Preservation of Physiological Responses to Hypoglycemia 2 Days After Antecedent Hypoglycemia in Patients With IDDM

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OBJECTIVE — To assess the effects of short-term antecedent hypoglycemia on responses to further hypoglycemia 2 days later in patients with IDDM.

RESEARCH DESIGN AND METHODS — We studied eight type I diabetic patients without hypoglycemia unawareness or autonomic neuropathy during two periods at least 4 weeks apart. On day 1, 2 h of either clamped hyperinsulinemic (60 mU · m^{-2} · min^{-1}) hypoglycemia at 2.8 mmol/l or euglycemia at 5.0 mmol/l were induced. Hyperinsulinemic hypoglycemia was induced 2 days later with 40-min glucose steps of 5.0, 4.0, 3.5, 3.0, and 2.5 mmol/l. Catecholamine levels and symptomatic and physiological responses were measured every 10–20 min.

RESULTS — When compared with the responses measured following euglycemia, the responses of norepinephrine 2 days after hypoglycemia were reduced (peak, 1.4 ± 0.4 [mean ± SE] vs. 1.0 ± 0.3 nmol/l [$P < 0.05$]; threshold, 3.4 ± 0.1 vs. 2.9 ± 0.1 mmol/l glucose [$P < 0.01$]). The responses of epinephrine (peak, 4.0 ± 1.4 vs. 3.5 ± 0.8 nmol/l [$P = 0.84$]; threshold, 3.8 ± 0.1 vs. 3.6 ± 0.1 mmol/l glucose [$P = 0.38$]), water loss (peak, 194 ± 34 vs. 179 ± 47 g^{-1} · m^{-2} · h^{-1} [$P = 0.73$]; threshold, 2.9 ± 0.2 vs. 2.9 ± 0.2 mmol/l glucose [$P = 0.90$]), tremor (peak, 0.28 ± 0.05 vs. 0.37 ± 0.06 root mean square volts (RMS V) [$P = 0.19$]; threshold, 3.2 ± 0.2 vs. 3.1 ± 0.2 mmol/l glucose [$P = 0.70$]), total symptom scores (peak, 10.6 ± 2.1 vs. 10.8 ± 1.9 [$P = 0.95$]; threshold, 3.3 ± 0.2 vs. 3.6 ± 0.1 mmol/l glucose [$P = 0.15$]), and cognitive function (four-choice reaction time: threshold, 2.9 ± 0.2 vs. 3.0 ± 0.2 mmol/l glucose [$P = 0.69$]) were unaffected.

CONCLUSIONS — The effect on hypoglycemic physiological responses of 2 h of experimental hypoglycemia lasts for 1–2 days in these patients with IDDM. The pathophysiological effect of antecedent hypoglycemia may be of shorter duration in IDDM patients, compared with nondiabetic subjects.

Loss of the ability to detect impending hypoglycemia (hypoglycemia unawareness) is a distressing and potentially dangerous complication of insulin treatment in the management of IDDM. It increases the risk of severe hypoglycemic episodes (1), and affected patients are prevented from entering programs of intensified insulin therapy. Its development is associated with a long duration of diabetes (2) and periods of tight glycemic control (3,4). Single or repeated episodes of short-duration experimental hypoglycemia result in impaired physiological responses to subsequent hypoglycemic episodes in both diabetic and nondiabetic subjects (5–10). It has been proposed that hypoglycemia unawareness is caused by an acquired deficiency in the sympathoadrenal response as a result of repeated clinical episodes of hypoglycemia (4). This proposition has gained support from the finding that preventing hypoglycemic episodes restores both symptomatic awareness and physiological responses to hypoglycemia (11–13).

Boyle et al. (14) have shown that 56 h of hypoglycemia increases cerebral glucose uptake during subsequent episodes in normal subjects. They have also demonstrated similar changes in IDDM subjects with hypoglycemia unawareness and tight glycemic control (15). However, although these data indicate that the pathophysiology of hypoglycemia unawareness is probably linked to changes in cerebral glucose transport, the clinical effects of short-term hypoglycemia remain unclear.

