The effect of selective $\beta_1$-blockade on glucose thresholds for release of counterregulatory hormones and symptoms in insulin-dependent diabetes mellitus

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To evaluate the effect of $\beta_1$-blockade (metoprolol) on the plasma glucose thresholds initiating counterregulatory hormone responses and symptoms of hypoglycemia, we used a modified glucose clamp technique to produce a standardized gradual glucose decline from 5.0 to 2.0 mmol/l in nine patients with insulin-dependent diabetes mellitus (IDDM) (HbA1c range 6.7–10.3%, duration of diabetes 5–18 years, autonomous neuropathy present in three of the patients). The responses were studied once with metoprolol and once with placebo, in random order. With the $\beta_1$-selective blockade, epinephrine release was triggered at a significantly higher (p<0.02) plasma glucose level (3.5 mmol/l) than it was with placebo (3.0 mmol/l). Metoprolol did not change thresholds for growth hormone (3.7/3.5 mmol/l), cortisol (2.9/2.9 mmol/l), glucagon (2.8/2.8 mmol/l) or for pancreatic polypeptide (2.8/2.7 mmol/l). The peak responses of epinephrine and growth hormone were significantly higher (p<0.01) with the $\beta_1$-blockade. Metoprolol did not change the thresholds for neuroglycopenic and autonomic symptoms. Six out of the seven patients who answered yes to having hypoglycemia did so at a higher blood glucose with metoprolol than without. In our study, the $\beta_1$-selective blockade altered the responses of counterregulatory hormones, but it did not change the thresholds for hypoglycemic symptoms.

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During decline in plasma glucose concentration, two important mechanisms guard against progression into serious hypoglycemia: the counterregulatory hormones epinephrine, glucagon, growth hormone and cortisol, and the neuroglycopenic and autonomic symptoms making patients aware of their low blood glucose level (1).

The use of non-selective $\beta$-blockade in diabetic patients may induce bradycardia, increase blood pressure and impair the glucose recovery rate during hypoglycemia (2, 3). This is not reported for $\beta_1$-selective blockade (4, 5), and it is generally agreed that $\beta_1$-selective blockers are preferable when $\beta$-blockade is needed in insulin-treated patients (6, 7).

The effects of $\beta_1$-selective blockade on counterregulatory hormones and symptoms have been studied in diabetic patients with bolus injections of insulin bringing about a rapid decline in plasma glucose (2, 4, 5). Furthermore, Kerr et al. (8) have studied $\beta$-blockade in normal subjects during euglycemia (4.5 mmol/l) and hypoglycemia (2.5 mmol/l), but such methods do not reveal the more subtle changes in the defense and awareness of hypoglycemia. Especially changes in the plasma glucose thresholds will not be disclosed.

Recent studies indicate that plasma glucose thresholds and hypoglycemia responses are influenced by the degree of glycemic control (9), autonomic neuropathy (10) and sex (11). Potentially, changes in glucose thresholds may have considerable consequences for insulin-treated diabetics.

Our study was undertaken to examine whether plasma glucose thresholds for counterregulatory hormones and symptoms are influenced by the $\beta_1$-selective blocker metoprolol. Since plasma pancreatic polypeptide is considered a reliable marker of vagal activity (12), this hormone was measured as well.

Patients and methods

Nine patients (six males) with insulin-dependent diabetics mellitus (IDDM) were studied on two occasions, once with and once without $\beta_1$-selective blockade. The two studies were performed at least two weeks apart in the men and approximately four weeks apart in the women, depending on their menstrual cycle. Their median age was 26 years (range 24–49) and the duration of diabetes was from 5 to 18 years (median 14). With the exception of one patient who was 15%
overweight, they were all within 110% of normal body weight (13). Four patients were receiving conventional insulin therapy with two daily injections of soluble and intermediate acting insulin, and five were receiving multiple injection therapy. HbA1c on the day with $\beta_1$-blockade was 8.5% (range 6.7–10.2), and on the day without $\beta_1$-blockade 8.5% (range 7.0–10.3) (non-diabetic range 4.5–6.5). On standard testing, according to Ewing and Clarke (14), three of the patients had definite autonomic neuropathy. All three had a pathological heart rate response to standing up and to deep breathing. In addition, one of them also had a pathological heart rate response to the Valsalva manoeuvre. A fourth patient had, as a single defect, a pathological heart rate response to standing up, and was thus classified as having early autonomic neuropathy. None of the nine patients had a pathological blood pressure response on any of the tests carried out by us, nor did they have nephropathy or proliferative retinopathy. With the exception of two women who were taking the contraceptive pill, none was taking medication other than insulin.

