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Research Article

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Hypoglycemia-associated Autonomic Failure in Insulin-dependent Diabetes Mellitus

Recent Antecedent Hypoglycemia Reduces Autonomic Responses to, Symptoms of, and Defense against Subsequent Hypoglycemia

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Abstract

We hypothesize that in patients with insulin-dependent diabetes mellitus (IDDM), recent antecedent iatrogenic hypoglycemia is a major cause of hypoglycemia-associated autonomic failure, a disorder distinct from classical diabetic autonomic neuropathy (CDAN), and that hypoglycemia-associated autonomic failure, by reducing both symptoms of and defense against developing hypoglycemia, results in recurrent iatrogenic hypoglycemia, thus creating a vicious cycle. We used the hyperinsulinemic (12.0 pmol · kg^{-1} · min^{-1}) stepped hypoglycemic clamp technique to assess autonomic and symptomatic responses to hypoglycemia and the insulin infusion test (4.0 pmol · kg^{-1} · min^{-1}) to assess defense against hypoglycemia on mornings before and after clamped afternoon hypoglycemia (~ 2.8 mmol/liter) and hyperglycemia (~ 11.1 mmol/liter) in patients with IDDM. Compared with nondiabetic subjects, IDDM with or without CDAN exhibited reduced epinephrine (P = 0.0222 and 0.0040) and pancreatic polypeptide (P = 0.0083 and 0.0056) responses to hypoglycemia. After afternoon hypoglycemia, lower plasma glucose concentrations were required to elicit autonomic and symptomatic responses during morning hypoglycemic clamps in patients without CDAN. At the 2.8 mmol/liter step, mean (±SE) epinephrine levels were 1,160±270 and 2,040±270 pmol/liter (P = 0.0060), pancreatic polypeptide levels were 14±2 and 49±11 pmol/liter (P = 0.0275), and total symptom scores were 22±3 and 41±7 (P = 0.0475) after afternoon hypoglycemia and hyperglycemia, respectively. During morning insulin infusion tests after afternoon hypoglycemia, nadir plasma glucose concentrations were 2.6±0.2 mmol/liter compared with 3.3±0.3 mmol/liter (P < 0.001) at the corresponding time points after afternoon hyperglycemia. Thus, we conclude: (*a*) elevated glycemic thresholds for autonomic responses to hypoglycemia are a feature of IDDM per se, not classical diabetic autonomic neuropathy; and (*b*) a single episode of afternoon hypoglycemia results in both elevated glycemic thresholds for autonomic and symptomatic responses to hypoglycemia and impaired physiological defense against hypoglycemia the next morning in IDDM. (*J. Clin. Invest.* 1993. 91:819–828.) Key words: hypoglycemia • epinephrine • pancreatic polypeptide • diabetes • autonomic failure

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Introduction

Recurrent iatrogenic hypoglycemia is a major problem for patients with insulin-dependent diabetes mellitus (IDDM)[1] (1, 2). It is now clear, from an analysis of a large experience with severe iatrogenic hypoglycemia in IDDM (3), that conventional risk factors, which are based conceptually on relative or absolute insulin excess alone, account for only a minority of episodes. Thus, risk factors that compromise physiological and behavioral defenses against mild to moderate hyperinsulinemia, which must occur from time to time because of the imperfections of current insulin replacement techniques, must play an important pathogenetic role (1, 4, 5). The latter risk factors include the clinical syndromes of defective glucose counterregulation (6–8), hypoglycemia unawareness (9–14), and elevated glycemic thresholds (lower plasma glucose concentrations required) for symptoms and activation of glucose counterregulatory systems during intensive therapy that effectively lowers overall plasma glucose concentrations (13, 15–17). These syndromes have been reviewed (1, 4, 5). Administration of a β-adrenergic antagonist may also be a risk factor in this category (18).

The prevention or correction of hypoglycemia is normally the result of both dissipation of insulin and activation of glucose counterregulatory systems. Whereas insulin is the dominant glucose-lowering factor, there are redundant glucose-raising (counterregulatory) factors, and there is a hierarchy among the glucoregulatory factors (1, 19). Among the several counterregulatory factors, glucagon and epinephrine (particularly in the absence of glucagon) play key roles. The secretion of glucagon and epinephrine, as well as insulin, is abnormal in IDDM. Absolute insulin deficiency is the proximate cause of IDDM. The hormone must be replaced, currently by subcutaneous injection or infusion, but the appearance of insulin in the circulation is unregulated and does not fall as plasma glucose levels decline. Selectively deficient pancreatic α-cell glucagon responses to decrements in plasma glucose are the rule in established IDDM (20–22). Thus, patients with IDDM, in contrast to nondiabetic persons, are largely dependent upon epinephrine to prevent or correct hypoglycemia (23). Selectively deficient adrenomedullary epinephrine responses to decrements in plasma glucose also develop in the majority of patients with relatively longstanding IDDM (21, 24). This is a critical abnormality in the setting of intermittent hyperinsulinemia and deficient glucagon responses. The superimposition of epinephrine deficiency results in a 25-fold or greater increase in the frequency of severe iatrogenic hypoglycemia, at least dur-

1. *Abbreviations used in this paper:* CDAN, classical diabetic autonomic neuropathy; IDDM, insulin-dependent diabetes mellitus.

ing intensive therapy (6–8). Such patients have defective glucose counterregulation (1, 6). Initially found to play a critical pathogenetic role in the syndrome of defective glucose counterregulation (6), reduced epinephrine responses to a given degree of hypoglycemia also occur in the syndromes of hypoglycemia unawareness (9, 12–14) and elevated glycemic thresholds during effective intensive therapy (13, 15, 16).

