

Reduced β -Adrenergic Sensitivity in Patients With Type 1 Diabetes and Hypoglycemia Unawareness

MARY T. KORYTKOWSKI, MD
 MARIAN MOKAN, MD
 THIEMO F. VENEMAN, MD

ASIMINA MITRAKOU, MD
 PHILIP E. CRYER, MD
 JOHN E. GERICH, MD

OBJECTIVE — We tested the hypothesis that impaired tissue sensitivity to catecholamines contributes to hypoglycemia unawareness in subjects with type 1 diabetes.

RESEARCH DESIGN AND METHODS — A total of 21 subjects with type 1 diabetes underwent a standardized insulin infusion protocol to produce a stepwise decrease in plasma glucose to 45-min plateaus of 4.3, 3.6, 3.0, and 2.3 mmol/l. Glycemic thresholds, maximum responses for adrenergic and neuroglycopenic symptoms, and counterregulatory hormones were determined. Patients were classified as hypoglycemia unaware if the initiation of adrenergic symptoms occurred at a plasma glucose level 2 SD below that of nondiabetic volunteers. β -Adrenergic sensitivity was measured as the dose of isoproterenol required to produce an increment in heart rate of 25 beats per minute above baseline (I_{25}) in resting subjects.

RESULTS — Subjects with type 1 diabetes and hypoglycemia unawareness experienced the onset of adrenergic symptoms at a lower plasma glucose level than did those with awareness (2.5 ± 0.1 vs. 3.7 ± 0.1 mmol/l, $P < 0.001$), whereas neuroglycopenic symptoms occurred at similar glucose levels (2.7 ± 0.2 vs. 2.8 ± 0.1 mmol/l). The plasma glucose levels for counterregulatory hormone secretion (epinephrine 2.9 ± 0.2 vs. 4.1 ± 0.2 mmol/l; norepinephrine 2.7 ± 0.1 vs. 3.2 ± 0.2 mmol/l; cortisol 2.5 ± 0.2 vs. 3.3 ± 0.2 mmol/l, $P < 0.01$) were also lower in subjects with unawareness. The maximal epinephrine ($1,954 \pm 486$ vs. $5,332 \pm 1,059$ pmol/l, $P < 0.01$), norepinephrine (0.73 ± 0.14 vs. 1.47 ± 0.21 nmol/l, $P = 0.04$), and cortisol (276 ± 110 vs. 579 ± 83 nmol/l, $P < 0.01$) responses were reduced in the unaware group. I_{25} was greater in unaware subjects than in subjects without unawareness (1.5 ± 0.3 vs. 0.8 ± 0.2 μ g), where I_{25} was not different from that of controls (0.8 ± 0.2 μ g).

CONCLUSIONS — We conclude that subjects with type 1 diabetes and hypoglycemia unawareness have reduced β -adrenergic sensitivity, which may contribute to their impaired adrenergic warning symptoms during hypoglycemia.

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Hypoglycemia unawareness occurs when appropriate autonomic warning signals (i.e., sweating, hunger, anxiety, palpitations, or tremor) do not exclude development of neuroglycopenia (i.e., confusion, dizziness, blurred vision, or weakness) (1). This failure to perceive autonomic hypoglycemic symptoms has been

proposed to contribute to an increased frequency of severe hypoglycemia in patients with type 1 diabetes (2). Hypoglycemia unawareness can also hinder attempts to achieve tight glycemic control with intensive insulin therapeutic regimens as recommended by the Diabetes Control and Complications Trial (3,4).

Duration of diabetes, antecedent episodes of hypoglycemia, and near-normal HbA_{1c} are known risk factors for hypoglycemia unawareness (1). Although the distinct physiological mechanisms underlying impaired responsiveness to hypoglycemia in type 1 diabetes are not known, several hypotheses have been proposed (5,6). One is the preservation of brain glucose uptake during hypoglycemia due to acceleration of cerebral glucose transport so that hypoglycemia occurs without neuroglycopenia (5). Another proposed mechanism for hypoglycemia unawareness is catecholamine depletion resulting from recurrent hypoglycemic episodes (6). However, unawareness has been observed in the presence of normal plasma catecholamine responses to hypoglycemia (7,8). Desensitization of the adrenergic receptor resulting from repeated episodes of hypoglycemia with chronic exposure to elevated catecholamine concentrations could explain this decrease in autonomic warning symptoms (9). A decreased number of cardiac β -adrenergic receptors are observed in streptozotocin-diabetic rats, which suggests that a down-regulation of catecholamine receptors may occur simply as a consequence of diabetes (10,11).