We have recently demonstrated that in nondiabetic subjects, some components of the physiological response to hypoglycemia remain impaired for up to 5 days after a period of experimental hypoglycemia (16). However, although an impaired autonomic response has been demonstrated in patients with diabetes on the day after antecedent hypoglycemia (7), the maximum duration of this effect is unknown. We wondered whether the effects of short-lived antecedent hypoglycemia would be as profound in patients with diabetes, all of whom had previously experienced clinical hypoglycemia. Thus, the aim of our study was to examine the effect of a single period of experimental hypoglycemia on the physiological response to a further episode 2 days later.

RESEARCH DESIGN AND METHODS

Subjects

Eight subjects (Table 1) gave written consent to participate in this study, which was approved by the Research Ethics Committee of the Northern General Hospital Trust, Sheffield, U.K. All patients had been diag-

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AUC, area under the curve; RMS V, root mean square volts.

Table 1—*Subject details*

Subject	Sex	Age	Duration of IDDM (years, months)	BMI (kg/m^2)	Glycemic control (% HbA_1)	Insulin dose ($U \cdot kg^{-1} \cdot day^{-1}$)
1	M	29	2, 1	28.9	9.5	0.35
2	M	20	3, 8	21.6	10.2	0.76
3	M	33	2, 7	20.2	9.3	0.58
4	M	19	14, 0	20.6	10.0	0.81
5	M	30	7, 2	22.8	10.2	0.84
6	F	37	4, 7	25.1	11.2	0.56
7	M	38	12, 7	24.2	11.6	0.61
8	M	37	3, 11	23.9	12.7	0.59

nosed with IDDM within the past 15 years and had no complications of diabetes. Their mean age (± SE) was 30 ± 2 years with a mean BMI of 23.4 ± 1.0 kg/m^2. They were using no medication other than insulin. Subject 8 used long-acting insulin preparations twice daily, and the other subjects used basal-bolus regimens. All had glycemic control above the normal range of 4.5–8.5% for HbA_1. No patients had any history of hypoglycemia unawareness or recurrent severe hypoglycemia. Autonomic neuropathy was excluded by use of a standard battery of tests (17,18).

Study design

Each patient was studied during 2 periods separated by at least four weeks. Before their first visit, patients were asked to avoid all biochemical hypoglycemia (blood glucose, <4 mmol/l) for 1 week by using regular home blood glucose monitoring and, if necessary, reducing their insulin dose by 10%. The pre-bedtime target was a glucose value of >10 mmol/l. At least two 3:00 A.M. readings were performed during the week. Any biochemical or symptomatic hypoglycemic episode resulted in that set of studies being deferred until hypoglycemia had been avoided for 1 week. Five patients were randomized to start with the control week studies, while three patients completed the intervention arm first.

Patients were admitted after a reduced dose of short-acting insulin before a light breakfast at home. Human soluble insulin (Actrapid; Novo Laboratories, Copenhagen, Denmark) was infused at a rate of 0–5 U/h until blood glucose was stable between 5–6 mmol/l, via a cannula sited in an antecubital vein of the nondominant arm. If necessary, 20% dextrose was given into the same vein via a continuous-flow peristaltic pump (IVAC 591, IVAC, San Diego, CA). Infusion rates were adjusted at 5–10 min intervals according to blood glucose measurements obtained from a retrograde cannula inserted in a dorsal hand vein of the same arm, with the hand kept in a heated box at 55°C. Blood glucose was measured by a glucose oxidase method (Yellow Springs Instrument, Yellow Springs, OH).

A hyperinsulinemic clamp was started at 1:00 P.M. (insulin infusion rate of 60 mU $\cdot m^{-2} \cdot min^{-1}$), the rate of dextrose infusion varying according to glucose measurements made every 5 min. After a 30-min equilibration period, blood glucose was clamped at either 5.0 or 2.8 mmol/l for 2 h. After stopping insulin, patients were given 20 g of intravenous dextrose and a carbohydrate-rich meal.

After discharge, patients restarted their normal subcutaneous insulin regimen with their evening meal and continued to avoid biochemical or symptomatic hypoglycemia until being readmitted the next evening at 9:30 P.M. A cannula was again sited in an antecubital vein of the nondominant arm, and insulin infused overnight to maintain blood glucose between 5 and 10 mmol/l. Patients were fasted from midnight.

