Preserved Sensitivity to β_2-Adrenergic Receptor Agonists in Patients with Type 1 Diabetes Mellitus and Hypoglycemia Unawareness

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Background and Objective: Use of β_2-adrenergic receptor agonists has been advocated for the treatment of hypoglycemia unawareness in type 1 diabetes. *In vitro*, however, hypoglycemia unawareness has been associated with reduced β_2-adrenergic sensitivity. Therefore, *in vivo* sensitivity to β_2-adrenergic receptor agonist stimulation was compared between type 1 diabetic patients with and without hypoglycemia unawareness and nondiabetic controls.

Methods: Ten type 1 diabetic patients with hypoglycemia unawareness, 12 type 1 diabetic patients with intact hypoglycemic awareness, and 11 healthy controls were enrolled. β_2-Adrenergic sensitivity was determined by measuring the forearm vasodilator response to intraarterial infusion of salbutamol. Salbutamol was infused in six increasing doses ranging from 0.003 to 1.0 $\mu g^1 \cdot min^{-1} \cdot dl^{-1}$. Forearm blood flow (FBF) was bilaterally measured by venous occlusion plethysmography. Diabetic patients received low-dose insulin before FBF measurements to ensure that experiments were carried out under normoglycemic conditions.

Results: At baseline, FBF was 1.9 ± 0.3 ml$^1 \cdot$min$^{-1} \cdot$dl^{-1} in controls, 2.3 ± 0.4 ml$^1 \cdot$min$^{-1} \cdot$dl^{-1} in patients with intact awareness, and 1.4 ± 0.1 ml$^1 \cdot$min$^{-1} \cdot$dl^{-1} in patients with hypoglycemia unawareness ($P = 0.048$ *vs.* aware patients). In response to salbutamol, FBF increased 9.1-fold in controls, 8.0-fold in patients with intact awareness, and 10.7-fold in patients with hypoglycemia unawareness ($P =$ NS). Heart rate increased in all groups due to systemic spillover of salbutamol but appeared blunted, considering a greater fall in mean arterial pressure in patients with hypoglycemia unawareness.

Conclusions: Sensitivity to β_2-adrenergic receptor agonist stimulation is preserved in type 1 diabetic patients with hypoglycemia unawareness. (*J Clin Endocrinol Metab* **91: 2878–2881, 2006**)

PATIENTS WITH TYPE 1 diabetes mellitus (T1DM) are at a continuous risk of iatrogenic hypoglycemia as a result of insulin treatment, especially in a setting of blunted glucose counterregulatory defenses and hypoglycemia unawareness. In turn, hypoglycemia unawareness occurs as a consequence of repeated iatrogenic hypoglycemic events and is closely linked to defects in hormonal counterregulation, in particular to the blunted adrenaline response [1]. To stimulate adrenergic action, treatment with β_2-adrenergic agonists has been advocated to prevent nocturnal hypoglycemia clinically [2] and support swift recovery from hypoglycemia [3]. However, the applicability of such treatment for patients with hypoglycemia unawareness might be offset when sensitivity to adrenergic stimulation is reduced. Various groups have reported reduced β-adrenergic sensitivity in hypoglycemia-unaware T1DM patients when compared with control and hypoglycemia-aware diabetic subjects, based on reduced cardiac chronotropic responses to isoproterenol [4–7]. Although reversal of reduced β-adrenergic sensitivity could be achieved when hypoglycemias were meticulously avoided [7], a single nocturnal hypoglycemic event was sufficient to decrease β-adrenergic sensitivity in T1DM patients with intact hypoglycemic awareness, whereas it increased in nondiabetic controls [8].

There is evidence from *in vitro* studies that the reduced β-adrenergic sensitivity associated with hypoglycemia unawareness is mediated through the β_2-adrenergic receptor. In hypoglycemia-unaware T1DM patients with a reduced chronotropic response to iv isoproterenol, β_2-adrenergic receptors on mononuclear leukocytes were found to express reduced affinity for isoproterenol [9]. In another study, 1 wk of intensive insulin treatment reduced β_2-adrenoceptor density on lymphocytes in T1DM, the magnitude of which correlated highly with the number of hypoglycemic events and with loss of hypoglycemic awareness [10]. These data contrast with an *in vivo* study investigating the effect of local perfusion with terbutaline, a β_2-adrenergic agonist, through a microdialysis catheter [11]. In that study, terbutaline-induced lipolysis and stimulation of adipose and skeletal muscle tissue blood flow were not found to differ between intensively treated (presumably hypoglycemia unaware) T1DM patients and control subjects. However, blood flow was assessed indirectly, using a model that can be applied only under the assumption of steady-state conditions [12].