Decreased sensitivity to adrenergic stimulation with the β -agonist isoproterenol has been observed in subjects with type 1 diabetes classified as having hypoglycemia unawareness by history (12,13). These studies have several limitations. One is the age difference between the hypoglycemia unaware and aware groups, which may have accounted for the observed differences in β -adrenergic sensitivity. Another is the unreliability of classifying subjects as unaware or aware based on recollection of prior hypoglycemic episodes (14,15). Nevertheless, avoidance of hypoglycemia has

From the University of Pittsburgh School of Medicine (M.T.K.), Pittsburgh, Pennsylvania; the Medical School of Komensky University (M.M.), Martin, Czechoslovakia; University Hospital Utrecht (T.F.V.), Utrecht, The Netherlands; the Second Department of Internal Medicine (A.M.), Propaedeutic, Athens University, Evangelismo Hospital, Athens, Greece; the Washington University School of Medicine (P.E.C.), St. Louis, Missouri; and the University of Rochester School of Medicine (J.E.G.), Rochester, New York.

Address correspondence and reprint requests to Mary T. Korytkowski, MD, Falk, Room 581, University of Pittsburgh Medical Center, 3601 Fifth Ave., Pittsburgh, PA 15213. E-mail: korytkowski@med1.dept-med.pitt.edu.

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Abbreviations: GCRC, General Clinical Research Center; I_{25} , dose of isoproterenol required to increase the heart rate 25 beats per minute above baseline.

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

Table 1—Clinical characteristics of subjects

	Diabetic subjects		Nondiabetic subjects
	Unaware	Aware	
Sex (F/M)	4/6	4/7	5/5
Age (years)	31.5 \pm 1.7	25.6 \pm 1.9	29.2 \pm 1.7
HbA _{1c} (%)	7.6 \pm 0.5*	9.4 \pm 0.6	4.8 \pm 0.05
BMI (kg/m ²)	24.0 \pm 0.7	23.7 \pm 1.3	24.8 \pm 1.2
Duration of diabetes (years)	16.1 \pm 3.0†	4.5 \pm 1.6	—
Insulin (U/day)	49.1 \pm 6.5	41.4 \pm 5.0	—
Insulin (U \cdot kg ⁻¹ \cdot day ⁻¹)	0.5 \pm 0.08	0.5 \pm 0.08	—

Data are means \pm SD. * P = 0.0003 vs. aware group; † P = 0.003 vs. aware group.

been reported to restore appropriate hypoglycemia awareness without restoration of normal catecholamine responses to hypoglycemia (16). These observations suggest that an alteration in sensitivity to catecholamines may be involved.

To more rigorously test the hypothesis that impaired tissue sensitivity to catecholamines contributes to hypoglycemia unawareness, we used a standardized step-wise hypoglycemia protocol to prospectively identify subjects with type 1 diabetes as having hypoglycemia unawareness or awareness before determination of β -adrenergic sensitivity with isoproterenol. Results of the isoproterenol testing in the diabetic subjects are compared with a group of age- and sex-matched nondiabetic volunteers.

RESEARCH DESIGN AND

METHODS — The protocol was reviewed and approved by the Institutional Review Board for Biomedical Research at the University of Pittsburgh and the General Clinical Research Center (GCRC) Review Committee. Informed written consent was obtained from each subject before participation in the study. All studies took place in the GCRC. All diabetic subjects participated in a previously reported study in which they were characterized as hypoglycemia unaware or aware (14).

Subjects

A total of 21 subjects with type 1 diabetes and 10 age-, weight-, and sex-matched nondiabetic control subjects were studied. The clinical characteristics of the study subjects are presented in Table 1. A physical examination, screening laboratory testing, and an electrocardiogram were performed in all subjects before inclusion. All participants had normal cardiovascular reflexes

and no evidence of autonomic neuropathy using previously established criteria (17). Objective testing of autonomic function included measurement of heart rate and blood pressure responses to standing; heart rate response to Valsalva; and heart rate variability during slow, deep breathing. The medical history included information regarding the ability of each subject to perceive hypoglycemic events as well as the frequency of these events. No subject reported symptoms of nocturnal diarrhea, gustatory sweating, gastroparesis, or sexual dysfunction.