Since these three clinical syndromes (defective glucose counterregulation, hypoglycemia unawareness, and elevated glycemic thresholds) segregate together, are associated with a high frequency of severe iatrogenic hypoglycemia, and share several pathophysiological features including reduced autonomic (particularly adrenomedullary but also parasympathetic and possibly sympathetic) responses to a given degree of hypoglycemia, we have suggested that they are examples of hypoglycemia-associated autonomic failure in IDDM, a disorder we distinguish from classical diabetic autonomic neuropathy (5). We emphasized that the pathogenesis of hypoglycemia-associated autonomic failure is not known, need not be the same in all three syndromes, and could be multifactorial in a given syndrome (5).

Based on our finding that a single < 2-h episode of afternoon hypoglycemia results in reduced symptomatic and autonomic responses to hypoglycemia the next morning in nondiabetic humans (25), coupled with conceptually similar data from other laboratories (26, 27), we propose that recent antecedent hypoglycemia is one potential mechanism of hypoglycemia-associated autonomic failure. Our overall hypothesis is that recent antecedent hypoglycemia is a major cause of hypoglycemia-associated autonomic failure in IDDM, and hypoglycemia-associated autonomic failure, by reducing both symptoms of and physiological defense against developing hypoglycemia, results in recurrent severe hypoglycemia thus creating a vicious cycle (5).

The present studies were performed to test two fundamental elements of this hypothesis. We used the hyperinsulinemic (12.0 pmol·kg^{-1}·min^{-1}) stepped hypoglycemic clamp technique (28) to assess autonomic (including adrenomedullary), symptomatic, and cognitive responses to hypoglycemia, and the insulin infusion (4.0 pmol·kg^{-1}·min^{-1}) test (6) to assess physiological defense against developing hypoglycemia on mornings before and after clamped afternoon hypoglycemia on one occasion and clamped afternoon hyperglycemia on another occasion in patients selected for the absence of classical diabetic autonomic neuropathy. Nondiabetic subjects and patients with classical diabetic autonomic neuropathy were also studied, each on a single occasion.

Methods

Subjects. 26 patients with IDDM selected for the absence (n = 15) or for the presence (n = 11) of classical diabetic autonomic neuropathy (CDAN), and 12 nondiabetic subjects consented to participate in these two studies that were approved by the Washington University Human Studies Committee and conducted on the Washington University General Clinical Research Center (GCRC). The clinical characteristics of the patients and their autonomic function test results and symptoms are listed in Table I. The nondiabetic subjects in study 1 included four women and two men with a mean±SD age of 24.2±2.6 yr and a mean body mass index of 24.0±3.0 kg/m^2. The nondiabetic subjects in study 2 included two women and four men with a mean age of 24.7±3.4 yr and a mean body mass index of 25.0±2.0 kg/m^2.

Patients with IDDM without CDAN had normal indices of heart rate variation during deep breathing (29) (Table I) and no clinical manifestations suggestive of autonomic neuropathy. Those with CDAN had both abnormal indices of heart rate variation (Table I) and at least one clinical manifestation of autonomic neuropathy.

Experimental design. The overall experimental design was the same for both studies. Patients with IDDM without CDAN were admitted to the GCRC for 5 d. Their diabetes was managed with intravenous regular insulin (Novolin; Novo-Nordisk, Bagsvaerd, Denmark) throughout with plasma glucose levels held high (11.1–16.7 mmol/liter, 200–300 mg/dl), except during the interventions described below. Mean background plasma glucose concentrations were 13.4±0.8 mmol/liter and 14.3±0.3 mmol/liter in study 1 and study 2, respectively. Hyperinsulinemic (12.0 pmol·kg^{-1}·min^{-1}, 2.0 mU·kg^{-1}·min^{-1}, of regular insulin intravenously) hyperglycemic (~ 11.1 mmol/liter) or hypoglycemic (~ 2.8 mmol/liter) clamps were performed between 1400 h and 1600 h on days 2 and 4 in random sequence. Mean plasma glucose concentrations during the second hour of these afternoon clamps were 11.3±0.4 and 2.7±0.2 mmol/liter in study 1 and 11.2±0.1 and 2.7±0.1 mmol/liter in study 2.

In study 1, hyperinsulinemic (12.0 pmol·kg^{-1}·min^{-1}, 2.0 mU·kg^{-1}·min^{-1}) stepped hypoglycemic clamps (28) were performed starting at ~ 0700 h, on days 3 and 5. In study 2, insulin infusion (4.0 pmol·kg^{-1}·min^{-1}, 0.67 mU·kg^{-1}·min^{-1}) tests (6) were performed starting at ~ 0700 h, on days 3 and 5. In both studies, patients with CDAN and nondiabetic subjects underwent the corresponding intervention (stepped hypoglycemic clamp or insulin infusion test) after an overnight fast on a single occasion. Only the patients were admitted to the GCRC (for maintenance of hyperglycemia with i.v. insulin overnight); nondiabetic subjects were studied as outpatients.

