Effects of previous glycaemic control on the onset and magnitude of cognitive dysfunction during hypoglycaemia in Type 1 (insulin-dependent) diabetic patients

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Summary. To determine whether the degree of previous glycaemic control may modify cognitive responses to hypoglycaemia, the glycaemic thresholds for, and magnitude of cognitive dysfunction as assessed by P300 event-related potentials as well as subjective and hormonal responses during hypoglycaemia were evaluated. Hypoglycaemia was induced by intravenous insulin infusion in 18 Type 1 (insulin-dependent) diabetic patients, 7 of whom were strictly controlled (HbA_{1c} : $6.3 \pm 0.3\%$; mean $\pm$ SEM; Group 1) and 11 of whom were poorly controlled (HbA_{1c} : $9.1 \pm 0.4\%$; Group 2). Within 60 min, mean blood glucose declined from 5.6 and 5.7 mmol/l (baseline) to a nadir of 1.6 and 1.8 mmol/l followed by an increase to 5.6 and 4.3 mmol/l after 120 min in Group 1 and 2, respectively. There was no significant difference between the groups in regard to P300 latency at baseline, but between 50 and 70 min a significant prolongation of this component was noted in Group 2 as compared with Group 1 at blood glucose levels between 1.6 and 2.3 mmol/l ($p < 0.05$). The glycaemic thresholds at which a significant increase of P300 latency over baseline was first noted were 1.6 ± 0.2 mmol/l in Group 1 and 3.5 ± 0.2 mmol/l in Group 2 ($p < 0.05$). The glucose thresholds at which this prolongation

was no longer demonstrable were 1.9 ± 0.1 mmol/l in Group 1 and 3.8 ± 1.4 mmol/l in Group 2, respectively ($p < 0.05$). The glycaemic threshold at which the P300 amplitude was first significantly reduced was 2.2 mmol/l in Group 2, whereas no such reduction was observed in Group 1. The glycaemic thresholds for the perception of subjective symptoms were 1.7 ± 0.2 mmol/l in Group 1 and 2.5 ± 0.2 mmol/l in Group 2 ($p < 0.05$), and those for the first significant rise of the counter-regulatory hormones were 2.3 ± 0.1 and 1.6 ± 0.2 mmol/l in Group 1 as well as 2.8 ± 0.1 mmol/l in Group 2 ($p < 0.05$). Thus, the glycaemic threshold for and magnitude of, cognitive dysfunction during hypoglycaemia are reduced in strictly-controlled as compared with poorly-controlled Type 1 diabetic patients. In the latter group, cognitive impairment may precede the onset of counter-regulatory hormone responses and symptom awareness. These findings support the concept of cerebral adaptation to previous low blood glucose levels.

Key words: Type 1 (insulin-dependent) diabetes mellitus, glycaemic control, hypoglycaemia, P300 event-related potentials, cognitive function.

Strict glycaemic control during intensive insulin therapy in diabetic patients may be associated with an increased risk of severe hypoglycaemia [1], blunted counter-regulatory hormone responses to a hypoglycaemic stimulus [2], and with attenuated recognition of hypoglycaemic symptoms [3]. Since the latter alteration may also be observed in patients with insulinoma, it was tempting to speculate that the brain may adapt to recurrent low blood glucose levels [4]. In fact, recent studies have demonstrated that prolonging the duration of mild hypoglycaemia resulted in diminished awareness of hypoglycaemic symptoms and improved reaction time in both healthy subjects [5] and Type 1 (insulin-dependent) diabetic patients [6]. Furthermore, in non-diabetic persons a single episode of hypoglycaemia caused a reduction of neuroendocrine and subjective

responses to subsequent hypoglycaemia the following day [7]. In contrast, the glycaemic threshold for cognitive dysfunction detected by neuropsychologic testing during hypoglycaemia did not differ between well-controlled patients and those with poor glycaemic control [8]. Moreover, diabetic subjects with previously strict glycaemic control and patients with insulinoma were more likely to develop electroencephalogram (EEG) abnormalities during hypoglycaemia than poorly-controlled patients, despite delayed hormonal and subjective responses [9]. Thus, the results of the two latter studies are not compatible with better cerebral protection against hypoglycaemia in strictly-controlled patients.

