

# Effect of Hypoglycemia on $\beta$ -Adrenergic Sensitivity in Normal and Type 1 Diabetic Subjects

ANDREAS FRITSCH, MD  
MICHAEL STUMVOLL, MD  
MATTHIAS GRÜB  
SONJA SIESLACK

WALTER RENN, PHD  
REINHOLD-MICHAEL SCHMÜLLING, MD  
HANS-ULRICH HÄRING, MD  
JOHN E. GERICH, MD

**OBJECTIVE** — The purpose of this study was to assess the potential role of reduced tissue sensitivity to catecholamines in the pathogenesis of hypoglycemia unawareness in patients with type 1 diabetes.

**RESEARCH DESIGN AND METHODS** — The effect of a single episode of hypoglycemia on  $\beta$ -adrenergic sensitivity was studied in 10 type 1 diabetic patients with apparently normal awareness of hypoglycemia (age  $29 \pm 5$  years, diabetes duration  $13 \pm 8$  years,  $HbA_{1c}$   $7.3 \pm 0.9\%$ ) and 10 age-matched healthy control subjects.  $\beta$ -Adrenergic sensitivity was measured with the isoproterenol test after a hyperinsulinemic euglycemic clamp and after a hyperinsulinemic hypoglycemic clamp.  $\beta$ -Adrenergic sensitivity was expressed as the dose of intravenous isoproterenol that increased the heart rate by 25 beats/min (IC25).

**RESULTS** — During hypoglycemia, diabetic subjects had an impaired plasma epinephrine response compared with that of the control subjects ( $16.7 \pm 5.0$  vs.  $40.1 \pm 6.8$  ng/ml,  $P = 0.02$ ). In control subjects, the IC25 was lower after hypoglycemia than after euglycemia ( $0.83 \pm 0.22$  vs.  $1.13 \pm 0.21$   $\mu$ g,  $P = 0.02$ ) indicating an increase in  $\beta$ -adrenergic sensitivity. In diabetic subjects, on the other hand, the IC25 was greater after hypoglycemia than after euglycemia ( $1.00 \pm 0.26$  vs.  $0.65 \pm 0.14$   $\mu$ g,  $P = 0.04$ ), indicating a decrease in  $\beta$ -adrenergic sensitivity.

**CONCLUSIONS** — In normal subjects, a single episode of hypoglycemia increases  $\beta$ -adrenergic sensitivity. In diabetic subjects, in contrast, hypoglycemia reduces  $\beta$ -adrenergic sensitivity. These results provide evidence that in type 1 diabetic patients, some maladaptation of tissue sensitivity to catecholamines contributes to the development of hypoglycemia unawareness. A unifying hypothesis is presented for the pathogenesis of hypoglycemia unawareness in type 1 diabetic patients incorporating the concepts of both a reduced catecholamine response and reduced adrenergic sensitivity.

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Hypoglycemia unawareness, defined as the lack of appropriate autonomic warning signals before the development of neuroglycopenia (1), occurs commonly in patients with type 1 diabetes. It is generally

characterized by 1) greater hypoglycemia being required to initiate autonomic warning symptoms and counterregulatory hormone secretion and 2) a reduction in the magnitude of both symptom and hormone responses

(2–4), perhaps most importantly that of epinephrine. The impaired counterregulatory hormone responses are related to both duration of diabetes and degree of glycemic control (2) and also usually involve the occurrence of episodes of hypoglycemia (5–9).

The pathophysiology of hypoglycemia unawareness remains incompletely understood. There is evidence that alterations in blood-brain glucose transport may be involved (1). In addition, reduced peripheral tissue sensitivity to catecholamines has been suggested to be involved in the decreased autonomic warning symptoms (1). Episodes of hypoglycemia unaccompanied by adrenergic symptoms have been reported to occur in type 1 diabetic patients with normal plasma catecholamine responses to hypoglycemia (10). Decreased  $\beta$ -adrenergic sensitivity has been found in patients with hypoglycemia unawareness diagnosed on the basis of self-reports (11,12). Moreover, strict avoidance of hypoglycemia, which can restore normal awareness of hypoglycemia in some patients (13), is not necessarily accompanied by restoration of normal epinephrine responses to hypoglycemia (14).

