Adaptation of Cognitive Function to Hypoglycemia in Healthy Men

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OBJECTIVE — Antecedent hypoglycemia reduces hypoglycemic counterregulation and symptoms, thereby provoking the hypoglycemia unawareness syndrome. The effects of antecedent hypoglycemia on hypoglycemia-induced cognitive dysfunction are less well established.

RESEARCH DESIGN AND METHODS — To determine whether antecedent hypoglycemia also reduces hypoglycemic cognitive dysfunction, we performed stepwise hypoglycemic clamp experiments (4.1, 3.6, 3.1, and 2.6 mmol/l) during a 6-h period in 30 young healthy men. A total of 15 subjects additionally received a 2.5-h antecedent hypoglycemic clamp (3.1 mmol/l) on the preceding day (prior-hypo group), whereas the other 15 subjects did not (control group). Cognitive function was assessed by auditory-evoked brain potentials (AEBPs) and reaction time during a vigilance task and short-term memory recall. Tests were performed during the stepwise hypoglycemic clamp at baseline and at each hypoglycemic plateau.

RESULTS — In both groups, performance on all measures of cognitive function deteriorated during stepwise hypoglycemia (all P < 0.01). However, after antecedent hypoglycemia, the hypoglycemia-induced decrease in the amplitude of the P3 of the AEBP was distinctly reduced compared with the control condition (P < 0.05). Also, short-term memory performance was less impaired in the prior-hypo group than in the control group (P < 0.005), and a minor hypoglycemic impairment of reaction time (P < 0.05) was evident in the prior-hypo group.

CONCLUSIONS — Data provide evidence that a single episode of mild antecedent hypoglycemia (3.1 mmol/l) attenuates several aspects of cognitive dysfunction during subsequent hypoglycemia 18–24 h later.

Hypoglycemia is the limiting factor in the management of type 1 diabetes (1). Tight glycemic control as a result of intensive insulin therapy has been shown to be accompanied by a 3-fold increase in the risk of severe hypoglycemia (2). Although specific treatment programs may help to decrease the risk of hypoglycemia (3), hypoglycemia still constitutes a severe problem for many patients with type 1 diabetes. Patients with well-controlled diabetes frequently show a reduced counterregulatory and symptomatic response to hypoglycemia that is often referred to as the "hypoglycemia unawareness syndrome" (4). Hypoglycemia itself seems to be the major factor in the pathogenesis of the syndrome because antecedent hypoglycemia has been shown to reduce hypoglycemic counterregulation and symptoms in type 1 diabetic patients (5–7) and in healthy subjects (8–16). However, unawareness may also result from a direct impairing influence of hypoglycemia on neurocognitive function (17–20). The effect of antecedent hypoglycemia on hypoglycemic cognitive dysfunction has been discussed controversially thus far (21,22). Several studies (6,7,14,23) failed to detect any significant effects, whereas others (15,24–26) indicated a preserving influence of antecedent hypoglycemia on cognitive function. The inconsistency appears to be related to methodical differences pertaining to the heterogeneity of the subjects studied, the small size of subject samples, and the cognitive function tests used. Some of these problems were overcome in the present study, which assessed the effect of a single episode of antecedent hypoglycemia on cognitive dysfunction during subsequent hypoglycemia in healthy men. We tested the hypothesis that antecedent hypoglycemia diminishes cognitive dysfunction during subsequent hypoglycemia. Indicators of cognitive functions were auditory-evoked brain potentials (AEBPs) and reaction time during a vigilance task and short-term memory recall. Because psychological factors such as fatigue or anxiety (22) may confound cognitive function testing during hypoglycemic clamp experiments, we also assessed the state of arousal by questionnaire and with physiological parameters such as blood pressure and heart rate.

RESEARCH DESIGN AND METHODS

Participants

A total of 30 healthy men participated in the experiments (means ± SEM, 26 ± 1 years of age; BMI 23.1 ± 0.6 kg/m²). Exclusion criteria were chronic or acute illness, taking medication of any kind, obesity, smoking, alcohol or drug abuse, and diabetes or hypertension in first-degree relatives. Each volunteer gave written informed consent, and the study was approved by the local ethics committee. All volunteers were requested to abstain from alcohol, not to perform any kind of exhausting physical activity, and to go to bed no later than 10:00 P.M. on the day before the experiments.