Analytical methods. Plasma glucose was measured with a glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma free insulin (30), C-peptide (30), glucagon (31), growth hormone (32), cortisol (33), and pancreatic polypeptide (34) were measured with RIAs. A single isotope derivative (radioenzymatic) method was used to measure plasma epinephrine and norepinephrine (35). Enzymatic methods were used to measure serum nonesterified fatty acids (NEFA) (36) and blood β-hydroxybutyrate (37), lactate (38), and alanine (39).

Symptoms (28) and cognitive functions (18) were assessed as described previously from our laboratory. Briefly, with respect to the former, subjects were asked to score, from one (absent) to seven (severe) 12 symptoms at the end of each glycemic step. These included five neurogenic (autonomic) symptoms (sweaty, heart pounding, shaky, hungry, and nervous); six neuroglycopenic symptoms (difficulty thinking, tired, dizzy, faint, tingling, and blurred vision); and one nonspecific symptom different in any way. Cognitive function tests, including measures of attention, line orientation, and both immediate and delayed paragraph recall, were performed during the final 15 min of each glycemic step.

Statistical methods. The data from study 1 were analyzed with a general linear models procedure repeated measures analysis of variance. Mean values in each group in study 2 were compared with a t test for paired data. The data are expressed as the mean plus or minus the SE except when the SD is indicated.

Results

Study 1: stepped hypoglycemic clamps

Comparison of IDDM with and without CDAN and nondiabetic subjects. During the stepped hypoglycemic clamps (without antecedent afternoon hypoglycemia), plasma glucose and insulin concentrations were comparable in all three groups (Fig. 1). Compared with those of nondiabetic subjects, plasma glucagon (P = 0.0001 and 0.0001, respectively) and epinephrine (P = 0.0222 and 0.0040, respectively), responses to hypo-

*Table I. Characteristics of Patients with Insulin-dependent Diabetes Mellitus with and without Classical Diabetic Autonomic Neuropathy (CDAN)**

	Study 1		Study 2	
	Without CDAN	With CDAN	Without CDAN	With CDAN
	n = 7	*n* = 6	*n* = 8	*n* = 5
Gender (female/male)	4/3	2/4	5/3	3/2
Age (yr)	29.7±2.3	27.2±2.6	27.6±6.2	26.8±2.2
Body mass index (kg/m^2)	24.2±0.8	23.1±1.7	25.6±3.2	22.7±1.5
Duration of IDDM (yr)	16.9±3.3	15.5±3.4	13.5±6.2	13.8±2.6
Insulin dose (U/d)	46.1±5.7	44.0±6.3	46.3±7.1	51.4±6.7
Glycated hemoglobin (%)‡	9.8±0.9	10.7±0.7	10.3±2.9	12.5±0.8
History of hypoglycemia unawareness	4	0	5	2
RR variation				
Standard deviation score (ms)	96.3±8.7	32.7±5.3	131.3±65.7	25.0±4.5
Expiration-to-inspiration index	1.29±0.04	1.10±0.02	1.44±0.18	1.07±0.02
Mean circular resultant	56.1±7.3	19.5±5.3	61.1±24.9	11.6±3.6
Systolic blood pressure (mmHg)				
Supine	121±4	123±5	117±10	130±4
Standing (5 min)	116±3	109±6	118±8	119±5
Symptoms of CDAN				
Orthostatic hypotension	0	3	0	4
Gastrointestinal	0	2	0	2
Genitourinary (impotence)	0	1	0	1
Abnormal sweating	0	5	0	3

* Mean±SE; ‡ normal < 6.3%.

glycemia were reduced in patients with IDDM with and without CDAN (Fig. 1). There were no significant differences between the glucagon and epinephrine responses of patients with or without CDAN. Plasma pancreatic polypeptide responses were also reduced (P = 0.0083 and 0.0056, respectively) in both IDDM groups, albeit more strikingly in those with CDAN (Fig. 2). Plasma cortisol, growth hormone, and norepinephrine levels were similar in all three groups (Fig. 2). NEFA responses were greater (P = 0.0006) in the patients without CDAN, and appeared to be slightly but not significantly, greater in those with CDAN compared with NEFA responses in nondiabetic subjects (Table II). There were no significant differences in the β-hydroxybutyrate or lactate (Table II) or alanine (data not shown) concentrations. Heart rates and blood pressure did not differ among the groups (data not shown). Patients with IDDM tended to have higher total, neurogenic, and neuroglycopenic symptom scores than nondiabetic subjects (Table III); this was significant for those with

