Effects of Autonomic Neuropathy on Counterregulation and Awareness of Hypoglycemia in Type 1 Diabetic Patients

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OBJECTIVE — The recent EURODIAB Study has identified autonomic neuropathy as an independent risk factor for severe hypoglycemia in patients with type 1 diabetes. We tested the hypothesis that counterregulatory catecholamine responses and awareness of hypoglycemia are impaired to a greater extent in type 1 diabetic patients with autonomic neuropathy (AN^+) than in those without autonomic neuropathy (AN^-).

RESEARCH DESIGN AND METHODS — We studied 22 type 1 diabetic patients (8 AN^+, 14 AN^-) matched for age, duration of diabetes, glycemic control, and history of hypoglycemic episodes. We also studied 33 nondiabetic control subjects using the stepped hypoglycemic clamp technique and determined glycemic thresholds and magnitudes of counterregulatory hormone responses and of hypoglycemia symptoms.

RESULTS — Both groups of diabetic patients had reduced awareness of hypoglycemia as evidenced by an elevated glycemic threshold for autonomic symptoms ≥2 SD above normal but neither the magnitude nor thresholds for symptoms differed in AN^+ patients and AN^- patients. Both groups also had impaired glucagon, epinephrine, norepinephrine, growth hormone and cortisol responses to hypoglycemia. However, in AN^+ patients compared with AN^- patients, magnitudes of epinephrine and norepinephrine responses (194 ± 49 vs. 784 ± 206 pmol/l, $P < 0.007$, and 316 ± 56 vs. 610 ± 87 pmol/l, $P < 0.02$, respectively) and epinephrine and norepinephrine glycemic thresholds (2.33 ± 0.10 vs. 2.82 ± 0.10 mmol/l, $P < 0.009$ and 2.34 ± 0.06 vs. 2.79 ± 0.10 mmol/l, $P < 0.008$, respectively) were impaired to a greater extent. This was associated with a 50% greater requirement of exogenous glucose to prevent more severe hypoglycemia during the 2.3 mmol/l glycemic plateau ($P < 0.002$). No differences were observed between other counterregulatory hormone responses in AN^+ and AN^- patients.

CONCLUSIONS — We conclude that in patients with type 1 diabetes, autonomic neuropathy further reduces counterregulatory catecholamine responses. Since this should increase the risk for severe hypoglycemia, one might consider safer therapeutic goals in these patients.

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Abbreviations: AN^+, with autonomic neuropathy; AN^-, without autonomic neuropathy; GH, growth hormone.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Hypoglycemia occurs frequently in patients with type 1 diabetes and is considered a major impediment for achieving optimal glycemic control with intensified insulin regimens (1). Conventional risk factors, such as missed meals, exercise, or alcohol ingestion, explain only a minority of hypoglycemic episodes (2). Consequently, it has been proposed that defective release of counterregulatory hormones may be important (3–10).

Early on, nearly all patients with type 1 diabetes lose their glucagon responses to hypoglycemia (11,12). Epinephrine responses then become critical for prevention of severe hypoglycemia (13). However, many patients with type 1 diabetes eventually develop combined deficiencies of epinephrine and glucagon (12), which is associated with a ~25-fold increased risk for severe hypoglycemia (3). Diminished epinephrine responses superimposed on the lack of a glucagon response increase the risk for severe hypoglycemia, not only by further reducing counterregulation of the actions of insulin but also by reducing awareness of hypoglycemia (7,14), so that affected patients no longer have adequate autonomic warning symptoms that previously prompted them to take preventive action (i.e., to eat before severe hypoglycemia occurred).