Taken together, these observations suggest that episodes of hypoglycemia may impair  $\beta$ -adrenergic sensitivity and that avoidance of hypoglycemia restores awareness of hypoglycemia by reversing impaired  $\beta$ -adrenergic insensitivity. To test this hypothesis, we therefore assessed the effect of a single 3-h episode of hypoglycemia on  $\beta$ -adrenergic sensitivity in type 1 diabetic patients and nondiabetic subjects using the isoproterenol test (IT) (15).

## RESEARCH DESIGN AND METHODS

### Subjects

The protocol was approved by the local ethics committee. There were 10 normal volunteers and 10 type 1 diabetic subjects studied. All participants gave their written informed consent. Their clinical characteristics are given in Table 1. None of the subjects were taking medications (other than insulin) except one of the diabetic subjects,

From the Department IV of Internal Medicine (A.F., M.S., M.G., S.S., W.R., R.-M.S., H.-U.H.), University of Tübingen, Tübingen, Germany; and the Departments of Medicine, Physiology, and Pharmacology (J.E.G.), University of Rochester School of Medicine, Rochester, New York.

Address correspondence and reprint requests to John E. Gerich, MD, University of Rochester School of Medicine, 601 Elmwood Ave., Box MED/CRC, Rochester, NY 14642.

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**Abbreviations:** CV, coefficient of variation; ECG, electrocardiogram; ELISA, enzyme-linked immunosorbent assay; IC25, dose of isoproterenol required to increase the heart rate by 25 beats/min; IT, isoproterenol test; MANOVA, multivariate analysis of variance.

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

Table 1—Characteristics of subjects studied

	Nondiabetic subjects	Type 1 diabetic subjects	P value
n	10	10	—
Sex (F/M)	4/6	3/7	—
Age (years)	25 $\pm$ 5	29 $\pm$ 5	0.1
BMI (kg/m <sup>2</sup> )	21.7 $\pm$ 2.6	23.8 $\pm$ 2.2	0.06
Duration of diabetes (years)	—	13 $\pm$ 8	—
HbA <sub>1c</sub> (%)	—	7.3 $\pm$ 0.9	—

Data are means  $\pm$  SD.

who was on thyroxine (75  $\mu$ g/day) replacement. Two diabetic and two nondiabetic subjects were moderate smokers; both did not smoke for at least 12 h before the ITs. Of the 10 type 1 diabetic subjects, 6 had previously undergone a stepwise hypoglycemic clamp procedure. The glycemic threshold for epinephrine response (blood glucose at which plasma epinephrine exceeded the mean basal plasma epinephrine by 2 SD) was normal (3.4  $\pm$  0.1 mmol/l) (2). The other four diabetic subjects had a diabetes duration of only 6  $\pm$  3 years and did not differ in age (31  $\pm$  3 years) and HbA<sub>1c</sub> (7.3  $\pm$  0.9%). None of the diabetic subjects had a history of hypoglycemia unawareness or frequent hypoglycemia as assessed by review of their daily self-blood glucose monitoring records and by direct questioning. None had experienced an episode of severe hypoglycemia within the last year, and in all, autonomic neuropathy was excluded by standard cardiovascular tests (heart rate variation at rest with spectral analysis, Valsalva maneuver, lying-to-standing, deep breathing) (16).

### Protocol

Subjects underwent a nocturnal hyperinsulinemic euglycemic and a hyperinsulinemic hypoglycemic clamp performed in random order 1–2 weeks apart, and on the following morning, an IT was performed to test  $\beta$ -adrenergic sensitivity.

Subjects were admitted to the university's clinical research unit at 1600. Diabetic patients had injected their last regular insulin before lunch. They had been instructed to reduce their insulin dose by 10% to avoid blood glucose levels <5 mmol/l for the 5 days before the study. Both groups of subjects had their last meal before admission at 1200. At 1700, indwelling catheters were inserted into an antecubital vein for infusion of glucose and insulin and into a dorsal vein, which was kept in a heated chamber for arterialized blood sampling. At 1800, an insulin infu-

sion with a constant rate of 1 mU  $\cdot$  kg<sup>-1</sup> body wt  $\cdot$  min<sup>-1</sup> was begun, and blood glucose was clamped at either 3 or 6 mmol/l for 180 min. Afterward, subjects had a standardized meal containing 48 g carbohydrates and were asleep by 2400. During the night, diabetic subjects were infused with insulin according to the algorithm of Mokan and Gerich (17) to maintain them as euglycemic overnight.