Induction of hypoglycemia

To categorize the subjects with type 1 diabetes according to their ability to detect hypoglycemia, all diabetic subjects were initially studied using a standardized step-wise hypoglycemia protocol. All intermediate and long-acting insulin was discontinued for 48 h before the hypoglycemia protocol, and subjects were managed with preprandial injections of regular insulin. Subjects were admitted to the GCRC on the night before the study. Near-normoglycemia (5–7 mmol/l [90–126 mg/dl]) was maintained overnight with an intravenous insulin infusion. Between 7:00 and 7:30 A.M., a hand vein was cannulated in a retrograde manner and maintained in a Plexiglas thermoregulated “hot-box” (65°C) for sampling of arterialized venous blood throughout the procedure. A deep antecubital vein was cannulated for infusion of insulin and glucose. After a 60-min equilibration period, a continuous intravenous infusion of insulin was begun (1 mU \cdot kg⁻¹ \cdot min⁻¹ for 270 min; 2 mU \cdot kg⁻¹ \cdot min⁻¹ for 60 min). A variable-rate glucose infusion (50% dextrose) was initiated and adjusted at 5-min intervals using the glucose-clamp technique to achieve target plateau plasma glucose concentrations of 4.3 (78),

3.6 (65), 3.0 (54), and 2.3 mmol/l (42 mg/dl), respectively. The plasma glucose was allowed to decrease 0.6 mmol/l (11 mg/dl) over 45 min, with maintenance of each plateau for 45 min before the next decrease.

Arterialized venous blood samples were drawn every 30 min for measurement of counterregulatory hormones (epinephrine, norepinephrine, and cortisol). A semi-quantitative symptom questionnaire composed of five autonomic (i.e., anxiety, palpitations, hunger, sweating, irritability, and tremor) and five neuroglycopenic (i.e., dizziness, tingling, blurred vision, difficulty thinking, and faintness) symptoms was administered every 15 min. Subjects scored the intensity of each symptom using a Likert scale quantified from 0 (none) to 5 (severe). The sum of symptom responses constituted the symptom score. Hypoglycemia unawareness was defined as the initiation of autonomic symptoms at a plasma glucose >2 SD below that of nondiabetic volunteers (14,20).

Isoproterenol testing

Subjects were again admitted to the GCRC at 7:00 A.M. after a 10-h overnight fast no sooner than 1 week after the hypoglycemia procedure described above. Subjects reporting a severe hypoglycemic event during the previous week were rescheduled for the study with instructions to avoid recurrent events. Subjects with type 1 diabetes were asked to withhold their morning insulin until completion of the study. A fasting plasma glucose was documented on all diabetic subjects before isoproterenol testing to verify the absence of hypoglycemia (plasma glucose <4.4 mmol/l [80 mg/dl]) or significant hyperglycemia (≥ 11.1 mmol/l [200 mg/dl]).

At 7:30 A.M., an antecubital vein was cannulated and maintained with an infusion of 0.9% normal saline. Subjects were attached to a continuous cardiac monitor. After 30 min of rest in a quiet, darkened room, baseline values for resting heart rate were determined as the mean of three separate readings obtained at 5-min intervals. Resting heart rate was determined from four consecutive R-R intervals during quiet breathing.

Sequential, incremental 1-ml intravenous injections of placebo (normal saline) or isoproterenol in ascending doses were administered. A 15-min interval was maintained between injections to allow the heart rate to return to baseline before each subsequent injection. The highest dose

Table 2—Glycemic thresholds and maximal response of counteregulatory hormones during hypoglycemia

	Unaware	Aware	P value
Epinephrine			
Threshold (mmol/l)	2.9 ± 0.2	4.1 ± 0.1	<0.001
Maximal response (pmol/l)	1,954 ± 486	5,332 ± 1,059	0.01
Norepinephrine			
Threshold (mmol/l)	2.7 ± 0.1	3.2 ± 0.2	<0.001
Maximal response (nmol/l)	0.73 ± 0.14	1.47 ± 0.21	0.04
Cortisol			
Threshold (mmol/l)	2.5 ± 0.2	3.3 ± 0.2	0.01
Maximal response (nmol/l)	276 ± 110	579 ± 83	0.09

Data are means ± SD.

tested was that which increased the heart rate 25 beats per min above baseline. This was defined as the I_{25} (18,19). This dose was repeated after the return to baseline conditions to verify the response.

Analytical methods

Plasma glucose was measured using a Yellow Springs Instruments glucose analyzer (Yellow Springs, OH). Cortisol was measured by a previously described radioimmunoassay (14). Plasma epinephrine and norepinephrine were measured using a single isotope derivative radioenzymatic method (14). HbA_{1c} was determined by a high-performance liquid chromatography method (Bio-Rad Diamat, Richmond, CA; normal range 4.3–6.1%).