Autonomic neuropathy can also reduce epinephrine responses to hypoglycemia (12,15–24). Although there is considerable evidence that the reduced epinephrine response during hypoglycemia in patients with type 1 diabetes is not usually due to classic autonomic neuropathy (6), it is presently unclear whether the presence of autonomic neuropathy has additive deleterious effects (12,16–21,23–27). The recent EURODIAB IDDM Complication Study of over 3,000 patients identified autonomic neuropathy as an independent risk factor for severe hypoglycemia (28). Nevertheless, several studies have failed to demonstrate an adverse effect of autonomic neuropathy on glucose counterregulation in patients with type 1 diabetes (25–27). To

a certain extent, this could be due to the fact that patient groups were often not matched for confounding variables, such as age, duration of diabetes, and glycemic control, and that different diagnostic criteria were used for the diagnosis of autonomic neuropathy. Moreover, the experimental designs have varied in their sensitivity to detect differences in glucose counterregulation (e.g., use of insulin tolerance tests versus standardized stepped hypoglycemic clamps).

The present study was therefore undertaken to test the hypothesis that autonomic neuropathy further impairs glucose counterregulation and awareness of hypoglycemia in patients with long-standing type 1 diabetes likely to have diminished glucagon and epinephrine responses to hypoglycemia. For this purpose, we used the stepwise hypoglycemic clamp technique to determine the glycemic threshold and the magnitude of hormonal and symptomatic responses to hypoglycemia in type 1 diabetic patients with autonomic neuropathy (AN^+) and without autonomic neuropathy (AN^-), who had a history of frequent episodes of severe hypoglycemia and who were well matched for age, duration of diabetes, glycemic control, and frequency of hypoglycemic episodes. Age-, sex-, and BMI-matched healthy volunteers served as control subjects.

RESEARCH DESIGN AND METHODS

Subjects

A total of 22 patients with type 1 diabetes who had documented frequent episodes of severe hypoglycemia while being treated with an intensified insulin regimen were selected for the study; 8 patients had symptomatic autonomic neuropathy (AN^+) and 14 patients had no signs or symptoms of autonomic neuropathy (AN^-). The diagnosis of autonomic neuropathy was established if, in addition to symptoms (i.e., diarrhea, sweating abnormalities, impotence), there were at least two pathologic findings of a standardized battery of cardiovascular autonomic function tests (heart rate variability during normal and deep breathing, heart rate response and blood pressure response to standing up, and Valsava maneuver) (29). Of the patients, seven AN^+ and three AN^- patients also had peripheral neuropathy determined by conduction velocity, vibration perception threshold, and temperature discrimination (30–32). Although all patients had a history of frequent episodes of severe hypoglycemia as defined by the Diabetes Control and Complications Trial (DCCT) (33), none had hypoglycemic episodes (plasma glucose <3.3 mmol/l) for at least 3 days before the study. All patients had a history of diminished awareness of hypoglycemia (i.e., episodes of severe hypoglycemia unaccompanied by classic autonomic warning symptoms) and had a normal glomerular filtration rate determined by a 24-h urine collection. In addition, 33 nondiabetic control subjects were studied. Data of 10 of the 33 control subjects have been reported in a previous publication (34). The subjects' characteristics are give in Table 1. All participants gave informed written consent after the protocol had been approved by the local ethical committee.

Table 1—*Characteristics of subjects studied*

	Diabetic subjects		
	AN^+	AN^-	Control subjects
Sex (M/F)	4/4	8/6	19/14
Age (years)	34.5 ± 1.9	36.4 ± 1.6	32.4 ± 1.34
BMI (kg/m^2)	20.2 ± 0.66	25.1 ± 0.6	25.1 ± 0.7
Duration of diabetes (years)	22.6 ± 1.4	20.8 ± 1.3	—
HbA_{1c} (%)	7.7 ± 0.3	7.4 ± 0.3	5.5 ± 0.15
Severe hypoglycemia (episodes/year)	16.4 ± 4.4	14.2 ± 3.2	—

Data are means ± SEM.