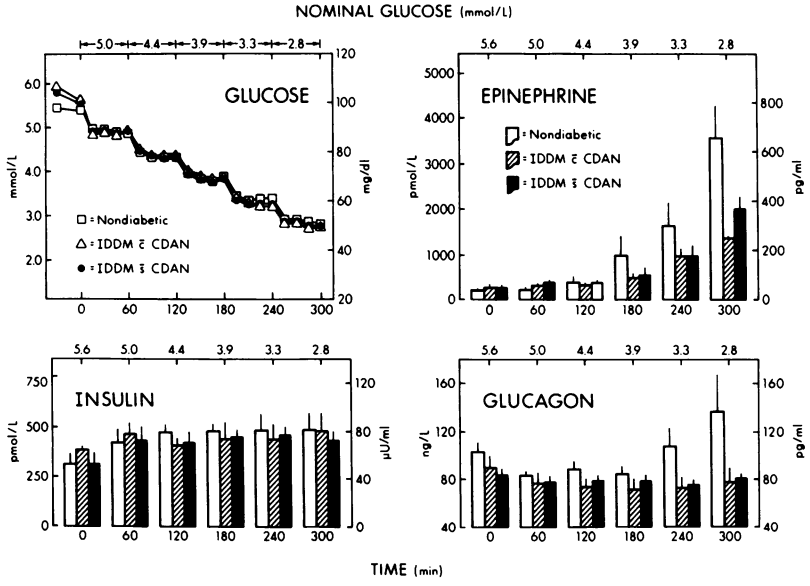

Figure 1. Mean±SE plasma glucose, insulin, epinephrine, and glucagon concentrations during hyperinsulinemic, stepped hypoglycemic glucose clamps in nondiabetic subjects (*open squares and columns*), patients with IDDM with CDAN (*open triangles and cross-hatched columns*), and patients with IDDM without CDAN (*closed circles and columns*). Standard errors not shown lie within the symbols.

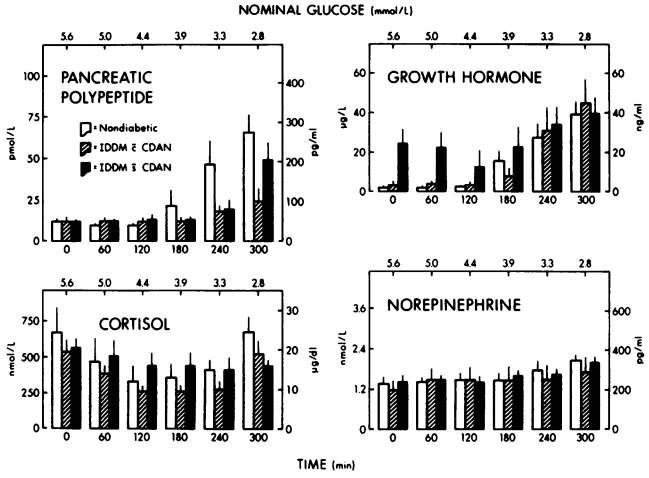

Figure 2. Mean±SE plasma pancreatic polypeptide, cortisol, growth hormone, and norepinephrine concentrations during hyperinsulinemic, stepped hypoglycemic glucose clamps in nondiabetic subjects (*open columns*), patients with IDDM with CDAN (*cross-hatched columns*), and patients with IDDM without CDAN (*closed columns*).

CDAN (P = 0.0115, 0.0188, and 0.0139, respectively). Compared with patients without CDAN, patients with CDAN had lower total (P = 0.0323) and neurogenic (P = 0.0435) symptom scores. Paragraph recall, particularly delayed paragraph recall, deteriorated in both IDDM groups but not the nondiabetic subjects during hypoglycemia (P = 0.0080) (Table IV). There were no significant differences in attention or line orientation errors among the groups.

Effects of antecedent hypoglycemia in IDDM without CDAN. During morning, stepped hypoglycemic clamps after afternoon hyperglycemia or hypoglycemia plasma glucose and insulin concentrations were comparable (Fig. 3). However, plasma epinephrine (P = 0.0060, Fig. 3) and pancreatic polypeptide (P = 0.0275, Fig. 4) responses to morning hypoglycemia were reduced after afternoon hypoglycemia. Plasma cortisol, growth hormone, and norepinephrine levels were unaffected (Fig. 4). NEFA responses were reduced (P = 0.0374), but β-hydroxybutyrate and lactate (Table II) and alanine (data not shown) levels were unaffected, as were heart rates and blood pressures (data not shown). Total (P = 0.0475), neurogenic (P = 0.0061), and neuroglycopenic (P = 0.0218) symptom responses to morning hypoglycemia were also reduced substantially after afternoon hypoglycemia (Fig. 5, Table III). With respect to cognitive function (Table IV), there were more errors on the attention ($P < 0.05$) but not the line orientation test after afternoon hypoglycemia. Memory, particularly delayed paragraph recall, tended to be reduced after afternoon hypoglycemia, but this was not significant statistically.

Study 2: insulin infusion tests

During morning insulin infusion tests after afternoon hypoglycemia, in patients with IDDM without CDAN, the mean±SE nadir plasma glucose concentration was 2.6±0.2 mmol/liter

*Table II. Serum Nonesterified Fatty Acids and Blood β-Hydroxybutyrate, Lactate, and Alanine Concentrations (Study 1)**

		IDDM			
			Without CDAN		
Time	Nondiabetic	With CDAN	After hyperglycemia	After hypoglycemia	Nominal glucose
min					*mmol/liter*
Nonesterified fatty acids (mmol/liter)					
0	219±41	170±19	190±39	202±59	5.6
60	115±17	110±7	172±32	113±18	5.0
120	96±24	115±6	196±32	95±19	4.4
180	85±16	100±10	194±58	133±33	3.9
240	80±12	143±35	278±82	131±45	3.3
300	73±12	143±31	450±150	275±135	2.8
β-Hydroxybutyrate (mmol/liter)					
0	128±17	174±33	222±116	222±61	5.6
60	120±28	116±21	113±20	124±45	5.0
120	80±14	115±18	116±25	126±31	4.4
180	84±24	105±18	105±21	109±16	3.9
240	60±8	133±19	150±42	164±34	3.3
300	140±26	167±33	220±84	154±51	2.8
Lactate (mmol/liter)					
0	1,090±169	862±91	1,160±114	1,120±165	5.6
60	1,370±196	1,020±110	1,040±93	457±63	5.0
120	1,250±75	1,170±89	921±85	736±98	4.4
180	1,340±181	924±91	861±125	671±97	3.9
240	1,380±226	1,160±143	869±80	953±129	3.3
300	1,960±287	1,350±158	1,040±150	956±125	2.8

* Mean±SE.