At 7:00 A.M. a retrograde cannula was inserted into a dorsal hand vein of the nondominant arm, the hand was warmed as before, and a stepped hyperinsulinemic clamp was then started (insulin rate, 60 mU $\cdot m^{-2} \cdot min^{-1}$). The dextrose infusion rate was adjusted every 5 min as before. Blood glucose was held at 5 mmol/l until time 40 min, then lowered in 40-min steps to 4.0, 3.5, 3.0, and 2.5 mmol/l. At each step glucose fell for the first 20 min and was then clamped at target for 20 min, except for the 2.5 mmol/l plateau, which lasted for 40 min. Plasma glucose was then increased to 5 mmol/l over 20 min and maintained at that level for the final 20 min of each study. During the course of the morning, the following measurements were made at 10–20 min intervals.

Physiological measurements. Blood pressure and pulse were assessed by means of an Accutorr sphygmomanometer (Datascope Medical, Huntingdon, U.K.), employing an automated oscillatory analysis method with the cuff sited on the dominant arm.

Rates of sweating were measured using a 25-cm^2 ventilated chamber placed on the lower sternum (16). Calculation of the rate of water loss was based on measurements of humidity and ambient temperature as previously described (19).

Finger tremor was measured using a ring accelerometer placed on the index finger of the nondominant hand with the hand held outstretched and the forearm supported as previously described (16).

Symptom scores. Symptom scores were obtained by asking patients to rate a series of individual symptoms from 1 ("not present") to 7 ("very severe") (20,21). Symptoms used were: sweating, tremor, pounding heart, hunger (autonomic), difficulty speaking, confusion, drowsiness, clumsiness, odd behavior (neuroglycopenic), headache, nausea (malaise), and itching (dummy). Patients were also asked whether they felt their blood sugar level was low.

Four-choice reaction time test. Cognitive function was assessed by means of a serial four-choice reaction time test (22), measuring 500 responses for each test. The mean time for correct responses and the number of correct responses were recorded for each test. The measures of mean correct time and accuracy have been shown to change by <1% on repeated measures at euglycemia (23). Patients were trained on at least four occasions on the evening before each stepped clamp and again immediately before the clamp.

Endocrine measurements. Five milliliters of blood were added to a lithium-heparin tube containing 0.1 ml of EGTA-glutathione, and after separation, the plasma was stored at −80°C. Plasma epinephrine and norepinephrine were analyzed by high-performance liquid chromatography with electrochemical detection (24). Coefficients of variation for the assays were 8 and 4%, respectively.

Free plasma insulin was measured each hour during the stepped clamps, using a

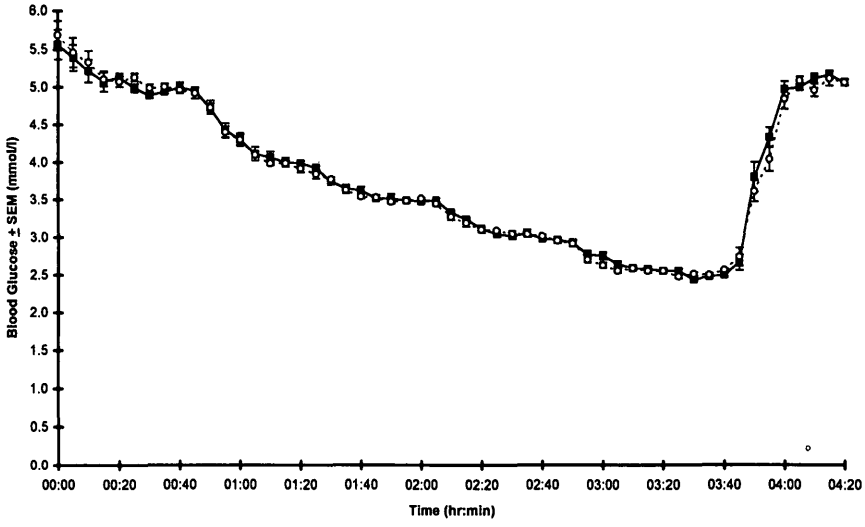

Figure 1—*Glucose values during morning hyperinsulinemic clamps 2 days after antecedent euglycemia (■) and hypoglycemia (□). Bars indicate SE.*

double-antibody radioimmunoassay method (25,26). Two milliliters of blood were added to a lithium-heparin tube and centrifuged immediately at 4°C and 2,400 rpm for 2 min; 0.5 ml of plasma was extracted and added to a refrigerated tube containing 0.5 ml of polyethylene glycol phosphate buffer. The sample was then vortexed and centrifuged at 4°C and 2,400 rpm for a further 30 min, and the supernatant was stored at −80°C until assayed.