Measurement of event-related potentials offers an objective technique to quantitatively assess cognitive brain

Table 1. Clinical characteristics of the patients with strict glycaemic control (Group 1) and poor glycaemic control (Group 2)

	Group 1 (n = 7)	Group 2 (n = 11)
Gender (male/female)	4/3	10/1
Age (years)	27 (17–44)	32 (22–50)
Height (cm)	174 (164–185)	178 (154–187)
Weight (kg)	76 (66–90)	78 (55–92)
Duration of diabetes (years)	13 (5–22)	15 (5–32)
HbA _{1c} (%)	6.5 (5.3–7.0) ^a	9.0 (7.5–12.3)
Mean blood glucose (mmol/l)	7.8 (6.1–10.2)	8.9 (5.2–13.8)
Insulin dose (IU/day)	48 (42–56)	44 (32–79)
Injections per day	4 (3–4)	4 (3–4)
Background retinopathy	3/7	5/11
Albuminuria ($\geq 20 \mu\text{g}/\text{min}$)	1/7	3/11
Peripheral neuropathy	1/7	4/11
Autonomic neuropathy	0/7	2/11

Data are expressed as medians (ranges) or numbers

^a $p < 0.05$ for Group 1 vs Group 2

function. The P300 component of these potentials is endogenously generated when the subject being tested discriminates and memorizes a given task-relevant stimulus. Its latency reflects the speed of information processing and is associated with attention and short-term memory [10]. Previous studies in healthy subjects have reported variable glycaemic thresholds for a significant increase in P300 latency during hypoglycaemia ranging from 2.6 to 4.0 mmol/l [11–14]. One study conducted in Type 1 diabetic patients showed that the amplitude of P300 decreased, while its latency remained unchanged at blood glucose levels down to 1.8 mmol/l [15], but the influence of previous glycaemic control has not been examined.

In the present study, we measured the P300 event-related potentials to compare the glycaemic thresholds for and the magnitude of, cognitive dysfunction during hypoglycaemia between Type 1 diabetic patients with previously strict as compared to poor glycaemic control. The onset of counter-regulatory hormone responses, symptom perception, and free insulin levels were also examined.

Subjects and methods

Patients

After informed consent had been obtained, 18 Type 1 diabetic patients who had been treated with multiple daily insulin injections participated in the study. They were grouped according to their HbA_{1c} levels on admission to the metabolic ward of the Diabetes Research Institute. Group 1 comprised seven patients with strict glycaemic control defined as HbA_{1c} ≤ 7.0 , while 11 patients with poor glycaemic control defined as HbA_{1c} $\geq 7.5\%$ were allocated to Group 2. The clinical characteristics of both groups are shown in Table 1. There were no significant differences between the groups with regard to any of the parameters listed, except for HbA_{1c}. A history of severe hypoglycaemia with unconsciousness was reported by two patients who experienced two episodes each and one patient with one episode in Group 1 and by two patients with four episodes each and two patients with one episode each in Group 2, respectively (NS).

Patients were excluded if they had serum C-peptide levels of more than 0.1 nmol/l after stimulation with 1 mg glucagon i. v., renal failure, proliferative retinopathy, previous history of vitreous haemorrhage, psychiatric disorders, ear diseases, ototoxicity or reported impaired hearing. None of the patients was receiving medica-

tion known to influence the central nervous system or the perception of hypoglycaemia.