To avoid the recently demonstrated reduction in hormonal and symptomatic responses 18 h, but not 24 h, after an episode of hypoglycaemia (15), the patients were instructed to measure their blood glucose five times during the day preceding the study. If hypoglycaemia (symptoms or a measured glucose level below 3.0 mmol/l) occurred during this period, the study was, for practical reasons, postponed for at least one week.

The patients were admitted to the metabolic research laboratory the evening before the study. The evening or night-time dose of intermediate acting insulin was omitted. The subjects were kept fasting and maintained euglycemic (5–7 mmol/l) overnight using an iv infusion of soluble insulin (Velosulin®, Novo Nordisk, Denmark). The blood glucose concentration was measured every second hour and the insulin infusion adjusted accordingly. The cannula for insulin infusion and for drawing blood samples for night-time glucose measurements was placed in the antecubital vein of the left arm.

The next morning a second cannula was inserted into a dorsal hand vein on the right arm. This arm was placed in a heated box (63°C) to arterioalize the venous blood (16). From then on this line was used for blood sampling and it was kept patent with 0.9% saline (0.5 ml/min). A constant infusion of soluble insulin (1 mU kg $^{-1}$ min $^{-1}$) and another of 24% glucose were connected to the indwelling antecubital cannula.

After a resting and stabilizing period of approximately 30 min, the tests were started between 09.00 and 10.30. In random order the patients were given either a bolus injection of 5 ml metoprolol (1 g/l) or 5 ml placebo (saline) iv followed throughout by a constant metoprolol/placebo infusion of 5 ml/h. Initially, the plasma glucose level was stabilized between 5.0 and 7.0 mmol/l for half an hour, and subsequently allowed to fall gradually for 240 min to 2.0 mmol/l (0.125 mmol/l per 10 min). All patients were told that the blood glucose would be lowered, but they did not know the level at any stage. If serious clinical symptoms of hypoglycaemia (tendency to fall asleep, diplopia, slurred or incoordinated speech, etc.) occurred before plasma glucose had reached 2.0 mmol/l, the insulin infusion was stopped and the blood glucose normalized by glucose infusion. Plasma glucose was measured repeatedly (see below) and the desired glucose level achieved by adjusting the glucose infusion accordingly.

Blood samples were drawn, pulse rate and blood pressure measured and a symptom questionnaire filled out every 5 min for the first half hour and thereafter every 10 min.

Written informed consent was obtained from each subject before participation. The protocol was approved by the regional ethics committee.

Determinants
HbA1c was assayed with HPLC (Diamat, Bio-Rad). Plasma glucose was measured bedside, using the glucose oxidase method, on a Beckman glucose analyzer. Blood for plasma epinephrine was collected on glutathione, centrifuged at 4°C, and analyzed by HPLC (17). Serum growth hormone was analyzed by IRMA, developed by the Hormone Laboratory, Aker University Hospital, with detection limit 0.3 mE/l and interassay precision 10% (18). Determination of serum cortisol was based on enhanced luminiscence (Amertile), the CV being 4.3%. Blood for determination of plasma glucagon was collected in ice-chilled tubes containing 10% trisylol/EDTA, centrifuged at 4°C and analyzed by RIA after extraction of plasma with ethanol. The detection limit is 4.5 pmol/l and interassay precision 12% (19). Serum pancreatic polypeptide was determined by RIA using antiserum rabbit anti-HPP, lot 615-1045B-248-19. The detection limit is 1.5 pmol/l and interassay precision 12.0% (20).

Blood samples for plasma metoprolol were drawn 5 min after bolus injection and every hour thereafter. Plasma metoprolol concentration was analyzed as described earlier (21).

Blood pressure and heart rate were measured automatically with a Dinamap vital signs monitor (Criticon, Diacor A/S).

Symptoms
Prior to the study the patients were instructed to fill in a visual analogue scale symptom questionnaire (22, 23). Consistent with categorization used by others (24), five neuroglycopenic symptoms (hunger, dizziness, faintness, tiredness and difficulty in thinking) and four autonomic symptoms (shakiness, sweating, palpitations and feeling of nervousness) were scored on a line from 0 (no symptom) to maximal 15 cm. The order of the symptoms was systematically changed on each
questionnaire. All the questionnaires had the following yes/no question: “Do you feel hypoglycemic?”.