Statistical analysis

All data were assessed for normality of distribution. Paired data were then compared by either Student's paired *t* test or Wilcoxon's signed ranks test as appropriate. Repeated data were similarly compared by repeated measures analysis of variance or Freidman's nonparametric repeated measures. The glycemic threshold for a physiological response was defined as the blood glucose value at which a rise in response of ≥2 SDs above baseline was observed on at least two successive measurements. Significant change for any symptom group was taken as a rise in score of ≥2 points above baseline on at least two successive readings. Baseline was taken as the last score at euglycemia. Significant change in performance during the four-choice reaction time test was taken as either a ≥2% increase in error rate or a ≥5% deterioration in mean correct response time. Baseline was again taken as the last stable result at euglycemia.

Results are expressed as means ± SE, unless otherwise indicated.

RESULTS

Glycemic control

Patients' HbA_1 results were similar at the beginning of each study week: 10.65 ± 0.39% for the control group vs. 10.30 ± 0.47% for the intervention group, $P = 0.10$ (laboratory normal range, 4.5–8.5%). There was no visit effect: 10.66 ± 0.42% for study week 1 vs. 10.29 ± 0.45% for study week 2, $P = 0.63$. No patients reported symptomatic or biochemical hypoglycemia in the week preceding each set of studies. All 3:00 A.M. home blood glucose readings were ≥7 mmol/l.

Blood glucose

Afternoon studies. Glucose values over the 2-h plateau periods were 4.96 ± 0.01 and 2.82 ± 0.01 mmol/l.

Morning stepped clamp studies. Means for glucose values against time were similar in the two study weeks (Fig. 1): $P = 0.13$. Values for the stepped plateaus were (control week first): 4.95 ± 0.05 vs. 5.01 ± 0.05 mmol/l; 4.04 ± 0.07 vs. 3.99 ± 0.04 mmol/l; 3.50 ± 0.04 vs. 3.50 ± 0.03 mmol/l; 3.02 ± 0.04 vs. 3.04 ± 0.03 mmol/l; 2.49 ± 0.03 vs. 2.50 ± 0.02 mmol/l.

Free insulin concentrations

Afternoon studies. Values during the 2-h afternoon plateaus were (control week first): $t = 0$ h, 81.2 ± 21.5 vs. 84.5 ± 18.1 mU/l; $t = 1$ h, 85.8 ± 19.5 vs. 67.9 ± 18.4 mU/l; $t = 2$ h, 77.2 ± 18.9 vs. 67.8 ± 15.8 mU/l ($P = 0.14$).

Morning stepped clamp studies. Values measured at hourly intervals were comparable in the two study weeks; these were (control week first): $t = 1$ h, 59.9 ± 11.1 vs. 75.5 ± 19.3 mU/l; $t = 2$ h, 61.3 ± 13.0 vs. 73.6 ± 20.9 mU/l; $t = 3$ h, 59.1 ± 15.6 vs. 69.5 ± 18.9 mU/l; $t = 4$ h, 53.3 ± 11.2 vs. 66.1 ± 15.7 mU/l ($P = 0.54$).

Catecholamines

Epinephrine and norepinephrine concentrations increased during morning hypoglycemia in each study period ($P < 0.0001$ for all responses) (Figs. 2 and 3). Peak and area under the curve (AUC) epinephrine responses to morning hypoglycemia were similar during the two studies (Tables 2