Study protocol

Isophane (NPH) insulin was discontinued 24 h before the study and regular insulin was injected before the evening meal on the day prior to the test. At 22.00 hours, an i. v. infusion of regular insulin (Actrapid HM ($n = 6$); Velasulin H ($n = 4$); Velasulin ($n = 4$) (Novo Nordisk, Mainz, FRG) and H-Insulin ($n = 4$) (Hoechst, Frankfurt, FRG) was started and continued overnight by means of a syringe pump. Capillary blood glucose levels were maintained between approximately 4.5–6.7 mmol/l by adjustments of the insulin dose based on hourly bedside measurements. After a 10-h overnight fast, at 08.00 hours the insulin infusion rate per hour was adjusted to 20 % of the total insulin injected on the day before NPH insulin was discontinued. The insulin infusion was stopped after approximately 50 min when the venous blood glucose concentration had decreased to below 2.5 mmol/l, and the blood glucose was allowed to increase spontaneously for the next 70 min. After 90 min, four (57 %) patients of Group 1 and six (55 %) patients of Group 2 received glucose i. v., because they showed an inadequate increase in blood glucose, with levels remaining below 2.2 mmol/l or had persisting unpleasant hypoglycaemic symptoms. During the 120-min test period venous blood glucose samples were taken at 10-min intervals from a 18 gauge venous cannula (Braun, Melsungen, FRG) inserted into an antecubital vein contralateral to the insulin infusion. Plasma glucagon, epinephrine, and norepinephrine were determined at 0, 20, 40, 50, 60, 70, and 80 min and free insulin was measured at 0 and 60 min. Furthermore, the patients were asked to report all symptoms they perceived during the test period. The following hypoglycaemic symptoms were considered: hunger, faintness, dizziness, tingling, blurred vision, difficulty in thinking, sweating, palpitations, nervousness, anxiety, and tremor.

Laboratory methods

Capillary or venous blood glucose were measured using the hexokinase method on the ACP 5040 autoanalyser (Eppendorf, Hamburg, FRG). Mean blood glucose on the day before the insulin infusion was started was calculated as the mean of five values (fasting, postprandial, before lunch, before supper, and at bedtime). HbA_{1c} was determined with the HPLC technique using a Diamat analysing system (Bio-Rad, Munich, FRG), the normal range being less than 6.3 %. Serum C-peptide was measured by RIA using a RIA-mat C-peptide (Byk-Mallinckrodt, Dietzenbach, FRG). Plasma glucagon was determined by use of the 30K antibody [16]. The reversed phase HPLC method with electrochemical detection was used to determine plasma epinephrine and norepinephrine (Beckman, München, FRG). The Insulin RIA 100 was employed for the measurement of serum free insulin (Pharmacia, Freiburg, FRG) using the method described by Kuzuya et al. [17].

Event-related potentials

The P300 component was determined using a Mistral E.P. System (Medelec, Old Woking, UK). The recording silver surface electrode was placed at the right mastoid, the reference electrode at the vertex (Cz), and the ground electrode at the forehead using a standard electrode paste. Auditory P300 recordings were obtained while the subjects performed a behavioural task (oddball paradigm). Each investigation included a series of two-tone pips with different pitch that were presented binaurally through headphones with an intensity of 80 decibel (dB) and interstimulus interval of 1 s. The patients were told to mentally count, during each P300 task, the infrequently emitted target pips of higher pitch (2000 Hz) which were randomly interspersed with a probability of 15 % among frequent pips of lower pitch (1000 Hz). At the end of the P300 task during which a total of 147 stimuli had been collected, the subjects were asked to report the total count of the infrequent pips. The duration of each recording

was less than 3 min. Artifacts were excluded using an automatic rejection system. The amplifier band pass was 0.1–30 Hz. The P300 component was recorded prior to the insulin-infusion test at baseline (0 min) and thereafter at 10–20-min intervals. To ensure reproducibility, each measurement was performed twice. The latency of the P300 waveform was determined from the maximum positivity averaged from the infrequent pips between 260 and 600 ms post-stimulus. In the case of two consistent peaks being recorded (P3a, P3b), the second component was computed. The P300 amplitude was defined as the difference between the average baseline voltage within 0–50 ms and the peak voltage of the corresponding latency.

Assessment of diabetic complications

Nerve conduction velocity (median and peroneal motor as well as median and sural sensory nerves), vibration perception threshold (metacarpal bone and medial malleolus), and thermal discrimination threshold (thenar eminence and foot) were determined as previously reported [18]. Peripheral neuropathy was defined as the presence of at least two abnormalities among these three tests in conjunction with neuropathic symptoms such as pain, paraesthesiae or numbness in the limbs.

Autonomic function tests were performed using a ProSciCard computer system (MediSyst, Linden, FRG). Cardiovascular autonomic neuropathy was defined as abnormal results for at least three out of the six following tests: heart rate variation at rest and during deep breathing, spectral power in the low-frequency and mid-frequency bands, maximum/minimum 30:15 ratio, and Valsalva ratio [19].