### Symptom questionnaire

A semiquantitative symptom questionnaire was administered before the clamp and at 30, 80, 150, and 180 min during the clamp. Subjects scored from 1 (none) to 7 (severe) in response to 9 autonomic (tremor, sweating, shivering, hunger, pounding heart, anxiety, nausea, feeling tearful), 10 neuroglycopenic (difficulty speaking, double vision, headache, drowsiness, blurred vision, tingling around the mouth, inability to concentrate, confusion, tiredness, weakness), and 10 dummy symptoms (hiccough, constipation, abdominal cramps, difficulty breathing, pain in legs, back pain, itching, bloating, yellow vision, heartburn) that were presented in random order (18). Total symptom score, as well as neuroglycopenic and autonomic symptom scores, were calculated for each time point as previously described (2).

### IT

The next morning between 0630 and 0930, subjects underwent an IT (15). Subjects were connected with a computer-assisted electrocardiogram (ECG) (NeuroDiag, Munich, Germany) for determination of cardiovascular responses. Blood pressure was recorded with an automatic device (DINAMAP; Critikon, Tampa, FL). Appropriately diluted isoproterenol was injected as a 5 ml bolus in a forearm vein through a three-way valve in doses of 0 (5 ml NaCl 0.9%) and 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, and 2.5  $\mu$ g of isoproterenol (isuprel; Sanofi, Brussels, Belgium). The interval between

each injection was 20 min. ECG was recorded on-line for 1 min before and for 5 min after each injection. The basal heart rate was defined as the mean of the 20 s before the first injection. The maximum heart rate was generally reached  $\sim$ 30–60 s after each injection and had returned to baseline before the end of the 5-min recording interval. The maximum heart rate was determined by searching for the shortest R-R interval after injection; extrasystoles were excluded by graphic analysis of heart rate recordings.  $\beta$ -Adrenergic sensitivity was expressed as the dose of isoproterenol that increased the heart rate by 25 beats/min. Blood pressure was recorded at baseline, at 2 min after injection, and at 5 min after injection, at which time heart rate had returned to baseline.

### Blood samples and analytical procedures

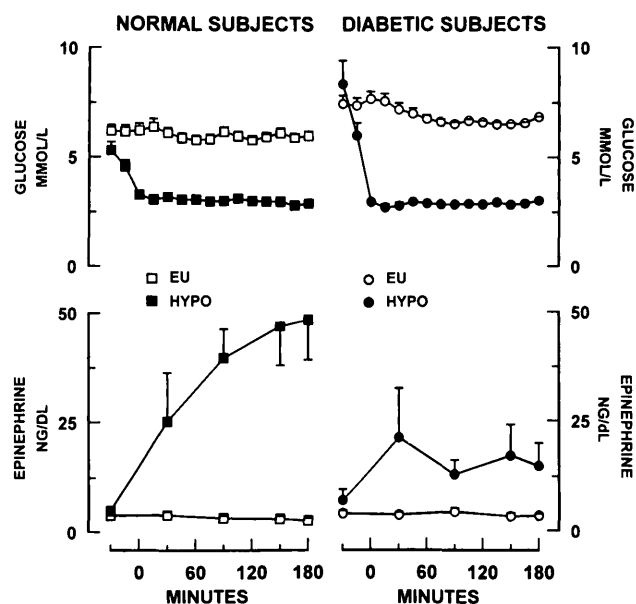
Plasma epinephrine, norepinephrine, free fatty acids, and insulin were measured at baseline and at 30, 90, 150, and 180 min during the clamps.

During the IT, blood glucose and free fatty acids were measured at 10-min intervals, 5 min before and 5 min after each injection. Plasma insulin was measured at baseline, before the 1  $\mu$ g injection, and at the end of the IT.

Blood glucose was determined bedside with a HemoCue blood glucose photometer (HemoCue AB, Angelham, Sweden) with an average difference of <0.1 mmol/l compared with a Beckman analyzer in the hypoglycemic range (19). Catecholamines were measured from EDTA plasma by high-performance liquid chromatography with electrochemical detection (ClinRep Analysis Kit; Recipe Pharma, Munich, Germany) with an interassay coefficient of variation (CV) of 2.9% for epinephrine and 3.2% for norepinephrine and an intra-assay CV of 2.5% for epinephrine and 2.8% for norepinephrine. Free fatty acids were measured from NaF plasma by enzyme-linked immunosorbent assay (ELISA) (Wako Chemicals, Hamburg, Germany) with a CV of 1.1–2.7%. Serum-insulin levels were measured by ELISA (Enzymun Test Insulin; Boehringer Diagnostica, Mannheim, Germany) with an interassay CV of 2.1–5.2% and an intra-assay CV of 1.7–4.6%.