Statistical methods
Glucose thresholds for each hormone or symptom are given as the plasma glucose level where responses were more than 2 sd above basal level, followed by an unequivocal, sustained increase. Some patients had an initial decrease or a spike in the first samples of cortisol and growth hormone, and in those cases we had to use seven nadir values as basic levels.

Demographic data are expressed as medians with range. The experimental results are given as means ± SEM. Differences in glucose thresholds and peak hormone levels, with and without metoprolol, were determined by the Wilcoxon rank sum tests. P<0.05 is considered significant.

Results
A gradual fall in plasma glucose levels was achieved in all nine patients on both occasions (Fig. 1). Two patients had not reported hypoglycemia when the insulin infusion was terminated at a plasma glucose of 2.0 mmol/l. The other seven patients did report hypoglycemia and had definite clinical signs of hypoglycemia before this level: according to the protocol, the insulin infusion was stopped before plasma glucose reached 2.0 mmol/l.

The glucose infusion rate was not accurately measured in two patients on the first day. In the remaining seven, the glucose infusion rate was lower when metoprolol was given, the difference being significant at blood glucose level of 4.0 mmol/l (Table 1).

Metoprolol
Five minutes after the metoprolol injection, the plasma concentration of metoprolol was 55±18.1 μg/l, and remained elevated during the infusion, with a mean level of 47.9±3.6 μg/l.

Pulse rate
During the metoprolol study, basal pulse rate was significantly lower (60±0.4 beats/min) than on the placebo day (67±0.5 beats/min) (p<0.05), thus documenting efficient β-blockade. Hypoglycemia associated tachycardia was not seen when metoprolol was given. On the placebo day, a more than 2 sd increase in pulse rate was seen in all patients (with a mean increase of 10 beats/min). The mean glucose threshold for the tachycardia response was 2.5±0.2 mmol/l.

Blood pressure
As with the pulse rate, the basal systolic and diastolic blood pressure was lower with metoprolol (114±1.2/64±0.4 mmHg) than without (119±1.0/66±0.4 mmHg) (p<0.05).

Symptoms
Only three neuroglycopenic symptoms (faintness, dizziness, hunger) and three autonomic symptoms (shakiness, sweating, palpitations) were answered consistently by all patients. The three symptoms of each group were added and the sum used in the statistical calculations. Metoprolol did not change the thresholds for any of the symptoms. The neuroglycopenic symptoms occurred earlier than the autonomic symptoms, with both metoprol (respective glucose thresholds 3.2±0.1 and 2.6±0.2 mmol/l) and placebo (respective glucose thresholds 3.1±0.2 and 2.7±0.2 mmol/l). This difference was significant (p<0.05) on the day with the β1-blockade.

On the yes/no question, six of the seven patients reporting hypoglycemia did so at a higher blood glucose level with metoprolol than without. The mean levels for the seven patients were 3.1±0.3 mmol/l and 2.8±0.2 mmol/l (not significant).

Hormones
There was a remarkable difference in glucose thresholds for hypoglycemia-induced release of the five hormones measured, especially when metoprolol was given. On that day, the glucose thresholds for epinephrine and growth hormone release were significantly higher (p<0.03/p<0.01) than for cortisol, glucagon and pancreatic polypeptide (Table 2). The threshold for epinephrine release was significantly higher (p<0.03) with metoprolol than without it. Without metoprol, four of the patients did not reach the defined 2 sd increment in growth hormone. For the other patients, there was a non-significant difference in the growth hormone threshold. The glucose thresholds for cortisol, glucagon and pancreatic polypeptide were unaffected by β1-blockade.

The peak responses of epinephrine and growth hormone were significantly higher (p<0.02/p<0.01) with β1-blockade than without (Fig. 1). The other differences in peak responses were not significant (Table 3).

Two of the patients with autonomic neuropathy had an attenuated pancreatic polypeptide, glucagon and epinephrine response to hypoglycemia, but with that exception, no consistent difference was detected between the four patients with and the five patients without autonomic neuropathy. The four patients with autonomic neuropathy, in particular, did not differ in glycemic thresholds compared with the rest of the group.

The release pattern of the hormones differed (Fig. 1). Thus, in most patients a gradual increase in epinephrine, growth hormone and cortisol was seen. In six of the nine patients the pancreatic polypeptide increment was remarkably sharp, like an on/off response.
Fig. 1. The effect of plasma glucose decrement on counterregulatory hormones and pancreatic polypeptide (mean ± SEM). —— Metoprolol
— Placebo.