Other than by *in vitro* [9, 10] or indirect [11] assessment, the sensitivity of the β_2-adrenergic receptor has not been tested quantitatively in T1DM with hypoglycemia unawareness by a validated *in vivo* technique. The purpose of the present study was to compare β_2-adrenergic sensitivity *in vivo* in T1DM patients with hypoglycemia unawareness to that in T1DM patients with normal hypoglycemic awareness and nondiabetic controls. To quantify β_2-adrenergic sensitivity, we measured the vasodilator action of salbutamol, a selective β_2-adrenergic receptor agonist, using the perfused forearm technique [13].

Subjects and Methods

Subjects

Written informed consent was obtained from 22 T1DM patients recruited from the outpatient clinic of our hospital and 11 healthy controls recruited by advertisement. All diabetic subjects were free of classical long-term diabetic complications, except background retinopathy. Autonomic neuropathy was excluded by normal responses to cardiovascular reflex tests (*i.e.* heart rate response to Valsalva maneuver, heart rate variability to deep breathing, and blood pressure responses to standing up and sustained handgrip) (14). The magnitude of hypoglycemic awareness was assessed on the basis of the score on a Dutch modification of a standardized hypoglycemia questionnaire (15). Patients with a score less than 3 (of maximal 10) were classified as being hypoglycemia aware (n = 12), and patients with higher scores were classified as hypoglycemia unaware (n = 10). Three of the latter had previously participated in a trial in which their inability to detect hypoglycemia was objectified by a hypoglycemic clamp test (16). All participants had a normal blood pressure and used no medication other than insulin or oral contraceptives, except for one nondiabetic and one diabetic subject who were on stable T_4 supplementation therapy for longer than 1 yr. Seven subjects were current smokers, one in the control group and three in each of the diabetic groups. All participants were requested to abstain from caffeine-containing substances and smoking for at least 48 h, from alcohol for at least 24 h, and from food intake at least 10 h before experiments took place. Diabetic subjects were asked to perform blood glucose self-measurements at least four times per day for at least 5 d preceding the experiment and to reduce the bedtime insulin dose by 20% on the preceding day to avoid nocturnal hypoglycemia. All patients checked capillary glucose at approximately 0200 h, and in case of nocturnal hypoglycemia, the test was canceled or postponed. The Radboud University Nijmegen Medical Centre Medical Ethics Committee approved the study.

Procedure

All experiments took place in the morning in a quiet, temperature-controlled room (23–24 C), with the subjects supine. The brachial artery of the nondominant arm was cannulated (Angiocath 20-gauge; Beckton Dickinson, Sandy, UT) under local anesthesia (Xylocaine 2%) for infusion of salbutamol (Ventolin; GlaxoSmithKline, Zeist, The Netherlands) and blood pressure monitoring (monitor 378341A; Hewlett Packard GmbH, Böblingen, Germany). Intraarterial infusion rates were calculated per deciliter forearm volume, measured by water displacement. In the diabetic subjects, an antecubital vein of the contralateral arm was cannulated for administration of insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) and glucose 20% (if necessary). After cannulation(s), a 30-min equilibration period was allowed to pass before baseline variables were obtained. A low-dose iv insulin infusion of 7–10 mU/min·m^{-2} body surface area was initiated to obtain normoglycemia in the diabetic subjects. Because of insulin's (modest) vasodilator effect (17), the insulin infusion was terminated as soon as plasma glucose levels fell to less than 7.0 mmol/liter; glucose 20% was given as necessary to prevent hypoglycemia.

The ensuing experimental procedure was similar for all participants. At constant flow rates, 5-min infusions of saline and incremental doses of salbutamol diluted in saline vehicle (0.003, 0.01, 0.03, 0.1, 0.3, 1.0 μg·min^{-1}·dl^{-1}) were subsequently administered intraarterially. Forearm blood flow (FBF) was measured during the final 2 min of each dosing step in both arms, using electrocardiogram-triggered mercury-in-silastic (Dow Corning, Midland, MI) strain gauge venous occlusion plethysmography, as described previously (13). The mean of six to eight FBF measurements was used for data analysis. Hand circulation was eliminated during FBF measurements by wrist cuffs inflated 100 mm Hg above systolic blood pressure. The succeeding salbutamol doses were interrupted once by a 15-min drug-free interval for deflation of the wrist cuffs to allow recovery of hand circulation.