Procedure

Stepwise hypoglycemic clamp experiments during a 6-h period were per-
formed in 30 subjects randomly allocated to 2 groups. One group additionally underwent an antecedent hypoglycemic clamp that lasted 2.5 h on the day preceding the stepwise hypoglycemia (prior-hypo group), whereas the other 15 subjects did not undergo the clamp study (control group). A moderate antecedent hypoglycemic level of \(-3.1 \text{ mmol/l}\) was chosen because, under this condition, antecedent hypoglycemia per se is not expected to induce acute cognitive disturbances that may interfere with the effects of subsequent hypoglycemia (27–30).

On the day of the antecedent hypoglycemic clamp study, the subjects in the prior-hypo group reported to the medical research unit at 1:30 P.M. They were instructed not to have breakfast on this day and to abstain from eating until the end of the clamp study. The experiments took place in a sound-attenuated room with the subjects sitting with their trunks in an almost upright position (\(-60^\circ\)) and their legs in a horizontal position on a bed. A cannula was inserted into a vein on the back of the hand, which was placed in a heated box (50–55°C) to obtain arterialized venous blood. A second cannula was inserted into an antecubital vein on the contralateral arm. Both cannulas were connected to long thin tubes that enabled blood sampling and adjustment of the rate of dextrose infusion from an adjacent room without being noticed by the subject. At 2:00 P.M., infusion of insulin (H-insulin; Hoechst, Frankfurt, Germany) began at a continuous rate of 1.5 mU \(\cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) for the subsequent 6 h. Arterialized blood was drawn at 5-min intervals to measure plasma glucose concentration. A 20% dextrose solution was simultaneously infused at a variable rate to control plasma glucose levels. Plasma glucose levels were reduced in a stepwise manner to achieve 4 plateaus of \(-4.1, 3.6, 3.1, \text{ and } 2.6 \text{ mmol/l}\). Each plateau was maintained for 45 min, and the next lower plateau was induced gradually within the next 45 min. Standard cognitive tests were performed at each of the hypoglycemic plateaus.

AEBPs and reaction time
AEBPs indexing different stages of the brain’s stimulus processing and reactions times were recorded while the subject performed a vigilance task (oddball paradigm). The task required the subject to detect target pips (1,200 Hz, duration 60 ms, intensity 64 decibel sound pressure level, probability 0.1) that were randomly interspersed among frequent standard pips of lower pitch (800 Hz), and the task contained \(-400 \text{ pips}\). Tone pips were presented binaurally through headphones with an interstimulus interval randomly varying between 1,000 and 3,000 ms (mean 2,000). The subject was instructed to press a button with the thumb of the dominant hand as quickly as possible whenever he recognized a target pip (reaction time). To avoid electroencephalogram (EEG) artifacts, the subject was asked to fixate his eyes on a centrally located dot and not to blink too often during task performance. Analyses of the AEBP focused on the P3 component (also termed “P300”), which is a large potential component evoked by the task-relevant target pips of the vigilance task. It typically peaks at \(-350-450 \text{ ms}\) post-stimulus over parietal cortical regions. The P3 is considered an indicator of the target processing within working memory (31), although the psychological significance of P3 is a matter of ongoing debates (32, 33). For AEBP assessment, the EEG (5-s time constant, 70 Hz/12-dB high-frequency roll off, 0.045 Hz/6-dB low-frequency roll off) was recorded continuously from frontal (Fz), central (Cz), and parietal (Pz) electrode loca-

![Figure 1](image)

**Figure 1**—Means ± SEM concentration of plasma glucose (A) and serum insulin (B) in the prior-hypo group (●) and the control group (○) during baseline and stepwise hypoglycemic clamp studies.
tions referenced to linked electrodes attached to the earlobes. An electrode at frontal parietal electrode location served as a baseline. For artifact recognition, the vertical EOG was monitored. Nonpolarizable silver-silver chloride electrodes 16 mm in diameter were used. EEG and EOG signals were amplified by a Nihon Kohden Neurofax 4421 G polygraph (Nihon Kohden, Japan) and digitized (CED 1401; Cambridge Electronic Design, Cambridge, U.K.) with a sampling rate of 385 Hz for off-line averaging of AEBPs. As previously described (34), data analysis of the recorded AEBPs calculated the latency and baseline-to-peak amplitude of the P3 component after the task-relevant target pip. P3 was defined with regard to the maximum positive amplitude from 280 to 740 ms poststimulus.

