Preserved Sensitivity to $\beta_2$-Adrenergic Receptor Agonists in Patients with Type 1 Diabetes Mellitus and Hypoglycemia Unawareness

Bastiaan E. De Galan, Pieter De Mol, Lianne Wennekes, Bas J. J. Schouwenberg, and Paul Smits

Departments of General Internal Medicine (B.E.D.G., B.J.J.S., P.S.) and Pharmacology-Toxicology (P.D.M., L.W., P.S.), Radboud University Nijmegen Medical Centre, 6500 HB, Nijmegen, The Netherlands

Background and Objective: Use of $\beta_2$-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 $\beta_2$-adrenergic sensitivity. Therefore, in vivo sensitivity to $\beta_2$-adrenergic receptor agonist stimulation was compared between type 1 diabetic patients with and without hypoglycemia unawareness and non-diabetic 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. $\beta_2$-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.min$^{-1}$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.min$^{-1}$dl$^{-1}$ in controls, 2.3 ± 0.4 ml.min$^{-1}$dl$^{-1}$ in patients with intact awareness, and 1.4 ± 0.1 ml.min$^{-1}$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 $\beta_2$-adrenergic receptor agonist stimulation is preserved in type 1 diabetic patients with hypoglycemia unawareness.

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Subjects and Methods

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 on 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 standardised hypoglycaemia questionnaire (15). Patients with a score less than 3 (of maximal 10) were classified as being hypoglycaemia aware (n = 12), and patients with higher scores were classified as hypoglycaemia unaware (n = 10). Three of the latter had previously participated in a trial in which their inability to detect hypoglycaemia 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 non-diabetic and one diabetic subject who were on stable 


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, Sunny, UT) under local anesthesia (Xylocaine 2%) for infusion of salbutamol (Ventolin; GlaxoSmithKline, Zeist, The Netherlands) and blood pressure monitoring (monitor 37841A; Hewlett Packard GmbH, Böblingen, Germany). Intravenous infusion rates were calculated per deciliter body surface area. Intraarterial infusion rates were given as necessary to prevent hypoglycaemia (Actrapid; Novo Nordisk, Bagsværd, 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 for 2.5 min, followed by 100 mU/min for 15 min in hypoglycaemia-unaware 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 hypoglycaemia.

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 · 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 A₁c (HbA₁c) 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 χ² 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 ± 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. HbA₁c and fasting plasma glucose values were lower and disease duration was longer in T1DM patients with hypoglycaemia 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 normoglycaemic range in the diabetic patients during FBF measurements.

The vascular response to salbutamol

Baseline values for FBF in the infusion arm were 1.9 ± 0.3 ml/min · dl⁻¹ in control subjects, 2.3 ± 0.4 ml/min · dl⁻¹ in hypoglycaemia-aware T1DM patients (P = NS), and 1.4 ± 0.1 ml/min · dl⁻¹ in hypoglycaemia-unaware patients (P = 0.048 vs. aware patients). Corresponding values in the noninfused arm were 1.9 ± 0.2, 2.0 ± 0.2, and 1.6 ± 0.2 ml/min · 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 ± 1.3 ml/min · dl⁻¹ in controls, 14.6 ± 1.3 ml/min · dl⁻¹ in hypoglycaemia-aware diabetic patients, and 13.8 ± 1.5 ml/min · dl⁻¹ in hypoglycaemia-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 HbA₁c and fasting plasma glucose values were lower and disease duration was longer in T1DM patients with hypoglycaemia 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 normoglycaemic range in the diabetic patients during FBF measurements.

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics</th>
</tr>
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<tbody>
<tr>
<td>Controls</td>
</tr>
<tr>
<td>No. (male/female)</td>
</tr>
<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
</tr>
<tr>
<td>Duration of diabetes (yr)</td>
</tr>
<tr>
<td>Insulin dose (U/kg)</td>
</tr>
</tbody>
</table>

Data are in number or means ± SD. ND, Not determined.

a P < 0.05 vs. controls.
b P < 0.05 vs. T1DM aware.
TABLE 2. Plasma glucose and insulin levels

<table>
<thead>
<tr>
<th>Glucose level (mg/dl)</th>
<th>Controls</th>
<th>T1DM aware</th>
<th>T1DM unaware</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>92 ± 8</td>
<td>243 ± 72a</td>
<td>211 ± 42b</td>
</tr>
<tr>
<td>Prior to test</td>
<td>92 ± 8</td>
<td>108 ± 16a</td>
<td>111 ± 17a</td>
</tr>
<tr>
<td>End of test</td>
<td>92 ± 8</td>
<td>125 ± 21a</td>
<td>131 ± 29a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insulin level (µU/ml)</th>
<th>Baseline</th>
<th>Prior to test</th>
<th>End of test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>9 ± 4</td>
<td>30 ± 43a</td>
<td>30 ± 20a</td>
</tr>
<tr>
<td>Prior to test</td>
<td>13 ± 5</td>
<td>27 ± 14a</td>
<td>29 ± 17a</td>
</tr>
<tr>
<td>End of test</td>
<td>10 ± 4</td>
<td>21 ± 12a</td>
<td>21 ± 14a</td>
</tr>
</tbody>
</table>

Data are means ± sd. To convert plasma glucose values to milli-
moles per liter, divide by 18; to convert plasma insulin values to
picomoles per liter, multiply by 6.

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 (AFBF).

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, respec-
tively ($P < 0.001$ 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% (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 re-
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 re-
sponses does not support a role for the β$_2$-adrenergic recep-
tor 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 microdi-
alysis (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, gran-
ulocytes, and monocytes of various subsets that differ ac-

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It is unlikely that the higher insulin levels in the two
diabetic groups affected our data. First, a significant vaso-
dilator effect of insulin requires 4-fold higher plasma con-
centrations 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 experi-
ments, 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 dura-
tion. The effect of the latter two parameters on β$_2$-adrenergic

![Fig. 1. Local FBF in the experimental arm during intraarterial infu-
sion of salbutamol. FBF increased in the experimental arms in all
groups ($P < 0.001$) but to similar extent ($P = NS$ between groups).](image)
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, β₂-adrenergic sensitivity may differ by sex, with women probably having a more profound responsiveness to β₂-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 β₂-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 TIDM patients with hypoglycemia unawareness.

Previous in vivo studies on β-adrenergic sensitivity in diabetes used the isoproterenol sensitivity test (4–8), in which insulinemia produces both sympathetic neural activation and vasodilation in 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 β₁- and β₂-receptors and an indirect baroreflex response to β₂-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 β₁-adrenergic receptor. In our study, the heart rate response to systemic spillover of salbutamol in TIDM 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 β₂-adrenergic agonist stimulation is not reduced in TIDM patients with hypoglycemia unawareness. This observation may be of potential value when treatment with β₂-adrenergic agonists is considered to support glucose counterregulation. Whether reduced β₂-adrenergic sensitivity in hypoglycemia unawareness is mediated through the β₁-adrenergic receptor or impairments in the baroreflex response pathway requires further study.

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Address all correspondence and requests for reprints to: Bastiaan E. De Galan, M.D., Ph.D., Department of General Internal Medicine 463, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB, Nijmegen, The Netherlands. E-mail: b.degalan@aig.umcn.nl. The authors have nothing to disclose and no conflicts of interest.

References