Analytical methods

Arterial plasma glucose levels were determined in duplicate by the glucose oxidation method (Beckman Glucose Analyzer II; Beckman, Fullerton, CA). Plasma insulin levels were determined by RIA (16). Hemoglobin A_{1c} (HbA_{1c}) was measured using HPLC (Bio-Rad Laboratories, Veenendaal, The Netherlands) with reference values of 4.8–6.2%.

Calculations and statistical analyses

Vasodilator responses to salbutamol were expressed as absolute FBF and as increase in FBF above baseline values (ΔFBF). The effects of salbutamol on FBF and hemodynamic variables were analyzed by repeated-measures ANOVA. Differences in means were tested by Student's *t* test. For data that had no normal distribution, the Wilcoxon signed rank test and Mann-Whitney *U* test were used to compare paired and unpaired data, respectively. The χ^2 test was used to compare the male-female distribution of the study population. The SPSS personal computer software package (version 12.0; SPSS, Chicago, IL) was used for statistical analyses. $P < 0.05$ was considered statistically significant. Data are presented as means $\pm$ SEM unless otherwise specified.

Results

Characteristics of the participants are shown in Table 1. As a group, the diabetic patients were slightly older and had higher heart rate and blood pressure at baseline than the control subjects. There was a preponderance of males among the unaware diabetic patients and a preponderance of females among the aware diabetic patients, but this difference did not reach statistical significance. HbA_{1c} and fasting plasma glucose values were lower and disease duration was longer in T1DM patients with hypoglycemia unawareness, compared with patients with intact awareness. Insulin levels were significantly higher in diabetic patients than controls at all time points (Table 2) but despite intermittent insulin infusion remained relatively stable for the duration of the experiment. Plasma glucose levels, albeit significantly higher than in controls, remained around the upper value for the normoglycemic range in the diabetic patients during FBF measurements.

The vascular response to salbutamol

Baseline values for FBF in the infusion arm were 1.9 $\pm$ 0.3 ml·min^{-1}·dl^{-1} in control subjects, 2.3 $\pm$ 0.4 ml·min^{-1}·dl^{-1} in hypoglycemia-aware T1DM patients (P = NS), and 1.4 $\pm$ 0.1 ml·min^{-1}·dl^{-1} in hypoglycemia-unaware patients (P = 0.048 *vs.* aware patients). Corresponding values in the noninfused arm were 1.9 $\pm$ 0.2, 2.0 $\pm$ 0.2, and 1.6 $\pm$ 0.2 ml·min^{-1}·dl^{-1} (P = NS). The lowering of plasma glucose levels in the diabetic patients did not affect FBF in either arm (data not shown). Maximal FBF responses to salbutamol in the infused arm were 14.9 $\pm$ 1.3 ml·min^{-1}·dl^{-1} in controls, 14.6 $\pm$ 1.3 ml·min^{-1}·dl^{-1} in hypoglycemia-aware diabetic patients, and 13.8 $\pm$ 1.5 ml·min^{-1}·dl^{-1} in hypoglycemia-unaware diabetic patients (Fig. 1), corresponding to 9.1-, 8.0-, and 10.7-fold increases, respectively ($P < 0.001$ for all groups). There were no statistically significant differences between the groups for

TABLE 1. Baseline characteristics

	Controls	T1DM aware	T1DM unaware
No. (male/female)	11 (6/5)	12 (4/8)	10 (7/3)
Age (yr)	28.3 $\pm$ 8.1	33.3 $\pm$ 11.5	38.8 $\pm$ 9.7[a]
BMI (kg/m^2)	24.2 $\pm$ 2.5	25.0 $\pm$ 1.9	23.8 $\pm$ 2.3
Heart rate (bpm)	58 $\pm$ 5	69 $\pm$ 9[a]	63 $\pm$ 9
MAP (mm Hg)	79 $\pm$ 11	90 $\pm$ 9[a]	93 $\pm$ 11[a]
HbA_{1c} (%)	ND	8.7 $\pm$ 1.2	7.6 $\pm$ 0.7[b]
Duration of diabetes (yr)		11.6 $\pm$ 5.2	19.7 $\pm$ 6.2[b]
Insulin dose (U/kg)		0.72 $\pm$ 0.24	0.68 $\pm$ 0.17

Data are in number or means $\pm$ SD. ND, Not determined.
[a] $P < 0.05$ *vs.* controls.
[b] $P < 0.05$ *vs.* T1DM aware.