Eye examinations were carried out by fundoscopy at the Department of Ophthalmology of the Düsseldorf University Clinic. Urinary albumin excretion rate was determined from 24-h samples by the immuno-nephelometric technique (Array Protein System, Beckman, Fullerton, Calif., USA).

Statistical analysis

All data are expressed by the arithmetical mean $\pm$ SEM, except for those of Table 1 which are given as medians and ranges. To minimize the risk of a type I error, those significant increases in the latency and reductions in amplitude of the P300 component which were noted first and last during the test period, were specified as the two primary end-points of interest. In the case of counter-regulatory hormones, the time at which the first significant rise over baseline (0 min) occurred, was chosen as end-point. Student's paired *t*-test was applied to differences between baseline (0 min) and the specified end-points. Significance levels were corrected for repeated testing using the Bonferroni method. The Mann-Whitney U test or the chi-squared test were used to analyse the data in Table 1. The *t*-test for two independent samples was applied to test the differences between the two groups of diabetic subjects studied. The level of significance was defined uniformly at $\alpha = 0.05$.

Results

Blood glucose, counter-regulation, and symptoms

The blood glucose levels and counter-regulatory hormone responses are shown in Figure 1. During the 120-min test period, mean blood glucose in Group 1 (strict control) declined from 5.6 ± 0.3 mmol/l at baseline (0 min) to a nadir of 1.6 ± 0.2 mmol/l after 60 min, followed by an increase to 5.6 ± 1.6 mmol/l after 120 min. The corresponding levels in Group 2 (poor control) were 5.7 ± 0.4 , 1.8 ± 0.1 , and 4.3 ± 0.7 mmol/l. No significant differences in blood glucose between either group were noted at any of the time-points tested. The glycaemic thresholds at which a significant increase of glucagon and norepine-

phrine over baseline (0 min) was first noted were 2.3 ± 0.1 mmol/l (50 min) in Group 1 and 2.8 ± 0.1 mmol/l (40 min) in Group 2, respectively ($p < 0.05$). There were no significant differences between the groups regarding the mean glucagon and norepinephrine levels during the test period. The area under curve (AUC) for glucagon was 6.7 ± 1.2 nmol $\cdot$ l⁻¹ $\cdot$ min in Group 1 and 6.3 ± 0.9 nmol $\cdot$ l⁻¹ $\cdot$ min in Group 2 (NS). The corresponding figures for norepinephrine were 109 ± 2.5 and 127 ± 13.0 nmol $\cdot$ l⁻¹ $\cdot$ min (NS). The glycaemic thresholds at which a significant rise of epinephrine over baseline was first observed were 1.6 ± 0.2 mmol/l (60 min) in Group 1 and 2.8 ± 0.1 mmol/l (40 min) in Group 2 ($p < 0.05$). After adjusting for the baseline values the mean epinephrine levels were significantly lower in Group 1 as compared to Group 2 at mean blood glucose levels of 2.2 ± 0.1 mmol/l (50 min) ($p < 0.05$). The AUC for epinephrine was significantly smaller in Group 1 than in Group 2 (60.7 ± 5.0 vs 84.8 ± 10.0 nmol $\cdot$ l⁻¹ $\cdot$ min; $p < 0.05$). The free insulin levels measured at 0 min and 60 min increased from 5.8 ± 0.7 to 16.4 ± 1.9 μ U/ml in Group 1 and from 8.5 ± 1.7 to 16.7 ± 1.6 μ U/ml in Group 2, respectively ($p < 0.05$), without significant differences between the groups. The blood glucose thresholds at which any symptom of hypoglycaemia was first perceived were 1.7 ± 0.2 mmol/l in Group 1 and 2.5 ± 0.2 mmol/l in Group 2 ($p < 0.05$).