Study design

Thresholds and magnitudes of counterregulatory hormone and symptomatic responses were quantified using the stepwise hypoglycemic clamp technique (34). In brief, all subjects were admitted the day before the experiment. Patients with type 1 diabetes were withdrawn from intermediate and long-acting insulin for 24 h and were managed by subcutaneous injections of regular insulin at each meal. The night before the study, patients were rendered euglycemic by an intravenous infusion of insulin. All subjects were given a standard dinner between 5:30 and 6:30 P.M. (30 kcal/kg, 50% carbohydrates, 35% fat, and 15% protein) and a standard snack (~4 h later) at bedtime (10 kcal/kg, 50% carbohydrates, 35% fat, and 15% protein). Between 7:00 and 7:30 A.M., a dorsal hand vein was cannulated retrogradely and maintained in a thermoregulated Plexiglas box (70°C) for sampling of arterialized venous blood. A deep antecubital vein was cannulated for infusion of insulin. After a 60-min equilibration period, a continuous infusion of insulin was begun (1 mU · kg^{-1} · min^{-1} for 270 min, followed by 2 mU · kg^{-1} · min^{-1} for an additional 60 min). In patients with type 1 diabetes and in the hypoglycemic experiment of control subjects, plasma glucose was clamped at sequential target glucose concentrations of 4.3, 3.7, 3.0, and 2.3 mmol/l using the glucose clamp technique (35) with plasma glucose measured every 5 min. The plasma glucose concentration was allowed to decrease ~0.7 mmol/l over 45 min, and a plateau was maintained for 45 min before the next decrease. In normal subjects on another occasion, the plasma glucose concentration was maintained between 5.0 and 5.6 mmol/l throughout the experiment to obtain control data. Arterialized venous blood samples were drawn every 30 min from −60 min to 360 min for determinations of plasma insulin, glucagon, epinephrine, norepinephrine, growth hormone, and cortisol.

A semiquantitative symptom questionnaire was administered every 15 min. Subjects scored from 0 (none) to 5 (severe) on each of the following symptoms: tremor, palpitation, anxiety, sweating, hunger, tingling, difficulty thinking, blurred vision, dizziness, drowsiness, faintness. Consistent with the categorization used by other investigators (36,37), the first six symptoms were considered autonomic and the other five were considered neuroglycopenic. The sum of each of these constituted the symptom score.

Analytic methods

Plasma glucose was immediately measured using a Beckman glucose analyzer (Fullerton, CA). Plasma free insulin (I^{125} RIA Kit, Incstar, Stillwater, MN, or Human Insulin Specific RIA Kit, Linco Research, St. Charles, MO) and glucagon (Glucagon-Kit Code 10904, Serono Diagnostics, Freiburg, Germany or I^{125} RIA Kit, Diagnostic Prod-

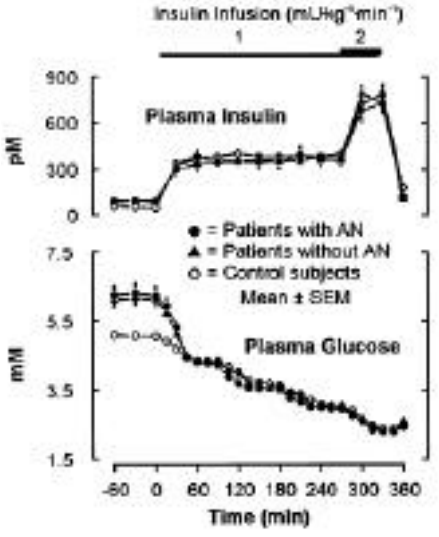

Figure 1—*Plasma insulin and glucose concentrations during stepped hyperinsulinemic-hypoglycemic clamp experiments.*

uct Corporation. Los Angeles, CA) were determined by commercially available radioimmunoassays. Plasma epinephrine and norepinephrine were measured by high-performance liquid chromatography with electrochemical detection (Chromsystems, Munich, Germany) or by a radioenzymatic method (Cat-A-Kit, Amersham, Buckinghamshire, U.K.). Cortisol was determined by fluorescence polarization immunoassay (TDx^R Cortisol, Abbot Diagnostika, Wiesbaden, Germany). Serum growth hormone (GH) was measured by an immunoradiometric assay (HGH MAIA-clone, Serono Diagnostics, Freiburg, Germany or HGH 100T Kit, Nichols Institute, San Juan Capistrano, CA).