### Statistics

Effects on  $\beta$ -adrenergic sensitivity were tested by multivariate analysis of variance (MANOVA) with repeated measures design



**Figure 1**—Blood glucose and plasma epinephrine levels during euglycemic and hypoglycemic clamps in nondiabetic and diabetic subjects.

containing the between-subjects factors group (type 1 diabetes versus normal subjects), the within-subjects factors time, the clamp (hypoglycemic versus euglycemic), and the interactions of these factors. Differences in hormone levels and symptom scores during the interventional clamps were tested analogously. The doses of intravenous isoproterenol required to increase the heart rate by 25 beats/min (IC25s) were compared with paired *t* tests.

Unless otherwise stated, data are means  $\pm$  SEM. *P* values calculated with MANOVA are marked with an asterisk.

## RESULTS

### Responses during hypoglycemic and euglycemic clamp

Plasma glucose and insulin levels were comparable during euglycemic and hypoglycemic clamps in both groups (Fig. 1 and

Table 2). Plasma glucose and epinephrine levels are shown in Fig. 1. During hypoglycemic clamp, plasma epinephrine increased to a greater extent in the nondiabetic subjects ( $P^* = 0.02$ ), whereas increases in plasma norepinephrine levels were comparable in both groups ( $P^* = 0.60$ ) (data not shown).

Both autonomic and neuroglycopenic symptoms increased during hypoglycemia, and there was no significant difference between nondiabetic and diabetic subjects ( $P^* = 0.25$ ) (Table 2). There was a correlation between levels of catecholamines and autonomic symptoms during hypoglycemic clamps in nondiabetic subjects ( $R = 0.68$ ,  $P = 0.03$ ) but not in diabetic subjects ( $R = 0.07$ ,  $P = 0.8$ ).

### IT

Baseline heart rates after euglycemic clamp were  $63 \pm 2$  and  $54 \pm 2$  beats/min in the

nondiabetic and diabetic subjects, respectively ( $P = 0.01$ ). Baseline heart rates after the hypoglycemic clamp were  $60 \pm 2$  and  $58 \pm 3$  beats/min in the nondiabetic and diabetic subjects, respectively ( $P = 0.6$ ). After the hypoglycemic clamps, heart rate decreased in the nondiabetic subjects ( $P = 0.01$ ) and increased in the diabetic subjects ( $P = 0.04$ ). In nondiabetic subjects, increases in heart rate during the IT were significantly greater after the hypoglycemic clamp ( $P^* = 0.047$ ) (Fig. 2). The IC25 was reduced after the hypoglycemic clamp ( $0.83 \pm 0.22$  vs.  $1.13 \pm 0.21$   $\mu$ g,  $P = 0.02$ ), indicating an increase in  $\beta$ -adrenergic sensitivity.

In contrast, in the type 1 diabetic subjects, increases in heart rate during the IT were significantly reduced after the hypoglycemic clamp ( $P^* = 0.042$ ) (Fig. 2). The IC25 was greater after the hypoglycemic clamp ( $0.65 \pm 0.14$  vs.  $1.00 \pm 0.26$   $\mu$ g,  $P = 0.04$ ), indicating reduced  $\beta$ -adrenergic sensitivity.