Event-related potentials

The mean responses in P300 latency elicited during the insulin-infusion test in the patients with strict glycaemic control (Group 1) and in those with poor control (Group 2) are shown in Figure 2. There was no significant difference between either group in P300 latency at baseline (0 min). However, after 50, 60, and 70 min at blood glucose levels of 1.6–2.3 mmol/l Group 1 showed a significantly less marked prolongation of this component than did Group 2 ($p < 0.05$). The glycaemic thresholds for the first significant rise in P300 latency over baseline were 1.6 ± 0.2 mmol/l (60 min) in Group 1 and 3.5 ± 0.2 mmol/l (30 min) in Group 2 ($p < 0.05$). The glucose thresholds at which this significant prolongation was no longer demonstrable were 1.9 ± 0.1 mmol/l (70 min) in Group 1 and 3.8 ± 1.4 mmol/l (90 min) in Group 2 ($p < 0.05$). The results of P300 amplitude are illustrated in Figure 3. The difference between both groups with regard to this parameter at baseline did not reach statistical significance. After adjusting for this difference, the P300 amplitude did not differ significantly between Group 1 and Group 2 during the test period. No significant reduction in P300 amplitude over baseline (0 min) was observed in Group 1. In contrast, the glycaemic threshold for the first significant decrease over baseline in P300 amplitude in Group 2 was 2.2 ± 0.1 mmol/l (50 min) ($p < 0.05$), and the threshold at which this reduction first disappeared was 2.5 ± 0.3 mmol/l (80 min).

There were neither significant differences in P300 latency and amplitude during the period studied between patients who received and those who did not receive glucose i.v. after 90–120 min nor between patients who were treated with human insulin and those who were treated

Fig. 1. Blood glucose levels and counter-regulatory hormone responses (mean $\pm$ SEM) during the insulin-infusion test in patients with strict glycaemic control (Group 1; $n = 7$, $\circ$) and in those with

poor glycaemic control (Group 2; $n = 11$, $\bullet$). * $p < 0.05$ for the level at which a significant rise vs baseline (0 min) was first noted; § $p < 0.05$ for Group 1 vs Group 2

Fig. 2. P300 latency (mean $\pm$ SEM) in response to hypoglycaemia in patients with strict glycaemic control (Group 1; $n = 7$, $\circ$) and in those with poor glycaemic control (Group 2; $n = 11$, $\bullet$). * $p < 0.05$ for the level at which a significant rise vs baseline (0 min) was first and last noted; § $p < 0.05$ for Group 1 vs Group 2

Fig. 3. P300 amplitude (mean $\pm$ SEM) in response to hypoglycaemia in patients with strict glycaemic control (Group 1; $n = 7$, $\circ$) and in those with poor glycaemic control (Group 2; $n = 11$, $\bullet$). * $p < 0.05$ for the level at which a significant rise vs baseline (0 min) was first and last noted

with porcine insulin. Furthermore, similar results without significant differences were obtained when comparing male and female patients as well as subjects with and without peripheral or autonomic neuropathy.

Discussion

In the present study, the average glycaemic thresholds at which cognitive dysfunction was first detected by a prolonged P300 latency during gradual decrease in blood glu-

cose were 1.6 mmol/l in strictly-controlled and 3.5 mmol/l in poorly-controlled Type 1 diabetic patients. In addition, cognitive dysfunction was significantly less severe at blood glucose levels of 1.6–2.3 mmol/l in patients with previously strict control when compared with those who were poorly controlled. Cognitive dysfunction during hypoglycaemia in the former group followed the activation of counter-regulatory hormones, whereas in the latter group it preceded the counter-regulation and the onset of symptom perception during hypoglycaemia. No significant dif-

ferences between the two groups were found regarding the insulin levels during hypoglycaemia.

The P300 component represents an index of information processing activity [10] which has been linked to variables such as expectancy, task difficulty, and memory [20]. It is termed endogenous because it depends on the cognitive context presented by a stimulus but is unaffected by the physical characteristics of the stimulus [21]. The P300 waveform is thought to be primarily generated in the medial temporal lobe including the hippocampus and amygdala [22], but recent evidence also implicates the vicinity of the auditory cortex as a possible P300 source location [23]. Since the technique of P300 measurement is objective and reproducible [24], it appears more suitable for rapid assessment of cognitive function than neuropsychological tests, which are more time-consuming, subjective and may be influenced by psychosocial factors such as the level of motivation or past academic experience [25]. The clinical utility of abnormal P300 response has been emphasized particularly in the characterization of dementia [20]. Regarding diabetes, no significant prolongation of P300 latency was noted in Type 2 (non-insulin-dependent) diabetic patients [26]. On the other hand, it has been demonstrated that this component may become abnormal in Type 1 diabetic patients without any history of severe hypoglycaemia [27].