*Table III. Total Neurogenic and Neuroglycopenic Symptoms Scores (Study 1)**

Time	Nondiabetic	IDDM			Nominal glucose
		With CDAN	Without CDAN		
			After hyperglycemia	After hypoglycemia	
min					*mmol/liter*
Total symptoms scores					
0	13±1	18±1	14±1	13±1	5.6
60	14±1	20±2	16±2	15±1	5.0
120	14±1	20±3	18±2	15±1	4.4
180	17±1	22±2	19±3	18±2	3.9
240	20±1	28±4	22±3	19±3	3.3
300	21±1	29±6	42±7	22±3	2.8
Neurogenic symptom scores					
0	6±0	10±1	7±0	7±0	5.6
60	7±0	12±4	8±1	7±0	5.0
120	7±0	10±2	9±1	7±0	4.4
180	9±1	11±2	9±1	8±1	3.9
240	11±1	14±3	10±1	10±1	3.3
300	12±1	16±4	22±4	10±1	2.8
Neuroglycopenic symptom scores					
0	6±0	7±0	6±0	5±0	5.6
60	6±0	8±1	6±1	6±0	5.0
120	6±0	8±1	7±1	7±1	4.4
180	7±1	10±1	9±2	8±1	3.9
240	7±1	12±2	10±2	8±2	3.3
300	7±1	11±2	16±2	11±2	2.8

* Mean±SE.

(46±4 mg/dl). At the corresponding time points during insulin infusion tests after afternoon hyperglycemia, the mean plasma glucose concentration was 3.3±0.3 mmol/liter (59±5 mg/dl) ($P < 0.001$) in the same patients. The corresponding values in the nondiabetic subjects were 3.5±0.2 mmol/liter (63±3 mg/dl); those in patients with CDAN were 3.3±0.2 mmol/liter (60±4 mg/dl). These values are illustrated in Fig. 6. Areas under the plasma glucose curves were also significantly ($P < 0.05$) smaller after afternoon hypoglycemia than those following afternoon hyperglycemia.

Discussion

The present data document that reduced adrenomedullary epinephrine (and islet pancreatic polypeptide) secretory responses to a given degree of hypoglycemia are a feature of IDDM per se, not CDAN, and that recent antecedent hypoglycemia reduces autonomic responses to, symptoms of and physiological defense against subsequent hypoglycemia in patients with IDDM.

The adrenal medullae can be conceptualized as sympathetic postganglionic neurons without axons (5, 40). They share a common embryological origin with sympathetic postganglionic neurons and express many of the same unique proteins, including the catecholamine biosynthetic enzymes, but release their products, including epinephrine, into the circulation to serve a hormonal function rather than into synaptic clefts to serve a neurotransmitter function. Thus, the adrenal medullae and the sympathetic nervous system comprise the sympathochromaffin (sympathoadrenal) system (41), which with the parasympathetic nervous system constitutes the autonomic nervous system. Clearly, therefore, reduced epinephrine and pancreatic polypeptide responses, markers of adrenomedullary and parasympathetic neural activation, respectively, to hypoglycemia (and reduced neurogenic symptom responses that likely reflect reduced sympathetic activation) that are the result of diabetes indicate a form of diabetic autonomic failure, albeit not necessarily classical diabetic autonomic neuropathy. We propose that a unique form of autonomic failure, which we term hypoglycemia-associated autonomic failure (5), plays an important role in the pathogenesis of iatrogenic hypoglycemia in patients with IDDM.

The data indicate that a single episode of afternoon hypoglycemia, of < 2 h duration, elevates glycemic thresholds for autonomic activation and symptoms of hypoglycemia the next morning in patients with IDDM selected for the absence of classical diabetic autonomic neuropathy. At comparable levels of hypoglycemia during morning, stepped hypoglycemic glucose clamps plasma epinephrine and pancreatic polypeptide responses and symptomatic responses were reduced after afternoon hypoglycemia compared with those after afternoon hyperglycemia in the same patients. Notably, both neurogenic and neuroglycopenic symptoms were reduced. These data extend earlier findings in nondiabetic humans (25–27) to patients with IDDM. They further extend those findings by demonstrat-