TABLE 2. Plasma glucose and insulin levels

	Controls	T1DM aware	T1DM unaware
Glucose level (mg/dl)			
Baseline	92 ± 8	243 ± 72^a	$211 \pm 42^{a,b}$
Prior to test	92 ± 8	108 ± 16^a	111 ± 17^a
End of test	92 ± 8	125 ± 21^a	131 ± 29^a
Insulin level (μU/ml)			
Baseline	9 ± 4	30 ± 43^a	30 ± 20^a
Prior to test	13 ± 5	27 ± 14^a	29 ± 17^a
End of test	10 ± 4	21 ± 12^a	21 ± 14^a

Data are means $\pm$ SD. To convert plasma glucose values to millimoles per liter, divide by 18; to convert plasma insulin values to picomoles per liter, multiply by 6.

[a] $P < 0.05$ *vs.* controls.
[b] $P < 0.05$ *vs.* T1DM aware.

either the maximal FBF response or the course of FBF ($P = 0.7$ by ANOVA). Comparable results were obtained when the data were expressed as absolute or relative changes in FBF from baseline (ΔFBF).

In both controls and hypoglycemia-aware T1DM patients, FBF in the noninfused arm increased 1.5-fold in response to the highest salbutamol dose to 3.0 ± 0.6 ml·min^{-1}·dl^{-1} in the first ($P = 0.007$) and 2.6 ± 0.3 ml·min^{-1}·dl^{-1} in the latter ($P = 0.043$), indicating a systemic effect. In T1DM patients with hypoglycemia unawareness, salbutamol did not affect FBF in the noninfused arm. Heart rate increased by 13 ± 2, 11 ± 2, and 9 ± 2 beats per minute (bpm) in controls, hypoglycemia-aware, and hypoglycemia-unaware T1DM patients, respectively ($P < 0.001$ for all groups), whereas mean arterial pressure (MAP) decreased in unaware diabetic patients (-5 ± 3 mm Hg, $P = 0.001$) and controls (-3 ± 2 mm Hg, $P = 0.005$) but not in patients with intact awareness. Despite within-group effects, the course of FBF in the noninfused arm and heart rate and MAP did not differ among the three groups when tested by ANOVA.

Stratification according to glycemic control and duration of disease

Stratification of the diabetic patients (n = 22) according to HbA$_{1c}$ value yielded a low-HbA$_{1c}$ group [mean HbA$_{1c}$ 7.1%
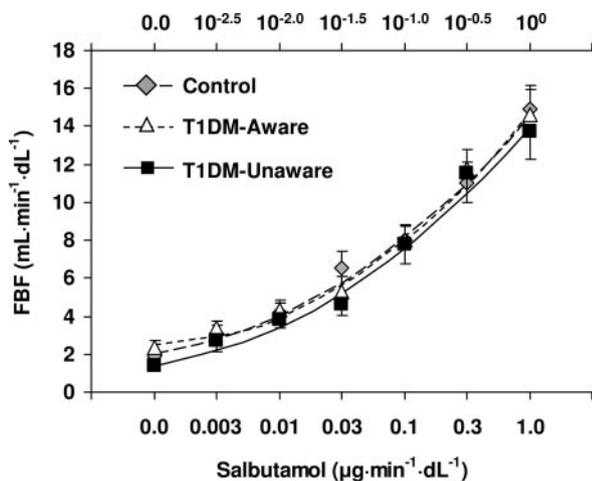

FIG. 1. Local FBF in the experimental arm during intraarterial infusion of salbutamol. FBF increased in the experimental arms in all groups ($P < 0.001$) but to similar extent ($P = $ NS between groups).

(range 6.0–7.9), n = 8], a middle-HbA$_{1c}$ group [8.1% (8.0–8.3%), n = 7], and a high-HbA$_{1c}$ group [9.5% (8.7–11.4%), n = 7]. In response to salbutamol, FBF increased from 1.7 ± 0.5 to 13.5 ± 1.4 ml·min^{-1}·dl^{-1} in the low-HbA$_{1c}$ group, from 1.6 ± 0.3 to 14.0 ± 2.0 ml·min^{-1}·dl^{-1} in the middle-HbA$_{1c}$ group, and from 2.4 ± 0.5 to 15.3 ± 1.7 ml·min^{-1}·dl^{-1} in the high-HbA$_{1c}$ group ($P = $ NS by ANOVA). When recalculating FBF responses according to disease duration, FBF increased from 1.9 ± 0.3 to 15.4 ± 1.3 ml·min^{-1}·dl^{-1} in patients with a mean disease duration of 9.9 (range 3–15) yr and from 1.9 ± 0.4 to 13.1 ± 1.4 ml·min^{-1}·dl^{-1} in patients with a mean disease duration of 20.6 (17–33) yr ($P = $ NS by ANOVA).