Considerable effort has recently been directed to elucidate the cerebral neurophysiological alterations that occur during hypoglycaemia in both healthy subjects and diabetic patients. They include changes in EEG [9, 15, 28], visual reaction time [13, 29, 30], neuropsychological performance [8, 31–35], cerebral glucose metabolism [36, 37] as well as visual [38, 39], brainstem auditory-evoked potentials [14, 40], and P300 event-related potentials [11–15, 41]. Studies that used the latter technique to define the glycaemic thresholds for cognitive dysfunction during hypoglycaemia in healthy subjects have yielded controversial results. De Feo et al. [11] reported that the P300 latency was prolonged after plasma glucose concentration had been lowered from 4.9 to 4.0 mmol/l. Since this change was observed prior to the counter-regulatory hormone release, they suggested that neuroglycopenia precedes the activation of counter-regulation. By contrast, two recent studies have demonstrated that a decline in plasma glucose to 2.6 mmol/l [13] and 3.0 mmol/l [14], respectively, was required to produce a significant prolongation of P300 latency that followed or paralleled the increase in symptom perception and counter-regulatory hormones. These discrepancies may be partly due to the different approaches of analysing the changes in the P300 potential or to distinct hypoglycaemic clamp procedures. Tallroth et al. [15] measured the P300 response in hypoglycaemic Type 1 diabetic patients. As in the present study, an i.v. insulin infusion was used to induce a fall in blood glucose from 6.4 mmol/l to an average nadir of 1.8 mmol/l. At this level, there was only a reduction in P300 amplitude over baseline, but no change in P300 latency could be demonstrated. However, that study did not discriminate the patients in relation to the previous degree of glycaemia, and the glycaemic threshold for the initial alteration of the P300 amplitude during the fall in

blood glucose has not been defined. In the present study, patients with near-normoglycaemic control did not experience a significant reduction in P300 amplitude at blood glucose levels down to 1.6 mmol/l, but in those with poor control, the threshold for such a reduction was 2.2 mmol/l. Since the glycaemic thresholds for the changes in P300 latency in the two groups could be defined at 1.6 and 3.5 mmol/l, the latency of P300 was more sensitive in detecting cognitive impairment during hypoglycaemia when compared with the amplitude. Furthermore, we have shown that in poorly-controlled patients, the prolongation in P300 latency may precede the hormonal counter-regulation and symptom perception. Such a sequence with cognitive dysfunction occurring as the first event during hypoglycaemia in poorly-controlled patients appears potentially hazardous by preventing them from recognizing hypoglycaemic symptoms. Thus, these patients may run an increased risk of prolonged hypoglycaemia. On the other hand, our results are also in line with studies suggesting that a delay in the hormonal counter-regulation of strictly-controlled diabetic patients may increase their risk of severe hypoglycaemia [2, 3].

Our findings of decreased blood glucose thresholds for cognitive dysfunction during hypoglycaemia in patients with near-normoglycaemic control are in apparent contrast with those recently reported by Widom and Simonson [8] using neuropsychological tests. They could not detect any differences with regard to the glycaemic threshold for cognitive impairment during hypoglycaemia down to 2.2 mmol/l between patients with a mean HbA_{1c} of 8.0 % and 11.8 %, respectively. It is not possible to explain this discrepancy on the basis of previous glycaemic control, because the percentage differences in HbA_{1c} between the strictly- and poorly-controlled groups were similar in both studies. The more likely explanations could be the different procedures of inducing hypoglycaemia (slow-fall hypoglycaemic clamp vs insulin-infusion test with a relatively fast fall in blood glucose) and the different techniques of assessing cognitive function (psychometric tests vs electrophysiologic techniques). Since slow-fall hypoglycaemic clamping with sustained hypoglycaemia may result in improved cerebral function and a decrease in symptom score [5, 6], and since learning effects may occur after repeated application of psychometric tests [8], we feel that the method chosen in the present study may yield more accurate and objective information on cognitive dysfunction during hypoglycaemia. Moreover, measurement of P300 seems to be more sensitive in detecting cognitive impairment during hypoglycaemia than psychometric tests. Attention and memory skills assessed by the word recall and digit span tests did not deteriorate at blood glucose levels down to 2.3 mmol/l in both well-controlled and poorly-controlled patients [8]. Furthermore, impairment in psychometric tests in response to hypoglycaemia in healthy subjects was triggered at the lowest glycaemic threshold when compared with the thresholds for counter-regulatory hormone release and subjective symptoms [34].