Short-term memory task
Short-term memory testing consisted of the consecutive presentation of 2 different word lists, each containing 15 words. The words belonged to 3 semantic categories: neutral words like “tree” and “field,” food-related words like “ham” and “eggs,” and emotional words like “mother” and “friend.” To enable repeated testing, 10 different lists were formed from a pool of words with each list including 5 words from each semantic category in random order. Words were presented orally at a rate of 1 word per second. After each list and a subsequent break of 1 min, the subject was required to verbally recall all of the words that he remembered from the preceding list within 1 min. The number of words correctly recalled at each testing was determined and summed across the 2 lists for all words presented as well as separately for each semantic category. Also, the number of false answers was determined (i.e., words that had been presented in a prior list or were never presented). However, the number of such errors was small, and respective analyses did not yield any conclusive information. Therefore, these data are not presented herein.

Indicators of the arousal state
A symptom and mood questionnaire was administered every 15 min. Subjects scored from 0 (none) to 4 (severe) on each of the following symptoms and moods: bodily discomfort, inner tension, anxiety, ability to concentrate, annoyance, fatigue, and excitability. The rating scores were averaged across the baseline phase and each of the hypoglycemic plateaus, respectively. Blood pressure and heart rate were measured automatically by a BC 40 (Bose-Prestige Automatic; Bosch und Sohn, Jüngingen, Germany), which simulated the Riva-Rocci procedure at baseline and each of the hypoglycemic plateaus before the cognitive tests.

Hormone measurements
Blood samples for measurements of counterregulatory hormones (cortisol,
growth hormone, epinephrine, and norepinephrine) were drawn at baseline and at the end of the 4 hypoglycemic plateaus (90, 180, 270, and 360 min) of the hypoglycemic clamp. Blood samples for insulin measurements were drawn every 30 min. Hormone concentrations were measured as previously described (35).

Statistical analysis Data are means ± SEM. Baseline values of all measurements were compared between the 2 groups by unpaired Student’s t test. Analysis of covariance (ANCOVA) for repeated measurements was performed to detect effects of plasma glucose and antecedent hypoglycemia, including the factors of plasma glucose (including the 4 glycemic plateaus) and group (prior-hypo vs. control) with baseline values serving as covariates. Only when main effects for the group factor or the group × plasma glucose interaction reached significance (P < 0.05) was post hoc analysis performed by ANCOVA at each of the glycemic plateaus. Also, measures of per-
performance on cognitive function tests, self-ratings, and counterregulatory hormones at the final hypoglycemic plateau were expressed as the difference from baseline. These difference values were subsequently subjected to stepwise forward multiple linear regression analysis with the changes in performance on cognitive function tests as dependent variables to determine their dependence on changes in self-ratings and counterregulatory hormones.

RESULTS

Glucose and insulin levels
During the antecedent hypoglycemic clamp, plasma glucose levels decreased to a steady-state level of 3.1 ± 0.1 mmol/l within the first 30 min of insulin infusion. This level was maintained for 2 h until the end of the clamp (data not shown). During the stepwise hypoglycemic clamp, plasma glucose levels decreased and serum insulin levels increased as expected with the time course of changes closely comparable between both groups (Fig. 1).

AEPB and reaction time
In both groups, amplitude of the P3 component significantly decreased during the stepwise hypoglycemic clamp (effect of plasma glucose across all electrode sites, P < 0.01). However, a significant group × plasma glucose interaction effect (P < 0.05) was evident, which indicates that the decrease in P3 amplitude during the clamp was distinctly smaller in the prior-hypo group than in the control group. Although the effects did not interact with topography, separate comparisons for each electrode site indicated the preserving effect of antecedent hypoglycemia on P3 amplitude to reach significance at Pz (P < 0.05) (Fig. 2A) and at Cz (P < 0.05) but not at Fz (P = 0.36).