Statistical analysis

The differences among groups were analyzed using analysis of variance followed by χ^2 analysis to examine between-group differences. After a significant omnibus test, Scheffé's post-hoc procedure was performed. Correlation analysis with analysis of covariance was performed for I_{25} (dose of isoproterenol required to increase the heart rate 25 beats per minute above baseline) with HbA_{1c}, diabetes duration, and age. Data are expressed as means ± SEM, unless otherwise specified.

RESULTS

Clinical characteristics

No significant differences were observed for age, sex, or BMI among the two groups of subjects with type 1 diabetes or normal subjects (Table 1). Subjects with hypoglycemia unawareness had a longer duration of diabetes and a lower HbA_{1c} than those with awareness (Table 1).

Responses to standardized hypoglycemia

Subjects with type 1 diabetes were classified as having hypoglycemia unawareness or awareness based on responses to the symptom questionnaire during the standardized hypoglycemia protocol. Hypoglycemia unawareness was defined as the onset of adrenergic symptoms at a plasma glucose level ≥ 2 SD below the level previously published for nondiabetic subjects (3.3 ± 0.06 mmol/l) (20). Using this criterion, the 10 subjects experiencing the onset of adrenergic symptoms at a plasma glucose ≤ 3.0 mmol/l (range: 2.2–3.0 mmol/l [40–54 mg/dl]) were classified as having hypoglycemia unawareness. The remaining 11 subjects experienced the onset of these symptoms at a plasma glucose ≥ 3.4 mmol/l (62 mg/dl; range: 3.4–4.4 mmol/l [61–79 mg/dl]) and were classified as having awareness.

The plasma glucose level at the onset of adrenergic symptoms was lower in the unaware group (2.5 ± 0.1 vs. 3.7 ± 0.1 mmol/l [47 ± 2 vs. 67 ± 2 mg/dl], $P < 0.001$) than in the aware group, where the onset of adrenergic symptoms was observed at a plasma glucose level similar to that observed in nondiabetic subjects (3.8 mmol/l [68 mg/dl]) (20). The maximal adrenergic symptom scores were similar in the unaware and aware groups (5 ± 1 vs. 7 ± 1 , $P = 0.13$). The onset of neuroglycopenic symptoms occurred at similar plasma glucose levels in unaware and aware subjects (2.7 ± 0.2 vs. 2.8 ± 0.1 mmol/l [49 ± 4 vs. 50 ± 2 mg/dl]), and maximal score (6 ± 1 vs. 7 ± 1) was not significantly different between the two groups.

The plasma glucose concentration at which counterregulatory hormone responses (epinephrine, norepinephrine, cortisol) occurred (i.e., the glycemic threshold) and the maximal hormonal responses to hypoglycemia were lower in the group with hypoglycemia unawareness (Table 2).

β -Adrenergic sensitivity

Subjects with hypoglycemia unawareness demonstrated evidence of impaired β -adrenergic sensitivity, with a significantly higher I_{25} than that of aware subjects (Fig. 1). The I_{25} of the aware group was almost identical to that of nondiabetic control subjects (Fig. 1). The I_{25} correlated with duration of diabetes ($r = 0.48$, $P = 0.025$), but not with age or HbA_{1c}. The differences observed between the diabetic subjects with hypoglycemia unawareness and those with awareness persisted when corrected for duration of diabetes ($P = 0.05$).