Discussion

Reduced β-adrenergic sensitivity has been reported in T1DM patients with hypoglycemia unawareness (9, 10). The present study was conducted to test the involvement of the β_2-adrenergic receptor *in vivo*. Therefore, the vasodilator response to local administration of salbutamol was compared among T1DM patients with hypoglycemia unawareness, T1DM patients with intact hypoglycemic awareness, and nondiabetic controls. Our finding of similar vasodilator responses does not support a role for the β_2-adrenergic receptor in reduced β-adrenergic sensitivity. When the data were expressed as fold increase from baseline, salbutamol elicited even higher responses in unaware diabetic patients than the other two groups, although these differences did not reach statistical significance.

Our data extend those obtained previously with microdialysis (11) but are at variance with *in vitro* studies reporting reduced β_2-adrenoceptor-mediated action in hypoglycemia-unaware T1DM patients on basis of reduced β_2-adrenergic receptor density or affinity for isoproterenol on white blood cells (9, 10). However, alterations in β_2-adrenoceptor density or binding affinity on white blood cells do not necessarily reflect those on other tissues, such as the vascular wall. In addition, white blood cells may not be a stable population, especially after stress hormone release. Lymphocytes, granulocytes, and monocytes of various subsets that differ according to receptor density and binding affinity from cells already in the circulation can be mobilized by catecholamines and cortisol (18). In contrast, the perfused forearm technique is a validated *in vivo* method to quantify tissue sensitivity to vasoactive substances (10). Moreover, our findings remained unaltered when the diabetic patients were stratified according to tightness of glycemic control as crude marker of (the risk for) hypoglycemia unawareness.

It is unlikely that the higher insulin levels in the two diabetic groups affected our data. First, a significant vasodilator effect of insulin requires 4-fold higher plasma concentrations than those achieved here (17); second, insulin does not exert its vasodilator effects by modulation of β-adrenergic receptors (19). Moreover, insulin levels were identical in the two diabetic groups throughout the experiments, and baseline FBF, although mutually different, was not dissimilar from that in controls.

Our study had some limitations. First of all, the groups were incompletely matched for sex, age, and disease duration. The effect of the latter two parameters on β_2-adrenergic

sensitivity is probably negligible: vasodilator responses to β-adrenergic stimulation are age independent (20), and stratification of our data according to disease duration did not reveal an effect of diabetes *per se*. However, β_2-adrenergic sensitivity may differ by sex, with women probably having a more profound responsiveness to β_2-adrenergic stimulation than men (21). In the present study, women were overrepresented in the hypoglycemia-aware diabetic group and underrepresented in the unaware diabetic group, although this difference did not reach statistical significance. A *post hoc* analysis of our data by gender did not reveal differences in β_2-adrenergic sensitivity between men and women in either the control or diabetic group or when all subjects were pooled (data not shown), although it should be acknowledged that our study was not designed for that purpose. Yet even when gender would have had an effect, a more balanced matching according to gender would have resulted in greater, not smaller, salbutamol responsiveness in the T1DM patients with hypoglycemia unawareness.

Previous *in vivo* studies on β-adrenergic sensitivity in diabetes used the isoproterenol sensitivity test (4–8), in which β-adrenergic sensitivity is expressed as the dose of iv isoproterenol that produces an increment in heart rate of 25 bpm over baseline values. This heart rate increment is the consequence of direct stimulation of cardiac β_1- (and β_2-) adrenoceptors and an indirect baroreflex response to β_2-adrenoceptor-mediated peripheral vasodilatation (22). When reconciling data from studies using the isoproterenol test with that of the current study, it seems plausible that hypoglycemia-associated reduction in β-adrenergic sensitivity is mediated by the β_1-adrenergic receptor. In our study, the heart rate response to systemic spillover of salbutamol in T1DM patients with hypoglycemia unawareness appeared blunted in view of the greater fall in blood pressure, which lends support to this suggestion. However, subtle impairments in baroreflex sensitivity, only to be detected by spectral analysis (23, 24) but not by conventional tests, have been found to correlate with diabetes duration. Because disease duration was longer in patients with hypoglycemia unawareness, compared with patients with intact awareness in both the current study and studies using the isoproterenol test (4–6), a contribution of reduced baroreflex sensitivity to the lower heart rate response cannot be excluded.

In conclusion, sensitivity to β_2-adrenergic agonist stimulation is not reduced in T1DM patients with hypoglycemia unawareness. This observation may be of potential value when treatment with β_2-adrenergic agonists is considered to support glucose counterregulation. Whether reduced β-adrenergic sensitivity in hypoglycemia unawareness is mediated through the β_1-adrenergic receptor or impairments in the baroreflex response pathway requires further study.

Acknowledgments

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