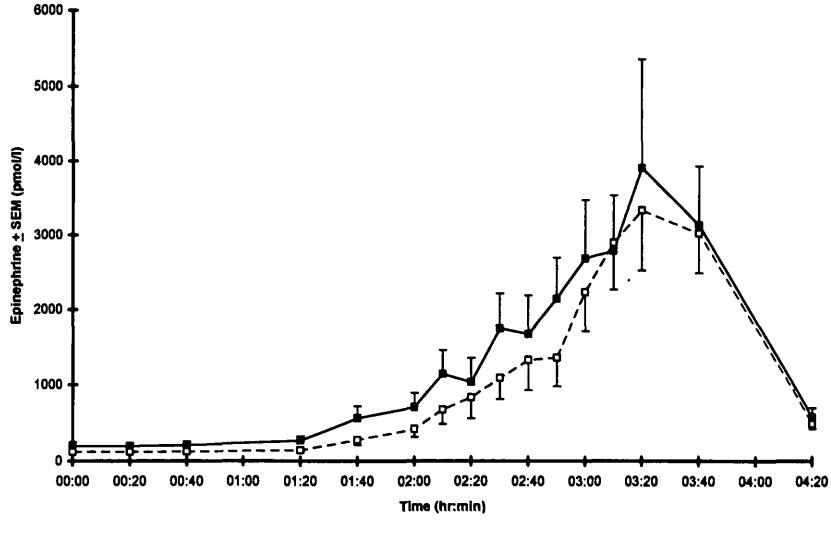

Figure 2—*Epinephrine responses during morning hyperinsulinemic clamps 2 days after antecedent euglycemia (■) and hypoglycemia (□). Bars indicate SE.*

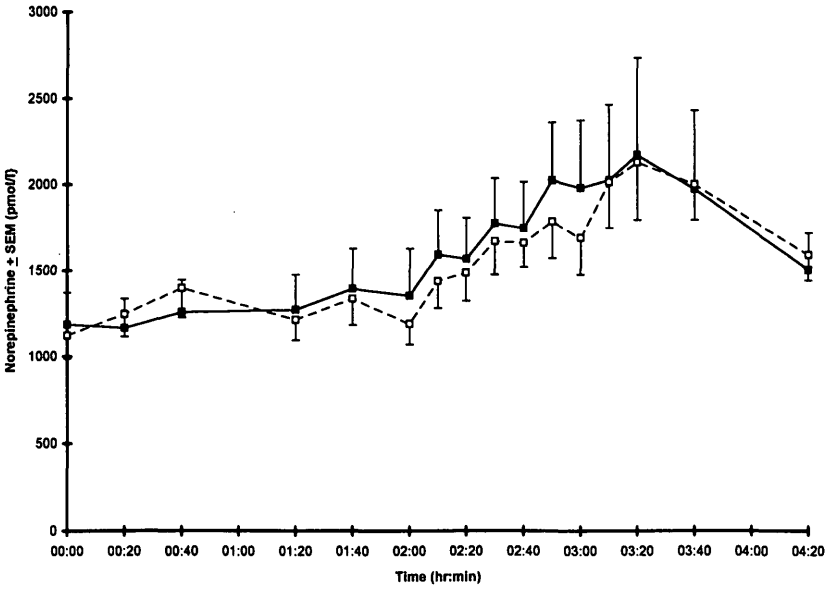

Figure 3—*Norepinephrine responses during morning hyperinsulinemic clamps 2 days after antecedent euglycemia (■) and hypoglycemia (□). Bars indicate SE.*

and 3), as were glycemic thresholds (Table 4). However, the norepinephrine response was reduced after hypoglycemia (Table 2) and started at a lower blood glucose concentration (Table 4), although the AUC response was unchanged (Table 3).

Physiological responses

Systolic and diastolic blood pressures did not change during morning hypoglycemia in either the control or intervention study (data not shown). Heart rate rose significantly during morning hypoglycemia in the control study only ($P < 0.05$; data not shown).

Rates of water loss increased during hypoglycemia in both the control ($P < 0.0001$) and intervention studies ($P < 0.0005$), the responses beginning at similar blood glucose concentrations (Table 4). Peak and AUC responses were also similar (Tables 2 and 3). The finger tremor response showed a similar pattern, with a significant rise occurring during each morning study ($P < 0.0001$), but with no differences observed in the peak or AUC response or in the threshold for response during the two morning studies (Tables 2–4).

Symptom scores

The glucose level at which patients first reported feeling hypoglycemic was 3.2 ± 0.2 mmol/l after euglycemia and 3.0 ± 0.2 mmol/l after hypoglycemia ($P = 0.25$). Total symptoms rose during hypoglycemia in each study ($P < 0.0001$), with similar thresholds (Table 4) and peak responses (Table 2). The observed rise in autonomic symptoms during hypoglycemia ($P < 0.0001$) in each study occurred at similar blood glucose concentrations (Table 4) and were of similar magnitude (Table 2). The rise in neuroglycopenic symptoms was also similar ($P < 0.01$ after euglycemia; $P < 0.0001$ after hypoglycemia).

Cognitive function

Cognitive function deteriorated at similar blood glucose concentrations in the two morning studies: 2.9 ± 0.2 mmol/l for the control group and 3.0 ± 0.2 mmol/l for the intervention group ($P = 0.69$).