The effect of previous glycaemic control on cerebral function determined by use of EEG was studied by Amiel et al. [9] who found that strictly-controlled diabetic pa-

tients and those with insulinoma developed EEG changes more frequently than did poorly-controlled patients. They concluded that the former two groups were more vulnerable to hypoglycaemia rather than more protected. In contrast, our results are compatible with the concept of cerebral adaptation to previous strict glycaemic control as has been previously described [42, 43]. Further evidence supporting this concept can be derived from animal studies which showed that chronic hyperglycaemia reduces [44] and chronic hypoglycaemia augments brain glucose transport [45]. Recent studies in humans suggest an increased cerebral glucose uptake during mild hypoglycaemia in both healthy subjects [36] and Type 1 diabetic patients [37]. Such an enhancement of glucose transport into the brain could result in cerebral protection from acute lowering of blood glucose. Hence, it is conceivable that a process of cerebral adaptation to prolonged periods of strict glycaemic control occurs in Type 1 diabetic patients. However, this does not signify that strictly-controlled subjects would necessarily become more resistant to recurrent hypoglycaemia, because possibly the same process may render them more susceptible to severe hypoglycaemia by blunting the hormonal counter-regulation.

Since in the present study venous blood glucose was measured during the insulin infusion, it may be argued that the arterio-venous difference could have been more pronounced in the strictly-controlled group due to a possibly higher insulin sensitivity in these patients. However, we feel that the determination of venous blood glucose did not negatively influence our results. After admission to hospital all patients were treated using their insulin regimens to optimize glycaemic control. Since the daily insulin dose and the mean blood glucose levels on the day before the insulin infusion was started were comparable in the strictly-controlled and poorly-controlled groups, it can be assumed that there were no relevant differences in insulin sensitivity between both groups. Since there were no differences regarding the average body weight, the glucose pool should be comparable in both groups. The average blood glucose decline during the insulin infusion was almost identical in the groups studied, and therefore, a comparable glucose clearance in peripheral tissue can be expected. Thus, the arterio-venous gradient should not have been different between both groups.

It may also be argued that the changes in P300 observed in the present study could be influenced by the insulin species or the prevailing insulin levels. In fact, a recent study has claimed different effects of porcine and human insulin on the P300 waveform during hypoglycaemia in healthy subjects [41]. However, we did not find any difference regarding the changes of this component between patients treated with human or porcine insulin. Moreover, the free insulin levels during hypoglycaemia did not differ between the strictly- and poorly-controlled patients. This is in agreement with the results of a recent study showing that hyperinsulinaemia did not influence cognitive dysfunction during hypoglycaemia [35]. Hence, there is no evidence for an effect of insulin per se or the insulin species on cognitive function in the present study.

Since two patients of the poorly-controlled group had cardiovascular autonomic neuropathy, it might be presumed that this could have influenced our results. However, these patients showed cognitive, hormonal, and subjective responses to hypoglycaemia that were similar to those of the remaining poorly-controlled subjects. Moreover, recent evidence suggests that inadequate counter-regulatory hormone response to and awareness of hypoglycaemia are not due to autonomic neuropathy [46, 47]. Hence, we did not consider abnormal autonomic tests as exclusion criteria.

In conclusion, we provide evidence of reduced glycaemic thresholds for cognitive dysfunction to hypoglycaemia in strictly-controlled Type 1 diabetic patients, while in those with poor glycaemic control, cognitive impairment is triggered at relatively high blood glucose levels and may precede the activation of counter-regulation and perception of subjective symptoms. Our results suggest that strictly-controlled patients can adapt to low blood glucose levels. Such an adaptation may result in preserved cerebral function during hypoglycaemia, but these patients may be less protected from prolonged hypoglycaemia due to a delayed hormonal counter-regulation. In poorly-controlled Type 1 diabetic subjects impaired cerebral function may interfere with the perception of subjective symptoms, possibly increasing the risk of severe hypoglycaemia.

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