*Table IV. Cognitive Function Scores (Study 1)**

Time	Nondiabetic	IDDM			Nominal glucose
		With CDAN	Without CDAN		
			After hyperglycemia	After hypoglycemia	
min					*mmol/liter*
Attention (errors)					
0	—	—	—	—	5.6
60	1±0	2±1	2±0	3±1	5.0
120	1±0	2±0	1±0	1±0	4.4
180	1±0	2±1	1±0	1±1	3.9
240	2±0	4±1	2±1	3±1	3.3
300	3±1	3±1	3±1	5±2	2.8
Line orientation (errors)					
0	—	—	—	—	5.6
60	1±0	1±0	2±1	2±1	5.0
120	0±0	2±0	1±0	1±0	4.4
180	1±0	1±0	2±0	2±0	3.9
240	1±0	1±0	1±0	2±0	3.3
300	1±1	1±0	2±1	2±1	2.8
Immediate paragraph recall (bits recalled)					
0	—	—	—	—	5.6
60	14±2	12±2	10±2	10±1	5.0
120	15±2	11±1	11±2	8±1	4.4
180	14±2	10±2	10±1	8±1	3.9
240	16±2	10±3	11±2	10±2	3.3
300	16±1	11±1	9±1	9±2	2.8
Delayed paragraph recall (bits recalled)					
0	—	—	—	—	5.6
60	13±2	12±2	9±1	8±2	5.0
120	12±2	11±1	10±2	8±2	4.4
180	11±2	10±2	8±2	6±2	3.9
240	15±1	7±3	8±2	7±2	3.3
300	14±2	7±2	5±2	5±2	2.8

* Mean±SE.

ing elevated glycemic thresholds for autonomic and symptomatic responses, as opposed to reduced responses to a single level of hypoglycemia. Finally, they provide a mechanism, reduced epinephrine responses in the setting of deficient glucagon responses, for the observation of Davis et al. (42) that the hepatic glucose production response to a second episode of hypoglycemia is reduced compared with that to an episode 1 h earlier in patients with IDDM.

Reduced symptomatic responses to falling plasma glucose concentrations after recent antecedent hypoglycemia could, in themselves, increase the risk of severe iatrogenic hypoglycemia in IDDM by compromising awareness of developing hypoglycemia and, thus, delaying the appropriate behavioral response (e.g., the consumption of food). However, in the setting of absent glucagon responses in IDDM, such reduced epinephrine secretory responses would be expected to further increase the risk of severe hypoglycemia by critically compromising physiological defense against developing hypoglycemia (6–8, 23). The present data document that expectation. During relatively low dose morning insulin infusions, plasma glucose concentrations fell to substantially lower levels after afternoon hypoglycemia compared with those at the corresponding time points during identical insulin infusion tests after afternoon hyperglycemia in the same patients with IDDM. This new finding indicates that recent antecedent hypoglycemia reduces not only autonomic responses to and symptoms of hypoglycemia, but it also reduces physiological defense against hyperinsulinemia and developing hypoglycemia in IDDM. It also provides additional support for the critical role of epinephrine, in the setting of imperfect insulin replacement and deficient glucagon responses, in the prevention of iatrogenic hypoglycemia in IDDM (1, 6–8).

Thus, these data provide direct support for two fundamental elements of our hypothesis that (*a*) recent antecedent hypoglycemia is a major cause of hypoglycemia-associated autonomic failure in IDDM; and (*b*) hypoglycemia-associated auto-

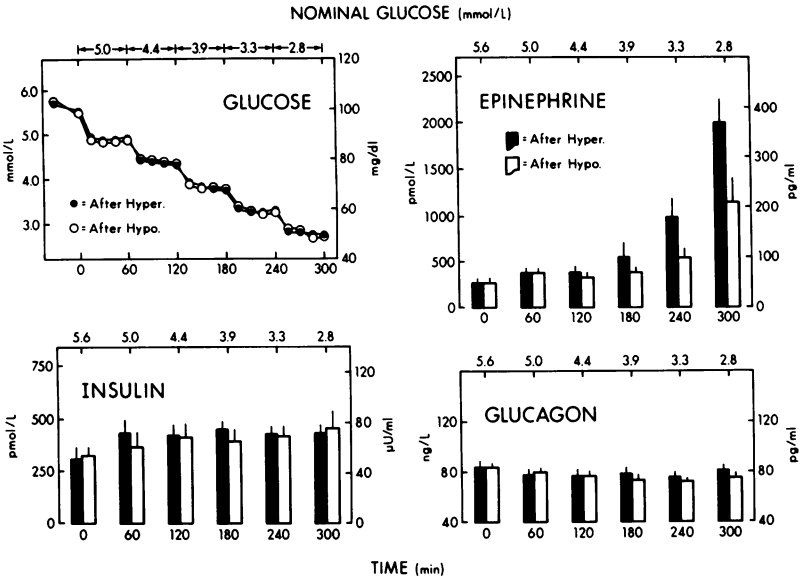

Figure 3. Mean±SE plasma glucose, insulin, epinephrine, and glucagon concentrations during hyperinsulinemic, stepped hypoglycemic glucose clamps in patients with IDDM without CDAN on mornings after afternoon hyperglycemia (*closed circles and columns*) and on mornings after afternoon hypoglycemia (*open circles and columns*). Standard errors not shown lie within the symbols.

nomic failure, by reducing both symptoms of and physiological defense against developing hypoglycemia, results in recurrent severe hypoglycemia thus creating a vicious cycle (5).