CONCLUSIONS — In this study, we explored the duration of the effect of short-term antecedent hypoglycemia in reducing the physiological response to subsequent episodes in patients with IDDM. When we measured these responses 2 days after a period of clamped hypoglycemia, we found that apart from a slight reduction in the rise in norepinephrine, there was no reduction in the peripheral physiological response, and that symptom scores and cognitive function were unaffected. The glycemic thresholds of the activation of these responses were no different from those measured after the euglycemic control arm. Thus, in this group of diabetic patients, the effect of antecedent hypoglycemia was barely apparent almost 2 days later, in contrast to the prolonged effect that has been reported in nondiabetic subjects (16).

A number of studies have shown that single or repeated episodes of clamped hypoglycemia cause significant reductions in the endocrine and symptomatic response to a subsequent period of hypoglycemia. These responses have generally been measured on the day after the last period of hypoglycemia. However, recent work has indicated that this alteration in response may be relatively long-lived in nondiabetic subjects. We have reported that 2 h of hypoglycemia in normal subjects impairs some components of the physiological response to subsequent episodes, including the rise in epinephrine, symptoms, and tremor, which are still present 5 days after the last period of hypoglycemia (16). Robinson et al. (27) have demonstrated reduced physiological responses to hypoglycemia in nondiabetic subjects 6 days after a 2-h period of hypoglycemia. The differences between these data and those of the present study suggest that the effect of single episodes of antecedent hypoglycemia may be greater and more long lasting in nondiabetic subjects.

The previous studies that have examined the effect of antecedent hypoglycemia

Table 2—*Peak response above baseline during hypoglycemia*

	Control study	Intervention study	Mean difference (95% CI)	*P*
Epinephrine (pmol/l)	3,970 ± 1,410	3,480 ± 790	480 (−1,440 to 2,400)	0.84
Norepinephrine (pmol/l)	1,370 ± 360	980 ± 29	390 (100 to 690)	0.04
Water loss ($g \cdot m^{-2} \cdot h^{-1}$)	194 ± 34	179 ± 97	6 (−80 to 111)	0.73
Finger tremor (RMS V)	0.28 ± 0.05	0.37 ± 0.06	−0.08 (−0.20 to 0.04)	0.19
Total symptoms	10.6 ± 2.1	10.8 ± 1.9	−0.1 (−4.8 to 4.5)	0.95
Autonomic symptoms	7.6 ± 1.6	6.3 ± 1.0	1.4 (−1.4 to 4.1)	0.31
Neuroglycopenic symptoms	4.0 ± 0.9	4.4 ± 1.0	−0.4 (−2.4 to 1.6)	0.69

Data are means ± SE, unless otherwise indicated.

Table 3—*Incremental AUC response during hypoglycemia*

	Control study	Intervention study	Mean difference (95% CI)	P
Epinephrine (pmol · h^{-1} · l^{-1})	3,650 ± 1,100	3,010 ± 700	640 (−870 to 2140)	0.35
Norepinephrine (pmol · h^{-1} · l^{-1})	1,180 ± 370	680 ± 270	490 (−90 to 1080)	0.30
Water loss (g/m^2)	114 ± 30	99 ± 38	15 (−83 to 127)	0.75
Finger tremor (RMS V/h)	0.27 ± 0.06	0.34 ± 0.08	−0.06 (−0.23 to 0.10)	0.43

Data are means ± SE, unless otherwise indicated.

in patients with diabetes have also done so on the following day. Dagogo-Jack et al. (7) found that the epinephrine, pancreatic polypeptide, and symptomatic responses were attenuated 18 h after a 2-h period of hypoglycemia. Growth hormone and cortisol responses were unchanged as was the impairment in cognitive function. Lingenfelser et al. (9) reported a reduced epinephrine, cortisol, and symptomatic response on the day after the last of three daily episodes of hypoglycemia induced by intravenous insulin bolus. These findings differ from those of the present study. They demonstrate a marked attenuation in a number of components of the physiological response measured on the day after a hypoglycemic episode, while we found only minimal changes 2 days later. The experimental data, therefore, suggest that for patients with diabetes, the effect of a single hypoglycemic episode in reducing subsequent physiological responses lasts for ~1–2 days.