Similarly, the increase in P3 latency observed in both groups with progression of hypoglycemia was less pronounced in the prior-hypo group than in the control group. Overall ANCOVA indicated significance for the group × plasma glucose interaction (P < 0.005) independent of topography. Separate comparisons at each electrode site indicated significance for this effect at Fz (13.4 ± 11.1 vs. 56.3 ± 8.6 ms; P < 0.005 for comparison at the last hypoglycemic plateau) and at Cz (12.0 ± 12.7 vs. 45.3 ± 8.0 ms; P < 0.05 for comparison at the last hypoglycemic plateau).

Reaction time to the target pips of the oddball task also increased in both groups during hypoglycemia (effect of plasma glucose, P < 0.001). Again, a significant group × plasma glucose interaction effect (P < 0.05) (Fig. 2B) indicated that, at the lowest hypoglycemic plateau, reaction time was less prolonged after the antecedent hypoglycemia than after the control condition (358.2 ± 13.4 vs. 389.2 ± 11.2 ms, P = 0.07).

Short-term memory recall
During the stepwise hypoglycemic clamp, recall performance deteriorated in both groups (effect of plasma glucose, P < 0.001), with this effect depending also on the presence of antecedent hypoglycemia (P < 0.005 for group × plasma glucose interaction) (Fig. 2C). At the lowest hypoglycemic plateau, subjects in the prior-hypo group remembered on average almost 4 words more than subjects in the control group (17.4 ± 0.8 vs. 13.6 ± 0.8; P < 0.005). Overall, ANCOVA did not indicate a significant difference in word categories. In a separate analysis of the different word categories, the difference between experimental groups reached significance for recall of emotional words (6.2 ± 0.6 vs. 4.8 ± 0.3; P < 0.01 for group × plasma glucose interaction) and of food-related words (6.2 ± 0.6 vs. 4.9 ± 0.4; P < 0.05 for group × plasma glucose interaction) but not for recall of neutral words (5.0 ± 0.7 vs. 3.9 ± 0.5; P = 0.24 for group × plasma glucose interaction).

Indicators of the arousal state and neuroendocrine counterregulatory hormones
Table 1 summarizes the results of the symptom and mood questionnaire and the blood pressure, heart rate, and counterregulatory hormone measurements. At baseline, none of the self-rated symptom and mood scores differed between the groups. Although scores of some psychological parameters changed with time during the clamp, none of them differed between the groups at the different hypoglycemic levels. Heart rate and systolic blood pressure increased and diastolic blood pressure decreased during the hypoglycemic clamp, but no difference was evident between the 2 groups. Counterregulatory hormone response to hypoglycemia was significantly reduced after antecedent hypoglycemia.

Multiple regression analysis
Table 2 summarizes correlation coefficients between changes in performance on cognitive function tests and the other self-rated symptom and mood scores were not significant.

CONCLUSIONS
This study assessed the effects of antecedent hypoglycemia on hypoglycemic cognitive dysfunction during

<table>
<thead>
<tr>
<th>Table 2—Correlations between the changes (plateau 4 - baseline) in performance on cognitive function tests, self-rated symptom and mood scores, and counterregulatory hormones</th>
<th>ΔP3 amplitude (at Pz)</th>
<th>ΔP3 latency (at Pz)</th>
<th>ΔReaction time</th>
<th>ΔWord recall</th>
</tr>
</thead>
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<tr>
<td>ΔConcentration</td>
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<td>0.42*</td>
<td>−0.42*</td>
<td>0.25</td>
</tr>
<tr>
<td>ΔFatigue</td>
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<td>0.05</td>
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<td>−0.39*</td>
</tr>
<tr>
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<td>ΔGrowth hormone</td>
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<td>0.09</td>
<td>−0.35</td>
</tr>
</tbody>
</table>

Data are correlation coefficients. *P < 0.05; †P < 0.01; ‡P < 0.005; §P < 0.01. Correlations between changes in performance on cognitive function tests and the other self-rated symptom and mood scores were not significant.
Hypoglycemia and cognitive function

Figure 3—Pearson’s correlation between the changes in serum cortisol concentration and the changes in performance of short-term memory recall at the final hypoglycemic plateau. Changes were defined by the difference from the respective baseline value. ●, Prior-hypo group; ○, control group.

subsequent hypoglycemia (i.e., the question of whether a cognitive adaptation to repeated hypoglycemia is involved). Cognitive dysfunction during hypoglycemia was found to be attenuated in men experiencing their second hypoglycemic episode within 24 h compared with the subjects who were not subjected to a mild antecedent hypoglycemia.