Statistical methods

The glycemic threshold in a subject for a given parameter was considered as the plasma glucose concentration at which the given parameter exceeded the 95% CI observed for that parameter at the corresponding time point in euglycemic control experiments after the adjustment of euglycemic and hypoglycemic baseline data to zero (34). If a glycemic threshold for a parameter in a subject could not be determined, as was the case for glucagon and symptoms of hypoglycemia in several subjects, the lowest plasma glucose concentration during the hypoglycemic clamp experiment was used as the glycemic threshold for statistical purposes. Means of increments above baseline of hormonal concentrations and symptom scores from 330 to 360 min were used for determination of the magnitude of responses. In patients with type 1 diabetes, a glycemic threshold below the 95% CI of the nondiabetic control group was considered as elevated (i.e., greater hypoglycemia required). One-way analysis of variance (ANOVA) was used to analyze differences between nondiabetic control subjects and type 1 diabetic patients with and without autonomic neuropathy. Subsequently, the Mann Whitney *U* test was used where appropriate. A P value <0.05 was considered statistically significant. Data are given as mean ± SEM.

RESULTS

Plasma glucose and insulin concentrations

During the stepwise hypoglycemic clamp test, plasma glucose and insulin concentrations were similar in all groups (Fig. 1).

Glucose counterregulatory hormones

Both AN$^+$ and AN$^-$ patients had significantly increased thresholds for all counterregulatory hormone responses (glucagon, epinephrine, norepinephrine, growth hormone, and cortisol) and reduced magnitudes of glucagon, epinephrine, norepinephrine, and cortisol compared with control subjects (Table 2, Fig. 2–4). The thresholds for epinephrine and norepinephrine responses were significantly greater in AN$^+$ patients than in AN$^-$ patients (2.33 ± 0.10 vs. 2.82 ± 0.10 mmol/l, $P < 0.009$ and 2.34 ± 0.06 vs. 2.79 ± 0.10 mmol/l, $P < 0.008$, respectively). Moreover, in AN$^+$ patients the magnitudes of epinephrine and norepinephrine responses were decreased by 75 and 50%, respectively, compared with AN$^-$ patients (194 ± 49 vs. 784 ± 206 pmol/l, $P < 0.007$, and 316 ± 56 vs. 610 ± 87 pmol/l, $P < 0.02$, respectively). Thresholds and magnitudes for glucagon, growth hormone, and cortisol responses of the two diabetic groups were not significantly different.

Both the glycemic thresholds and the magnitudes of epinephrine responses were negatively correlated with exogenous glucose infusion rates required to maintain the last hypoglycemic plateau at 2.3 mmol/l when results of all subjects were assessed ($r = -0.801$, $P < 0.001$; $r = -0.666$, $P < 0.001$, respectively) and when results of only the diabetic subjects were assessed ($r = -0.568$, $P < 0.01$; $r = -0.488$, $P < 0.03$, respectively). Thus during the last hypoglycemic plateau, AN$^+$ patients required nearly 50% more glucose than AN$^-$ patients (6.1 ± 0.3 vs. 4.2 ± 0.3 μmol · kg^{-1} · min^{-1}, $P < 0.002$), and AN$^+$ and AN$^-$ patients required ~3.5 and 2.5 times the exogenous glucose infusion of nondiabetic control subjects (1.7 ± 0.1 μmol · kg^{-1} · min^{-1}, $P < 0.001$).

Table 2—*Magnitudes and glycemic thresholds of counterregulatory hormones during stepped hyperinsulinemic-hypoglycemic clamp experiments in type 1 diabetic patients*