It is possible that we were unable to detect a real difference between the two arms of study because of the relatively small numbers of subjects. Some components of the physiological response to hypoglycemia, such as epinephrine, norepinephrine, and sweating, tended to be lower after clamped hypoglycemia, although only the norepinephrine rise was significantly different. However, although we cannot rule out a type 2 statistical error, the proportional differences we noted between the two arms of the study are considerably less than those reported in diabetic subjects the day after a previous hypoglycemic episode (7). Furthermore, other components of the physiologicial response, such as the rise in tremor and total symptom scores, were actually greater after hypoglycemia.

One reason why antecedent hypoglycemia had less effect on diabetic subjects could be an impaired physiological response to hypoglycemia before the start of the experiment. If patients started the study with a reduced physiological response to hypoglycemia, then the effect of a period of hypoglycemia might be less apparent. To try and ensure that patients had intact physiological responses to hypoglycemia initially, we recruited patients with moderate glycemic control and a relatively short duration of diabetes and excluded those with hypoglycemia unawareness. We did not measure the initial physiological response to hypoglycemia, but our study design included a control arm (i.e., after 2 h of euglycemia), which has previously been shown to leave the physiological response unchanged (5).

In the present study, the mean epinephrine response at a blood glucose of 2.5 mmol/l, measured 2 days after the euglycemic control, was 3,900 pmol/l. This compares with a mean epinephrine response during the control arm of 3,000 pmol/l in the study of Dagogo-Jack et al. (7) at approximately equivalent levels of hypoglycemia. The values of epinephrine we observed are also greater than the peak epinephrine concentrations of 3,000 pmol/l at a blood glucose of 1.7 mmol/l reported during the control arm of the study by Lingenfelser et al. (9). Allowing for some variation due to differences in the epinephrine assay, this is convincing evidence that the subjects in the present study did not have an initially impaired counterregulatory response.

An alternative explanation is that, since patients were allowed to return home between the initial glucose clamp and the slow-fall clamp 2 days later, spontaneous hypoglycemic episodes experienced at home may have led to an equally impaired physiological response in both arms. However, patients were instructed to maintain blood glucose at high levels with regular monitoring, including measurements during the night. Furthermore, our finding of reasonably brisk rises in epinephrine in both arms suggests that intercurrent hypoglycemia and a subsequently attenuated response did not influence the data.

It appears that prolonged or repeated hypoglycemia may induce an adaptive increase in glucose transporter numbers (28–30). It then follows that during a subsequent episode of hypoglycemia, cerebral glucose uptake is maintained, which provides a lesser stimulus to the autonomic response. There is as yet no direct evidence that the short-lived periods of hypoglycemia induced in the present study produce the same pathophysiological effect as hypoglycemia of longer duration. Nevertheless, if such a mechanism is responsible, it is conceivable that the duration of any adaptation could be different in diabetic subjects with previous exposure to hypoglycemia. Nondiabetic subjects with no prior experience of hypoglycemia might display a response of greater duration. Patients with a longer duration of diabetes may also respond differently to antecedent hypoglycemia.

Table 4—*Glycemic threshold for response to hypoglycemia (mmol^{-1} glucose)*

	Control study	Intervention study	Mean difference (95% CI)	P
Epinephrine	3.8 ± 0.1	3.6 ± 0.1	0.2 (−0.1 to 0.4)	0.38
Norepinephrine	3.4 ± 0.1	2.9 ± 0.1	0.5 (0.2 to 0.8)	<0.01
Water loss	2.9 ± 0.2	2.9 ± 0.2	0.0 (−0.7 to 0.7)	0.90
Finger tremor	3.2 ± 0.2	3.1 ± 0.2	0.1 (−0.4 to 0.6)	0.70
Total symptoms	3.3 ± 0.2	3.6 ± 0.1	−0.3 (−0.7 to 0.1)	0.15
Autonomic symptoms	3.1 ± 0.2	3.1 ± 0.1	0.1 (−0.4 to 0.5)	0.74
Neuroglycopenic symptoms	3.0 ± 0.2	3.0 ± 0.2	0.0 (−0.6 to 0.6)	0.94
Reaction time	2.9 ± 0.2	3.0 ± 0.2	−0.1 (−0.5 to 0.3)	0.69
Feeling hypoglycemic	3.2 ± 0.2	3.0 ± 0.2	0.2 (−0.3 to 0.8)	0.25

Data are means ± SE, unless otherwise indicated.

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