It has recently been suggested that psychological factors (e.g., anxiety or fatigue) may confound cognitive performance during hypoglycemic clamp experiments (22). Therefore, in the present study, we monitored self-rated symptom and mood scores as well as physiological indicators of arousal. None of these measures significantly differed between the groups with and without antecedent hypoglycemia. Nevertheless, small differences in fatigue and concentration that may have biased findings cannot be excluded. However, multiple regression analysis did not reveal any evidence that the preserving effects of antecedent hypoglycemia agree with previous data (14,15, 25,26,36). On the other hand, several other studies (6,7,23) failed to reveal similar effects of recurrent hypoglycemia. This inconsistency may be explained by differences in the study design. First, methods to assess cognitive dysfunction and related aspects of cognitive function are known to differ in sensitivity regarding the effects of hypoglycemia (22,37–40). Tests used in some foregoing trials may have been not optimal to reveal protective effects of antecedent hypoglycemia. Also, some foregoing studies were based on a fairly small or heterogeneous subject sample (6,7,14,23). Because the size of cognitive changes after antecedent hypoglycemia is only moderate, the statistical power of these studies may have been insufficient. Finally, for all measurements, cognitive dysfunction did not develop in the present study until a hypoglycemic level of 2.6 mmol/l was reached (i.e., after ~5 h of the clamp). This result agrees with previous data (27–30). Only at this plateau, when hypoglycemic disturbances of cognitive function were most pronounced, could the influence of antecedent hypoglycemia be discriminated. With this background, some foregoing experimental hypoglycemic conditions may have been just too mild to detect acute effects (7).

A potential mechanism underlying cerebral adaptation to hypoglycemia could be an increase in glucose extracted during hypoglycemia (41,42). An improved glucose uptake into the brain has been demonstrated after prolonged hypoglycemia in rats (43) and humans (11). Moreover, in rats (44,45), expression of glucose transporter (GLUT1 and GLUT3) molecules was increased after prolonged hypoglycemia. Improved central glucose uptake during hypoglycemia may prevent neuroglycopenia, thereby reducing the effect of hypoglycemia on cognitive function aside from a parallel weakening of neuroendocrine counterregulation and autonomic and neuroglycopenic symptoms.

In addition, weakened neuroendocrine counterregulation after antecedent hypoglycemia may contribute to the attenuated cognitive impairment during subsequent hypoglycemia. Many of the hormones involved in hypoglycemia counterregulation exert neurocognitive influences. For example, in healthy men, short-term elevation of cortisol levels impaired declarative memory performance (46) as well as event-related potential signs of sensory discrimination (47). Therefore, lower hypoglycemic cortisol release after antecedent hypoglycemia may have added to the preserving effects of antecedent hypoglycemia on short-term memory performance in the present study. This view is also supported by the strong association between the response of cortisol and performance on the word recall task to hypoglycemia (Fig. 3), although regression analysis cannot be used to prove a causal relationship.

It has been well established that recurrent severe hypoglycemic episodes adversely affect several aspects of cognitive function over the long term (17–20). With this background, the significance of a cognitive adaptation to hypoglycemia, as indicated herein, in the progress of central nervous system sequelae of diabetes remains to be defined. A cerebral adaptation to hypoglycemia has been regarded as maladaptive because of the weakened glucose counterregulation and resulting hypoglycemic unawareness, which increases the risk of severe hypoglycemia. On the other hand, adaptation to hypoglycemia in terms of increased glucose extraction may also be
beneficial because it not only reduces cognitive dysfunction but also may protect against structural brain damage during severe or recurrent hypoglycemia.

Acknowledgments — We thank Christiane Zinke and Steffi Baumann for their expert and invaluable laboratory assistance and Anja Otterbein for her organizational work.

References


Hypoglycemia and cognitive function