The present data also provide further support for our concept that hypoglycemia-associated autonomic failure in IDDM is distinct from classical diabetic autonomic neuropathy (5). In addition to patients with IDDM without classical diabetic autonomic neuropathy, nondiabetic subjects and patients with classical diabetic autonomic neuropathy were studied. The latter were selected on the basis of both reduced heart rate variation during deep breathing, a well-established indicator of autonomic neuropathy (29), and at least one clinical manifestation of classical diabetic autonomic neuropathy. Thus, they were clearly affected. Compared with nondiabetic subjects, both groups of patients with IDDM exhibited reduced adrenomedullary epinephrine responses to hypoglycemia. However, the epinephrine responses of the patients with and without classical diabetic autonomic neuropathy were virtually indistinguishable. Thus, reduced adrenomedullary epinephrine secretory responses to hypoglycemia are a feature of IDDM per se, not of classical diabetic autonomic neuropathy. (This is consistent with evidence that classical diabetic autonomic neuropathy is largely an axonal lesion [43, 44], probably the result of nerve fiber loss.) While this conclusion is at variance with that of Hilsted et al. (45), the data are not greatly dissimilar. Hilsted et al. (45) found substantial plasma epinephrine elevations during hypoglycemia in IDDM patients with autonomic neuropathy and documented severe sympathetic neural hypofunction, although the epinephrine levels were slightly lower than those in unaffected patients. Our data are remarkably similar in this regard. However, in contrast to Hilsted et al., we clamped plasma glucose concentrations to assure that the hypoglycemic stimulus was identical in all three study groups. With this technique, the epinephrine responses to hypoglycemia were found to be substantially reduced in patients with and without classical diabetic autonomic neuropathy compared with those of nondiabetic subjects.

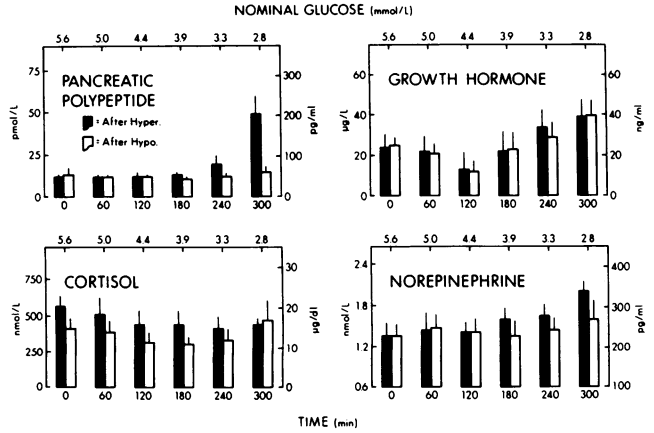

Figure 4. Mean±SE plasma pancreatic polypeptide, cortisol, growth hormone, and norepinephrine concentrations during hyperinsulinemic, stepped hypoglycemic glucose clamps in patients without classical diabetic autonomic neuropathy on mornings after afternoon hyperglycemia (*closed columns*) and on mornings after afternoon hypoglycemia (*open columns*).

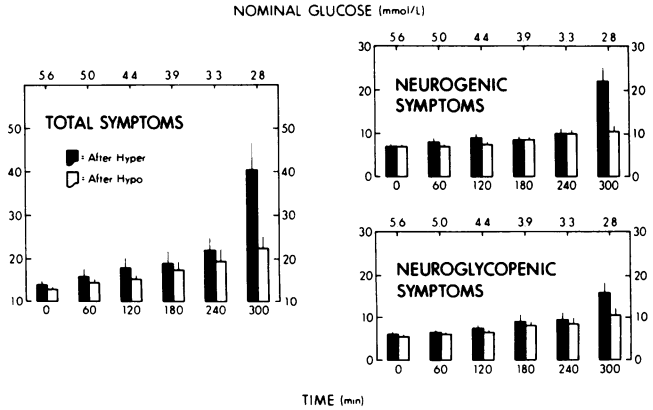

Figure 5. Mean±SE total, neurogenic, and neuroglycopenic symptoms scores during hyperinsulinemic, stepped hypoglycemic glucose clamps in patients without classical diabetic autonomic neuropathy on mornings after afternoon hyperglycemic (*closed columns*) and on mornings after afternoon hypoglycemia (*open columns*).

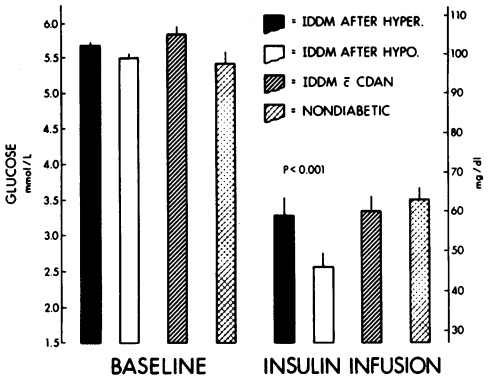

Figure 6. Mean±SE baseline and nadir plasma glucose concentrations during morning insulin infusion tests after afternoon hypoglycemia (*open columns*) and at the corresponding time points during morning insulin infusion tests following afternoon hyperglycemia (*closed columns*). Corresponding values in patients with classical diabetic autonomic neuropathy (*cross-hatched columns*) and in nondiabetic subjects (*double cross-hatched columns*) are shown.

If the adrenomedullary epinephrine response to hypoglycemia is not reduced further in classical diabetic autonomic neuropathy, as the present data indicate, one would not expect physiological defense against developing hypoglycemia to be further compromised in affected patients. The present data document this expectation. During relatively low dose insulin infusions, plasma glucose concentrations fell to similar levels in patients with and without classical diabetic autonomic neuropathy. Thus, a technique sufficiently sensitive to detect impaired defense against hyperinsulinemia in the form of hypoglycemia-associated autonomic failure induced by antecedent hypoglycemia did not disclose impaired defense in classical diabetic autonomic neuropathy. These data provide direct support for the view, initially espoused by Hilsted et al. more than a decade ago (45), that classical diabetic autonomic neuropathy is not a risk factor for iatrogenic hypoglycemia in IDDM (3, 46).