Hormones	Patients with type 1 diabetes		Control subjects
	AN$^+$	AN$^-$	
n	8	14	33
Glucagon			
Magnitude (pg/ml)	−62 ± 15*	−33 ± 6*	179 ± 22
Glycemic threshold (mmol/l)	2.23 ± 0.02*	2.25 ± 0.02*	3.75 ± 0.09
Epinephrine			
Magnitude (pmol/l)	194 ± 49*†	784 ± 206*	4,329 ± 409
Glycemic threshold (mmol/l)	2.33 ± 0.10*†	2.82 ± 0.10*	3.77 ± 0.05
Norepinephrine			
Magnitude (pmol/l)	316 ± 56*‡	610 ± 87§	1,397 ± 158
Glycemic threshold (mmol/l)	2.34 ± 0.06*†	2.79 ± 0.10*	3.55 ± 0.09
Cortisol			
Magnitude (nmol/l)	22 ± 47*	122 ± 59$	311 ± 36
Glycemic threshold (mmol/l)	2.50 ± 0.10*	2.47 ± 0.10*	3.30 ± 0.06
Growth hormone			
Magnitude (ng/ml)	20.7 ± 5.8	20.6 ± 3.2	20.8 ± 2.1
Glycemic threshold (mmol/l)	2.75 ± 0.13*	2.99 ± 0.15*	3.71 ± 0.07

Data are means ± SEM. *$P < 0.001$ vs. control subjects; †$P < 0.01$ vs. AN$^-$, ‡$P < 0.02$ vs. AN$^-$, §$P < 0.003$ vs. control subjects.

Symptom scores

Both groups of diabetic patients had comparably reduced autonomic and neuroglycopenic symptom scores (Table 3). Because of diminished symptoms, glycemic thresholds for autonomic symptoms could be compared in only 4 of 8 AN^+ patients (2.33 ± 0.05 mmol/l) and in 5 of 14 AN^- patients (2.45 ± 0.06 mmol/l, $P = 0.263$). Overall, autonomic symptoms were significantly correlated with the magnitude of both plasma epinephrine ($r = 0.516$, $P < 0.001$) and norepinephrine ($r = 0.374$, $P < 0.005$) responses. However, among the diabetic subjects, there was no correlation between autonomic symptoms and the magnitude of either epinephrine ($r = -0.024$, $P > 0.9$) or norepinephrine ($r = -0.377$, $P > 0.08$) responses.

CONCLUSIONS — The present study confirms that counterregulatory hormone responses and awareness of hypoglycemia are reduced in patients with long-standing type 1 diabetes, even in the absence of detectable autonomic neuropathy (6). Moreover, the data provide evidence that autonomic neuropathy selectively worsens plasma epinephrine and norepinephrine responses to hypoglycemia without apparently affecting awareness of hypoglycemia. Accordingly, we found that compared with type 1 diabetic AN^- patients carefully matched for age, duration of diabetes, glycemic control, or antecedent hypoglycemic episodes, AN^+ patients had lower plasma epinephrine and norepinephrine responses but similar cortisol and growth hormone responses and similar autonomic and neuroglycopenic symptoms. Our observations are consistent with most prior studies (12,16–21,23,24). Although these studies provide evidence that autonomic neuropathy might have deleterious effects on counterregulatory plasma catecholamine responses, in most of these (12,16–21), standardized hypoglycemia was not used to ensure that the hypoglycemic stimulus for catecholamine responses were uniform. Moreover in these studies, subjects were not matched for diabetes duration or glycemic control or no such information was given, factors now known to influence catecholamine responses and awareness of hypoglycemia (1,8). Thus the results of these studies cannot be considered conclusive.

To our knowledge, only three prior studies have examined the influence of autonomic neuropathy on catecholamine