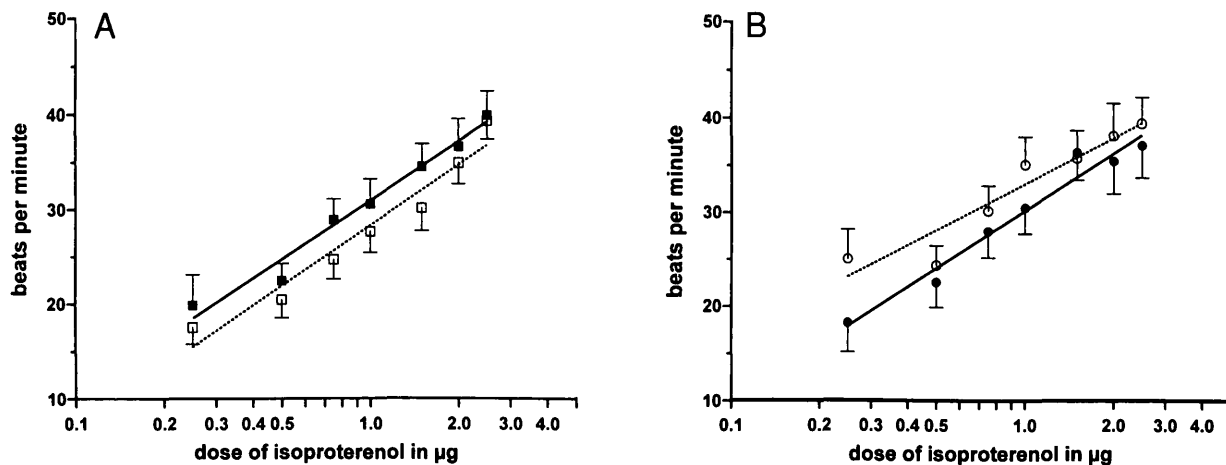
The increase in heart rate during the IT was not linearly correlated either with age in nondiabetic and diabetic subjects ( $R = 0.01$ ,  $P = 0.95$ ) or with HbA<sub>1c</sub> ( $R = 0.19$ ,  $P = 0.6$ ) and diabetes duration ( $R = 0.58$ ,  $P = 0.08$ ) in diabetic subjects. There was also no correlation between levels of catecholamines during hypoglycemic clamps and the IC25 during the IT in either group ( $R = 0.15$ ,  $P = 0.5$ ).

There was a significant rise in blood pressure in all subjects ( $P < 0.001$ ) during the IT. There were, however, no significant differences between blood pressure during ITs after hypoglycemia or euglycemia ( $P = 0.9$ ) or between diabetic or nondiabetic subjects ( $P = 0.9$ ). The rise in blood pressure from baseline during the IT did not differ after hypoglycemia or euglycemia ( $P = 0.4$ ) or between diabetic or nondiabetic subjects ( $P = 0.7$ ). In the nondiabetic subjects, during the IT, systolic and diastolic blood pressures averaged  $114 \pm 3$  and  $64 \pm 2$  mmHg after euglycemia and  $116 \pm 4$  and

**Table 2**—Metabolic and symptomatic responses during euglycemic and hypoglycemic clamps

	Nondiabetic subjects				Diabetic subjects			
	Euglycemic		Hypoglycemic		Euglycemic		Hypoglycemic	
	Baseline	Clamp	Baseline	Clamp	Baseline	Clamp	Baseline	Clamp
Blood glucose (mmol/l)	6.0 $\pm$ 0.2	6.0 $\pm$ 0.2	5.9 $\pm$ 0.2	3.0 $\pm$ 0.3†	6.0 $\pm$ 0.2	6.8 $\pm$ 0.1	6.0 $\pm$ 0.2	2.9 $\pm$ 0.0†
Insulin (pmol/l)	189 $\pm$ 28	463 $\pm$ 49†	135 $\pm$ 23	357 $\pm$ 29†	190 $\pm$ 31	569 $\pm$ 106†	218 $\pm$ 41	568 $\pm$ 105†
Epinephrine (ng/dl)	3.8 $\pm$ 0.4	3.2 $\pm$ 0.4	4.8 $\pm$ 0.7	40.7 $\pm$ 6.8†	4.7 $\pm$ 0.7	3.8 $\pm$ 0.5	7.1 $\pm$ 2.6	16.7 $\pm$ 5.0*
Autonomic symptoms	0.2 $\pm$ 0.13	1.0 $\pm$ 0.11†	0.2 $\pm$ 0.13	4.7 $\pm$ 0.82†	0.5 $\pm$ 0.13	0.63 $\pm$ 0.11	0.6 $\pm$ 0.13	2.93 $\pm$ 0.82†
Neuroglycopenic symptoms	0.3 $\pm$ 0.3	1.03 $\pm$ 0.26*	0.2 $\pm$ 0.13	3.05 $\pm$ 0.7†	0.4 $\pm$ 0.3	0.75 $\pm$ 0.26	1.2 $\pm$ 0.13	3.18 $\pm$ 0.7†

Data are means  $\pm$  SEM. \* $P < 0.05$ , † $P < 0.001$  during clamp vs. baseline.