Both the reduced adrenomedullary epinephrine secretory response to a given degree of hypoglycemia in IDDM per se and the further reduced epinephrine response to hypoglycemia after antecedent hypoglycemia in IDDM appear to be threshold abnormalities. Although lower plasma glucose concentrations were required to elicit a comparable epinephrine response, low glucose levels did elicit a response. This was also the case for the reduced pancreatic polypeptide response and the reduced symptomatic responses after antecedent hypoglycemia. In contrast, the reduced glucagon response to hypoglycemia, which characterizes established IDDM (20, 21), appears to be absolute. The lowest glucose level tested did not elicit even a suggestion of a glucagon response in the patients with IDDM under any study condition. This implies that the mechanisms of the deficient glucagon response and of the deficient epinephrine, pancreatic polypeptide, and symptomatic responses to hypoglycemia in IDDM are different.

Patients with IDDM exhibited higher symptom scores than nondiabetic subjects at the lower plasma glucose concentrations. This observation confirms previous evidence that glycemic thresholds for symptoms are reduced (i.e., at higher plasma glucose concentrations) in patients with poorly or moderately controlled IDDM (13, 17, 18). Interestingly, the cognitive function of memory, most noticeably in the measure of delayed paragraph recall, deteriorated in both groups of patients with IDDM, but not in the nondiabetic subjects, at the lower plasma glucose levels. This finding suggests that the glycemic thresholds for cognitive dysfunction, like those for symptoms, are also reduced in patients with moderately controlled IDDM.

Although neuroglycopenic, as well as neurogenic responses to hypoglycemia were found to be reduced after antecedent hypoglycemia, aside from attention cognitive function was not significantly affected. While memory, particularly delayed paragraph recall, appeared to be reduced at the intermediate glycemic steps after afternoon hypoglycemia, this was not significant statistically. This may have been the result of our experimental design. The lowest glycemic step tested (2.8 mmol/liter) is approximately at the glycemic threshold for cognitive dysfunction in normal subjects (47) (although apparently below the glycemic threshold for cognitive dysfunction in patients with moderately or poorly controlled IDDM as just discussed); a lower glycemic step might have disclosed an effect of antecedent hypoglycemia. Furthermore, the cognitive tests used may lack sufficient sensitivity and precision to detect such an effect with small sample sizes. Clearly we cannot reject the possibility of a type II statistical error. Nonetheless, taken at face value the present data suggest that recent antecedent hypoglycemia may not elevate glycemic thresholds for cognitive dysfunction in IDDM. This is a fundamentally important issue because of its mechanistic implications. If antecedent hypoglycemia elevates glycemic thresholds for autonomic responses to and symptoms of subsequent hypoglycemia but not glycemic thresholds for cognitive dysfunction, the phenomenon is not easily explained by increased fractional glucose extraction by the brain after antecedent hypoglycemia, as suggested by data from animals (48, 49) and proposed elsewhere (5, 50). Unfortunately, published data on this important point are conflicting. Evidence that the glycemic thresholds for electroencephalogram activation (51), neuroglycopenic symptoms (52, 53), and cognitive dysfunction (52, 53) during hypoglycemia are not elevated in tightly controlled IDDM has been presented. However, others have presented evidence that the glycemic thresholds for neuroglycopenic, as well as neurogenic symptoms (13), and those for cognitive dysfunction (54) are elevated in such patients. Veneman and Mokan (55) recently found elevated glycemic thresholds for cognitive dysfunction, as well as neuroglycopenic symptoms after an episode of nocturnal hypoglycemia in nondiabetic subjects. We have found elevated glycemic thresholds for neuroglycopenic, as well as neurogenic symptoms in relatively well-controlled IDDM (13), IDDM with defective glucose counterregulation (13), nondiabetic subjects (25), and patients with IDDM (present data) after an episode of hypoglycemia. Thus, this issue remains controversial.

The extent to which the elevated glycemic thresholds for symptoms and autonomic responses to hypoglycemia demonstrated here to follow recent antecedent hypoglycemia are germane to such changes in thresholds during effective intensive therapy of IDDM (15, 16, 56) is unknown. However, since the latter is associated with an increased frequency of iatrogenic hypoglycemia (1, 3, 6, 7), the present findings may well be relevant to that phenomenon.

In summary, the data presented document that hypoglycemia-associated autonomic failure, a disorder distinct from classical diabetic autonomic neuropathy, can be induced by recent,

short-term antecedent hypoglycemia in patients with IDDM. The data provide direct support for two fundamental elements of the concept that recent antecedent iatrogenic hypoglycemia is a major cause of hypoglycemia-associated autonomic failure and that hypoglycemia-associated autonomic failure, by reducing both symptoms of and physiological defense against developing hypoglycemia, results in recurrent severe hypoglycemia, thus creating a vicious cycle (5). Thus, hypoglycemia-associated autonomic failure may well be a major risk factor for iatrogenic hypoglycemia in IDDM.

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