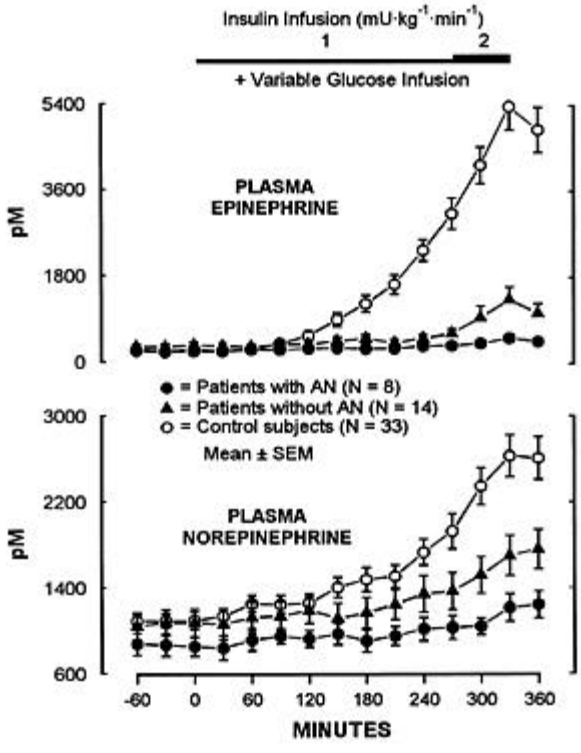

Figure 2—*Plasma epinephrine and norepinephrine concentrations during stepped hyperinsulinemic-hypoglycemic clamp experiments.*

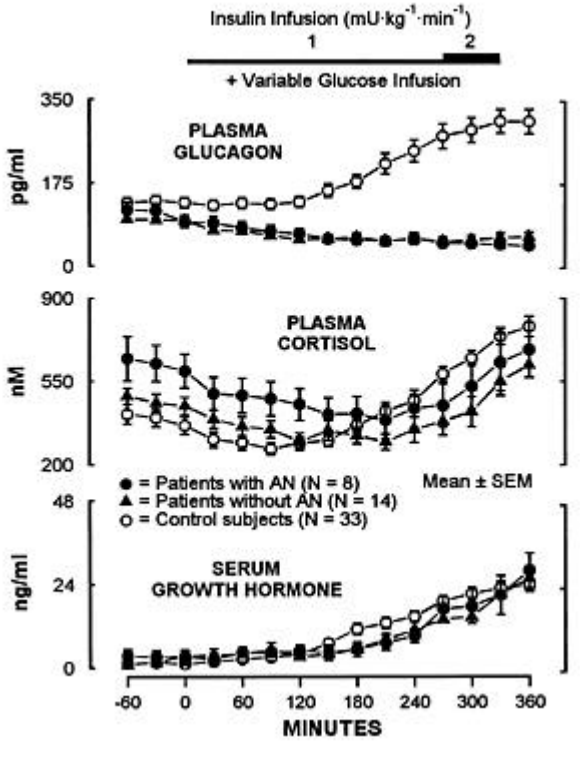

Figure 3—*Plasma glucagon, cortisol, and growth hormone concentrations during stepped hyperinsulinemic-hypoglycemic clamp experiments.*

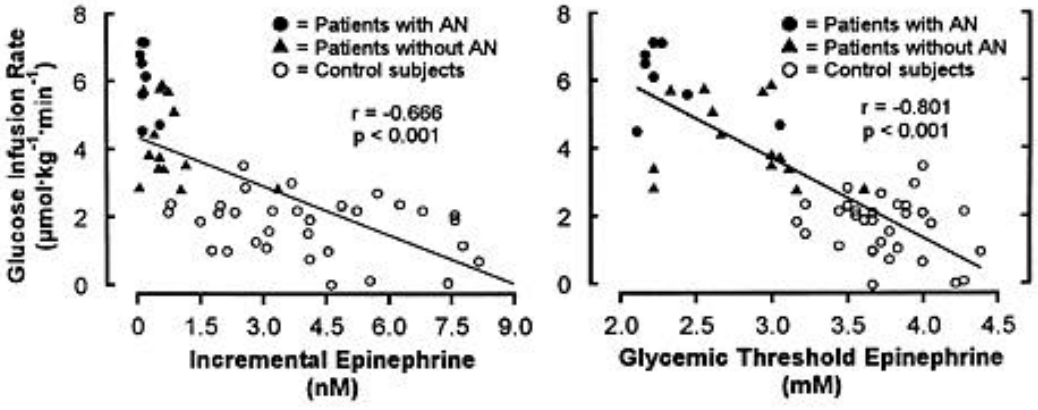

Figure 4—*Correlation between increments and glycemic thresholds of epinephrine and exogenous glucose requirements during the last hypoglycemic plateau (2.3 mmol/l) of stepped hyperinsulinemic-hypoglycemic clamp experiments.*

