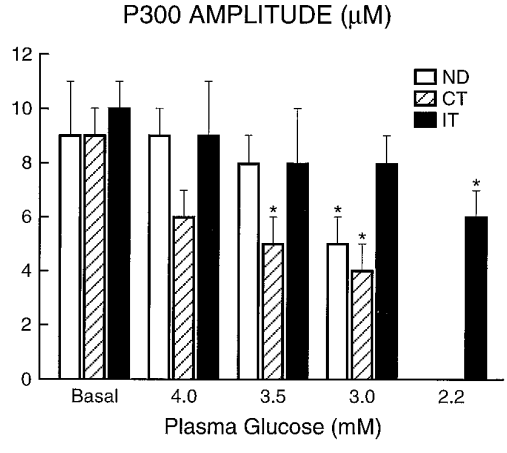

FIG. 4. P300 amplitude during each hypoglycemic plateau in nondiabetic, conventionally treated IDDM, and intensively treated IDDm subjects. *Asterisks* indicate significant amplitude reduction compared to baseline ($P < 0.05$).

have yielded conflicting results (19–22, 28, 29). It has been suggested that intensively treated IDDM patients may be more, rather than less, vulnerable to neuroglycopenia than poorly controlled counterparts when conventional electroencephalogram recordings are used as an end point (22). In contrast, studies using neuropsychological tests to assess cognitive performance during hypoglycemia in IDDM patients have found either no change (19, 21) or a lowering of the plasma glucose level required to provoke cognitive dysfunction in patients treated intensively (20, 28, 29). It should be emphasized that these discrepancies may be more apparent than real because the various tests of cognitive function are likely to involve specific brain regions that may have different glucose requirements.

In the current study, brain function was assessed using P300, an event-related potential elicited during discrimination of signals with a low subjective probability. The P300 waveform, unlike the electroencephalogram, which measures the spontaneous electrical output of the brain, is generated by the active cognitive processing of stimulus infor-

mation and is not affected by the physical characteristics of the stimulus. It requires the active participation of the subject and involves higher brain centers, particularly the hippocampus, and the parahippocampal gyrus (30). Although the P300 may be influenced by such external factors as the probability of the stimulus and the relevance and difficulty of the task, and may only be relevant for specific cognitive functions, it is useful in analyzing effects in which the subject acts as his/her own control (31). In this context it has been shown to be sensitive to small decrements in circulating glucose (23, 32). These changes appear to be produced by hypoglycemia *per se* because the infusions of multiple counterregulatory hormones have no effect on P300 under conditions of euglycemia (33).

Our observation that intensively treated IDDM patients require a greater and poorly controlled, conventionally treated IDDM patients require a smaller decrement in glucose concentration to alter P300 event-related potentials is consistent with data cited above suggesting the development of a CNS adaptation depending on the intensity of insulin treatment (14–18, 25, 26). Our data are consistent with those of an earlier report that also used the P300 to monitor changes in cognitive function during hypoglycemia in IDDM patients (34). However, in that study the assessment of glycemic thresholds for P300 changes was limited by the fact that hypoglycemic stimulus was brief, the magnitude of stimulus was not controlled, and the measurements of P300 were made under rapidly changing, nonsteady state conditions. Recent studies suggest that there is a delay before changes in plasma glucose alter measurement of P300 (34).

In summary, both ends of the spectrum of glycemic control of IDDM influence in a similar way not only the level of glucose required to trigger counterregulatory responses, but also the responsiveness of at least some brain functions to mild hypoglycemia. Although the nature of this association is uncertain, its existence may be more than just a coincidence. It is conceivable that the same molecular mechanisms affected regions of the CNS responsible for glucose sensing (35, 36) as well as others involved in cortical event-related potentials (30). It should also be noted that although P300 was preserved during mild to moderate experimental hypoglycemia in intensively treated IDDM patients, they are at much higher risk of severe hypoglycemia in real life than conventionally treated patients. Therefore, this treatment-induced adaptation may have a clinically adverse effect if plasma glucose falls to values well below those that can be compensated for by changes in brain glucose metabolism.

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