**Figure 2**—A: Increase of heart rate during IT (semilogarithmic scale) in nondiabetic subjects. □, IT after euglycemia; ■, IT after hypoglycemia. B: Increase of heart rate during IT (semilogarithmic scale) in diabetic subjects. ○, IT after euglycemia; ●, IT after hypoglycemia.

65 ± 2 mmHg after hypoglycemia. In the diabetic subjects, systolic and diastolic blood pressures averaged 116 ± 3 and 63 ± 2 mmHg after euglycemia and 115 ± 3 and 62 ± 2 mmHg after hypoglycemia.

Blood glucose and insulin levels during the IT were not significantly different after the hypoglycemic or euglycemic clamps in normal subjects (Table 3). Plasma free fatty acids during the IT were greater after hypoglycemic clamp in normal subjects ( $P^* = 0.035$ ). In diabetic subjects, blood glucose was clamped during the IT with no difference observed in blood glucose, insulin, and free fatty acid levels after hypoglycemia or euglycemia. However, blood glucose and insulin levels were significantly ( $P^* < 0.001$ ) greater in diabetic subjects compared with those of the nondiabetic subjects.

**CONCLUSIONS** — The present studies were undertaken to test the hypothesis that hypoglycemia could diminish  $\beta$ -adrenergic sensitivity and thus provide at least a partial explanation for the induction of reduced awareness of hypoglycemia by hypoglycemia that occurs in patients with type 1 diabetes (1,20,21) and insulinoma patients (22). For this purpose, we assessed the effects of a single episode of nocturnal hypoglycemia on  $\beta$ -adrenergic sensitivity in nondiabetic volunteers and in type 1 diabetic subjects with apparent normal awareness of hypoglycemia. We used a standard IT in which the IC25 is taken as an index of  $\beta$ -adrenergic sensitivity (15).

After the episode of nocturnal hypoglycemia, normal volunteers increased their  $\beta$ -adrenergic sensitivity as manifested by a

decrease in the IC25 from 1.13 ± 0.21 to 0.83 ± 0.22  $\mu\text{g}$  ( $P < 0.05$ ). This is consistent with our finding of a greater increment in plasma fatty acid levels during the IT after the hypoglycemic clamp. These results suggest that antecedent hypoglycemia, or at least a single episode, which has been shown to reduce plasma epinephrine responses and awareness, does not normally reduce  $\beta$ -adrenergic sensitivity and may in fact increase it. This could involve upregulation of  $\beta$ -receptors in normal volunteers due to increases in plasma cortisol during hypoglycemia (23), since glucocorticoids have been shown to increase  $\beta$ -receptor gene expression in smooth muscle cell cultures (24).

It had been previously reported that after a brief hypoglycemia in normal volunteers induced by a bolus injection of insulin,  $\beta$ -adrenergic sensitivity is decreased (25). In that study, however, hypoglycemia was not standardized, and circulating epinephrine levels were still markedly increased during the IT. These elevated catecholamine levels

could have desensitized cardiac  $\beta$ -adrenoceptors (26).

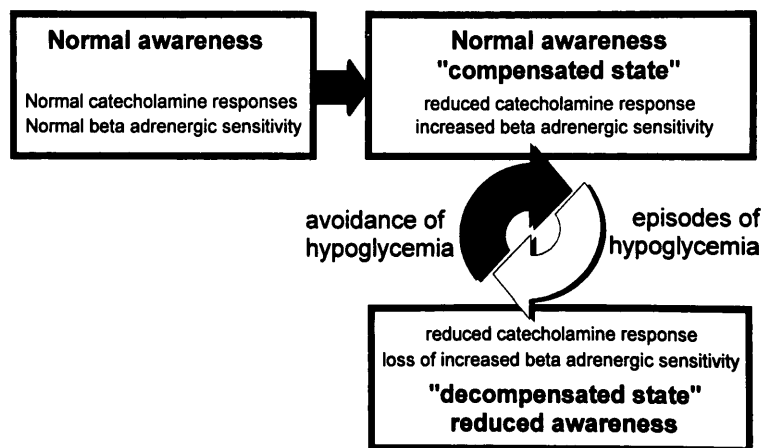
In our control experiments (nocturnal euglycemic hyperinsulinemic clamps), the diabetic subjects actually had somewhat greater  $\beta$ -adrenergic sensitivity than the nondiabetic subjects, as reflected by their respective IC25 (0.65 ± 0.14 vs. 1.13 ± 0.21  $\mu\text{g}$ ,  $P = 0.07$ ). These same subjects had reduced plasma epinephrine responses during the hypoglycemic clamps. These findings suggest that the diabetic subjects may have been compensating for a reduced epinephrine response with increased  $\beta$ -adrenergic sensitivity to maintain normal awareness of hypoglycemia. Indeed, despite reduced epinephrine responses, autonomic symptoms during the hypoglycemic clamps were normal in the diabetic subjects.