responses and awareness of hypoglycemia in patients with type 1 diabetes, using the standardized stepwise hypoglycemia clamp technique. Of these, two studies of Bolli and colleagues (23,24) found that autonomic neuropathy further impaired plasma epinephrine responses and further diminished autonomic symptoms during hypoglycemia. In contrast, Dagogo-Jack et al. (27) did not find a significant difference in epinephrine responses between AN^+ and AN^- patients. However, it is of note that in this study during the glycemic plateau of greatest hypoglycemia, AN^+ patients had plasma epinephrine responses that were one-third less than those in AN^- patients. Moreover in AN^+ patients, plasma free fatty acid responses, which are thought to primarily reflect catecholamine responses (39), were two-thirds less than those in AN^- patients, and AN^+ patients had lower autonomic symptom scores than AN^- patients. Thus, it is conceivable that either the small number of subjects studied or the statistical method used to evaluate the data resulted in insufficient power to detect a difference in epinephrine responses.

In the present study, we found no difference in autonomic symptom scores in contrast with Dagogo-Jack et al. (27), Bottini et al. (23), and Fanelli et al. (24); all of whom found that patients with autonomic neuropathy had reduced autonomic symptoms. It should be pointed out, however, that our patients were matched for frequency of hypoglycemia and had been free of hypoglycemia for only 3 days before the study and that Fanelli et al. (24) also found no difference in autonomic symptoms when subjects were studied before long-term avoidance of hypoglycemia. Furthermore in our patients, an appreciable number had few symptoms at the lowest plasma glucose level studied. It is therefore possible that recent hypoglycemia in our subjects and in those of Fanelli et al. (24) without autonomic neuropathy may have obscured detection of a difference in symptoms of hypoglycemia, and that we might have distinguished between groups had greater hypoglycemia been used and if subjects had not been matched for frequency of antecedent hypoglycemia. Consequently, the results of our study cannot be taken as definite evidence that AN^+ patients do not have diminished autonomic symptoms.

Finally, it is worthy of emphasis that in the present studies, requirements of exogenous glucose to prevent more severe hypoglycemia were nearly 50% greater in AN^+ patients than in AN^- patients and that these glucose requirements were inversely correlated with counterregulatory epinephrine responses. These results indicate that reduction in epinephrine responses had rendered the AN^+ patients more vulnerable to the hypoglycemic actions of insulin and, in agreement with the well-accepted view that epinephrine is the primary counterregulatory hormone once glucagon responses to hypoglycemia are absent (40), should cause such patients to be at increased risk for severe hypoglycemia.

In conclusion, the present study indicates that autonomic neuropathy further impairs counterregulatory plasma catecholamine responses in patients with type 1 diabetes and further reduces their defense against hypoglycemia. More conservative therapeutic goals might therefore be indicated in such affected individuals.

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Table 3—*Magnitudes and glycemic thresholds of autonomic and neuroglycopenic symptoms during stepped hyperinsulinemic-hypoglycemic clamp experiments in AN^+ and AN^- type 1 diabetic patients and in nondiabetic control subjects*

Hormones	Patients with type 1 diabetes		Control subjects
	AN^+	AN^-	
n	8	14	33
Autonomic symptoms			
Magnitude (score)	2.5 ± 0.7*	2.4 ± 0.4*	9.2 ± 0.8
Glycemic threshold (mmol/l)†	2.27 ± 0.03*	2.32 ± 0.04*	3.29 ± 0.06
Neuroglycopenic symptoms			
Magnitude (score)	1.5 ± 0.4*	2.6 ± 0.5*	5.9 ± 0.7
Glycemic threshold (mmol/l)	2.26 ± 0.04*	2.45 ± 0.04*	2.92 ± 0.07

Data are means ± SEM. *$P < 0.001$ vs. control subjects; †since glycemic thresholds could not be determined in 4 of 8 AN^+ and 9 of 14 AN^- subjects, their thresholds were assumed to be at the lowest glucose level achieved.

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