Hypoglycemia, counter regulation, and beta blockade in IDDM
Table 1. Mean (+SEM) glucose infusion rates (mg min⁻¹) during hypoglycemia in seven diabetic patients.

<table>
<thead>
<tr>
<th>Plasma glucose (mmol/l)</th>
<th>+ Metoprolol</th>
<th>− Metoprolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>36.0 (± 17.3)</td>
<td>49.7 (± 32.2) NS</td>
</tr>
<tr>
<td>4.5</td>
<td>34.3 (± 12.9)</td>
<td>67.4 (± 27.8)</td>
</tr>
<tr>
<td>4.0</td>
<td>42.9 (± 1.1)</td>
<td>122.9 (± 35.0) p &lt; 0.05</td>
</tr>
<tr>
<td>3.5</td>
<td>71.4 (± 33.8)</td>
<td>131.4 (± 32.3) NS</td>
</tr>
<tr>
<td>3.0</td>
<td>90.0 (± 31.3)</td>
<td>100.0 (± 31.1) NS</td>
</tr>
<tr>
<td>2.5</td>
<td>56.7 (± 27.5)</td>
<td>70.0 (± 30.4) NS</td>
</tr>
</tbody>
</table>

Table 2. Mean (+SEM) plasma glucose thresholds (mmol/l) triggering the counterregulatory hormones, pancreatic polypeptide, symptoms, and pulse rate increase.

<table>
<thead>
<tr>
<th></th>
<th>+ Metoprolol</th>
<th>− Metoprolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>3.5 (± 0.2)</td>
<td>3.0 (± 0.1)  p &lt; 0.02</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>3.7 (± 0.1)</td>
<td>3.5 (± 0.2)  NS</td>
</tr>
<tr>
<td>Cortisol</td>
<td>2.9 (± 0.2)</td>
<td>2.9 (± 0.2)  NS</td>
</tr>
<tr>
<td>Glucagon</td>
<td>2.8 (± 0.1)</td>
<td>2.8 (± 0.2)  NS</td>
</tr>
<tr>
<td>Pancreatic polypeptide</td>
<td>2.8 (± 0.2)</td>
<td>2.7 (± 0.2)  NS</td>
</tr>
<tr>
<td>Neuroglycopenic</td>
<td>3.2 (± 0.1)</td>
<td>3.1 (± 0.2)  NS</td>
</tr>
<tr>
<td>Autonomic symptoms</td>
<td>2.6 (± 0.2)</td>
<td>2.7 (± 0.2)  NS</td>
</tr>
<tr>
<td>Pulse increase</td>
<td>absent</td>
<td>2.5 (± 0.2)</td>
</tr>
</tbody>
</table>

Table 3. Mean (+SEM) peak concentrations for the counter-regulatory hormones and pancreatic polypeptide during hypoglycemia.

<table>
<thead>
<tr>
<th></th>
<th>+ Metoprolol</th>
<th>− Metoprolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine (nmol/l)</td>
<td>4.0 (± 0.5)</td>
<td>2.6 (± 0.5)  p &lt; 0.01</td>
</tr>
<tr>
<td>Growth hormone (µg/l)</td>
<td>24 (± 2.4)</td>
<td>14 (± 2.3)   p &lt; 0.01</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>707 (± 63)</td>
<td>661 (± 84)   NS</td>
</tr>
<tr>
<td>Glucagon (pmol/l)</td>
<td>45 (± 8.9)</td>
<td>39 (± 6.9)   NS</td>
</tr>
<tr>
<td>Pancreatic polypeptide (pmol/l)</td>
<td>334 (133)</td>
<td>67 (109) NS</td>
</tr>
</tbody>
</table>

In four of the patients, there was a substantial fall in growth hormone, both with and without metoprolol, at the end of the study, in spite of a still falling plasma glucose. As expected, the glucagon release was low (Fig. 1), but it was detectable in all patients and did show a hypoglycemia-induced increase.

Discussion

In the present study we found that in diabetic patients a β₁-selective blockade with metoprolol does affect the plasma glucose threshold for epinephrine release during hypoglycemia. To our knowledge, this has not been reported before. However, the symptoms of hypoglycemia were not significantly affected.

The patients were not homogeneous with respect to duration of diabetes, metabolic control, age, sex or autonomic neuropathy. However, they served as their own controls, and the two study days were comparable with regard to menstrual phase for the women (25) and level of HbA1c (9, 23).