In contrast to the results in nondiabetic volunteers, an episode of hypoglycemia reduced  $\beta$ -adrenergic sensitivity in the diabetic subjects, as reflected by the increase in their IC25 from 0.65 ± 0.14 to 1.00 ± 0.26  $\mu\text{g}$  ( $P < 0.05$ ). This opposite response in

**Table 3**—Results of IT after hypoglycemia and euglycemia

	Nondiabetic subjects		Diabetic subjects	
	After hypoglycemia	After euglycemia	After hypoglycemia	After euglycemia
Blood glucose (mmol/l)	4.8 ± 0.2	4.9 ± 0.6	7.9 ± 0.4	8.5 ± 0.4
Plasma insulin (pmol/l)	78 ± 7	74 ± 0.7	165 ± 0.41	151 ± 30
Plasma FFA (mmol/l)	0.65 ± 0.08	0.52 ± 0.07*	0.64 ± 0.08	0.61 ± 0.1
IC25	0.83 ± 0.22	1.13 ± 0.21*	1.00 ± 0.26	0.65 ± 0.14*

Data are means ± SEM. \* $P < 0.05$  (paired  $t$  test) hypoglycemia vs. euglycemia. IC25: normal versus diabetic subjects after hypoglycemia,  $P = 0.6$  (unpaired  $t$  test); after euglycemia,  $P = 0.07$  (unpaired  $t$  test). FFA, free fatty acid.



**Figure 3**—Hypothesis for the pathogenesis of hypoglycemia unawareness in type 1 diabetic patients.

the diabetic subjects suggests that some maladaptation of  $\beta$ -adrenergic sensitivity to hypoglycemia may play a role in the development of hypoglycemia unawareness.

Regarding the mechanisms for this phenomenon, one possibility is that a plasma catecholamine response of a certain threshold magnitude during hypoglycemia is necessary to produce the enhanced  $\beta$ -adrenergic sensitivity observed in the nondiabetic volunteers, and once diabetic patients have responses below this threshold, they are unable to increase their  $\beta$ -adrenergic sensitivity. Loss of this ability could ultimately lead to development of unawareness with repeated episodes of hypoglycemia.

Another possibility is that loss of the ability to increase  $\beta$ -adrenergic sensitivity after hypoglycemia is merely another early component of the hypoglycemia unawareness syndrome occurring in diabetes, e.g., chronic hyperglycemia could alter  $\beta$ -adrenergic sensitivity. A third possibility is that the failure of hypoglycemia to increase  $\beta$ -adrenergic sensitivity was somehow related to the fact that the diabetic subjects were already compensating for reduced epinephrine response with enhanced  $\beta$ -adrenergic sensitivity. Repeated episodes of hypoglycemia could exhaust this compensatory mechanism.

The first and second possibilities proposed above do not provide an explanation for the reversal of hypoglycemia unawareness with avoidance of hypoglycemia, since plasma catecholamine responses are not increased under these circumstances. The third possibility does offer a potential explanation.

Based on these considerations, we offer the following hypotheses for the pathogen-

esis of hypoglycemia unawareness (Fig. 3). Initially, as an intrinsic element of type 1 diabetes related to its duration, there is a progressive reduction in plasma catecholamine responses to hypoglycemia (27). At this stage, hypoglycemia unawareness does not occur because of an increase in  $\beta$ -adrenergic sensitivity. However, with repeated episodes of hypoglycemia, this compensatory response is lost, and at this stage hypoglycemia unawareness ensues. This loss could be limited to a subpopulation destined to develop hypoglycemia unawareness or could be generalized. Elimination of frequent hypoglycemic episodes can lead to recovery of the ability to increase  $\beta$ -adrenergic sensitivity and restoration of awareness.

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