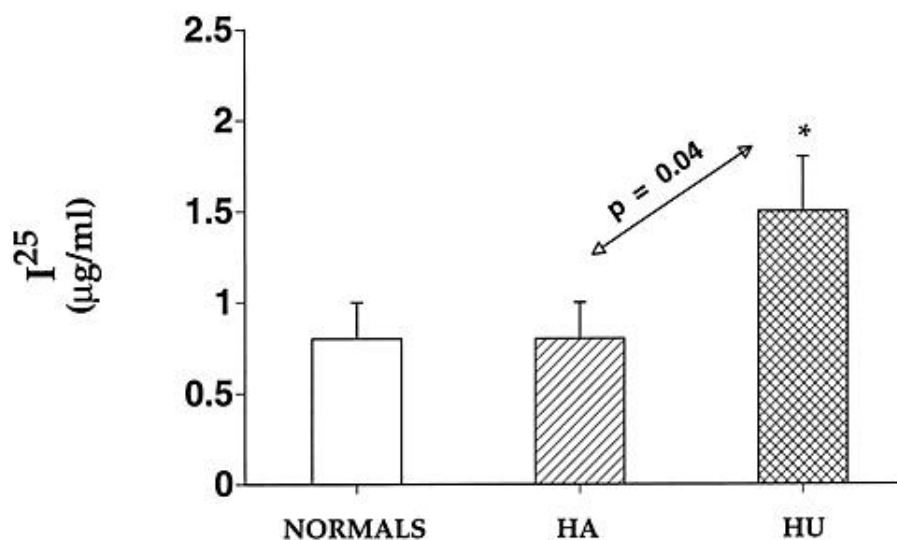


Figure 1— I_{25} in normal subjects, patients with hypoglycemia awareness (HA), and patients with hypoglycemia unawareness (HU).

No adverse events were observed during the isoproterenol infusions. The following transient symptoms were reported with equal frequency by subjects in all three groups: flushing, palpitations, shortness of breath, pounding behind the eyes, generalized warmth, and anxiety.

CONCLUSIONS — In the present study, subjects with type 1 diabetes objectively identified as having hypoglycemia unawareness using a standardized hypoglycemia protocol were observed to have an impairment in β -adrenergic sensitivity, as determined by heart rate responses to incremental doses of the β -agonist isoproterenol. This provides evidence that in subjects with type 1 diabetes and hypoglycemia unawareness, impaired responsiveness to endogenous catecholamines contributes to the risk for severe hypoglycemic events.

In identifying subjects with type 1 diabetes who are at risk for severe hypoglycemic events, a prior history of such an episode is a strong predictor for recurrence. Although this risk can be modified with patient education regarding insulin administration and food intake, there is an interest in developing prospective screening methods for identifying subjects with type 1 diabetes who are at risk for severe hypoglycemia, especially when using intensive insulin regimens.

Interviews directed at determining whether a patient has hypoglycemia unawareness or awareness, although important, are an inaccurate method of identifying individuals at risk for severe hypoglycemic events (14,15). Approximately half ($n = 5$) of the subjects with unawareness in this study reported awareness of hypoglycemic symptoms.

Isoproterenol testing has been used in the past as a reliable and reproducible means of evaluating the efficacy of β -adrenergic blocking agents (18,19). It has since been used in other clinical situations, including the evaluation of patients with syncope (21), panic disorders (22), and more recently, hypoglycemia unawareness in diabetes (12,13,23). Although not affected by body weight, a decrease in β -adrenergic sensitivity with advancing age has been observed with isoproterenol testing (12,24).

In this study, the I_{25} results observed in the nondiabetic subjects, as well as those in diabetic subjects with hypoglycemia awareness, are similar to those previously reported by Cleaveland et al. (18) in young

healthy subjects (I_{25} : 0.88 μ g/ml). However, our results are lower than those reported by Berlin and colleagues (12,13,23) in subjects with type 1 diabetes. The previously described age-related decrease in β -adrenergic sensitivity with age may have contributed to this discrepancy between the present and previous studies in subjects with type 1 diabetes (23). The narrow age range of subjects participating in this protocol may have obviated any age-associated decline in β -adrenergic sensitivity. The average ages of our subjects with unawareness and awareness were 31.5 ± 1.7 and 25.6 ± 1.9 years, whereas the ages of subjects in the prior study were 46 ± 3 and 34 ± 2 years, respectively (13).

The authors of this prior report performed isoproterenol testing in a small subset ($n = 5$ in each group) of subjects with type 1 diabetes from their larger study (23). These 10 subjects were classified as hypoglycemia unaware or aware based on symptomatic and hormonal responses to the acute induction of hypoglycemia (23). A difference in β -adrenergic sensitivity was again observed between the two groups, but the subjects with unawareness were again significantly older than aware subjects (43 ± 3 and 32 ± 2 years). Together with the small number of subjects studied, this 11-year age difference between the two groups may have contributed to the observed difference in I_{25} (12,18), thus confounding the authors' conclusion that subjects with type 1 diabetes and hypoglycemia unawareness have reduced β -adrenergic sensitivity.

We conclude that subjects with type 1 diabetes carefully identified as having hypoglycemia unawareness using a standardized hypoglycemia protocol have evidence of reduced β -adrenergic sensitivity as measured during an isoproterenol stimulation test. Together with a decrease in counterregulatory hormone responses to hypoglycemia, this may contribute to reduced adrenergic warning symptoms during hypoglycemia.

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