Four of the patients had some signs of autonomic neuropathy, but did not differ in glucose threshold values from the others, irrespective of β-blockade. However, since we did not include patients with neuropathy of such an advanced degree, one at which Hoeldtke et al. (10) found necessary to be of predictive value for reduced epinephrine secretion and hypoglycemic unawareness, our small number of patients does not rule out an effect of autonomic neuropathy on glucose threshold values.

The large range in HbA1c was the result of one patient with values of 10.1% and 10.4%. This patient, who did not have autonomic neuropathy, had glucose threshold values some 0.5 mmol/l above the group mean, consistent with the finding of Amiel et al. (9) and Boyle et al. (23). The other eight patients had HbA1c values between 6.7% and 8.6%, and at this narrow HbA1c range no correlations between glycemic control and glucose thresholds were seen.

The plasma glucose thresholds for hormonal release or recognition of symptoms are not easy to define. Some authors prefer to choose the threshold as the glucose level at which a predetermined hormonal increment above basal is reached, as 2 sp above basal levels, or merely from visual inspection of the curves (9, 22). All these methods were applied on our data, but regardless of the threshold criteria chosen the main conclusions were the same.

The threshold values differ between studies, depending on the threshold criteria and on the study designs. However, the main trends (26), such as the order of responses, are comparable. Thus, Mitrakou et al. (24), using glycemic plateaus to study the thresholds in healthy subjects, have reported threshold values similar to those found by us, with the clear exception of glucagon. This higher threshold for glucagon (lower blood glucose concentration) in the diabetic group was expected, due to the duration of disease in our patients (27).

The effects of acute β₁-blockade on hormone levels may differ from those following treatment for an extended period. It is postulated that prolonged treatment with β-blockade may increase the density of β-adrenoreceptors, or change processes below the receptor level (28). If so, one may expect a glucose threshold gap between metoprolol and placebo to narrow rather than to widen during chronic treatment with a β-blocking agent.

With metoprolol, the peak epinephrine concentrations were significantly higher than with placebo. This is in agreement with previous findings (6, 8), and is thought to be a result of decreased epinephrine clearance (29).

The earlier epinephrine response is difficult to explain, but one possibility could be the disappearance of inhibition at the β-receptor level. When the receptors are
blocked for epinephrine feedback inhibition, the concentration will increase earlier and reach a higher level.

In spite of the earlier and higher response in epinephrine levels, as well as a reduced pulse rate, the thresholds for neither autonomic symptoms nor neuroglycopenic symptoms were influenced by metoprol. Accordingly, $\beta_1$-selective blockade appears not to mask hypoglycemic symptoms.

In our experimental setting, the hypoglycemic symptoms may be different from those experienced by the patients in daily life, and many patients spontaneously reported that they were much more sensitive to hypoglycemic symptoms when physically active. This may explain the patients' problems with answering whether they felt hypoglycemic or not.

In six of the seven patients reporting hypoglycemia, this occurred earlier with $\beta_1$-blockade than without, and concurred with the threshold for neuroglycopenic symptoms. This is difficult to explain, but may indicate that the $\beta_1$-blockade made the patients more physically relaxed, and thus able to recognize the neuroglycopenic symptoms earlier.

In prolonged experimental hypoglycemia in normal subjects and diabetic patients, the growth hormone secretion has been reported both to be sustained and to decrease (30, 31). Our patients reached the same high concentrations in growth hormone as that found by others, but four had a final and unequivocal decrease, both with and without metoprol, in spite of a falling blood glucose. Unfortunately, the blood glucose in our study was not allowed to remain low enough for us to see the extent and duration of this growth hormone decline.

When metoprol was given, growth hormone increased significantly higher than on the placebo day. This is in agreement with the concept of an $\alpha$-mediated stimulation and a $\beta$-mediated inhibition of growth hormone release from the pituitary, intensified by the higher catecholamine level (32).

The pancreatic polypeptide response showed a low and stable basal level until the threshold point, followed by a surprisingly steep and sustained increment. This is in contrast to the gradual increase seen for epinephrine, indicating that the centers regulating the pancreatic polypeptide release (which is mostly under vagal control) and the epinephrine release, respond differently to the falling blood glucose. Whether a similar discrepancy in response also applies to other cholinergic and autonomic functions, like sweating and tachycardia, is not known.

$\beta$-blockers may worsen insulin resistance (33) and metoprol is known to inhibit insulin clearance in diabetic patients (6). In our study, the first effect was the most important with regard to the blood glucose, since the glucose infusion rate was lower when metoprol was given. With the current focusing on insulin resistance and possible atherogenic effects of insulin (34), one may accordingly speculate that unwanted metabolic consequences may follow prolonged $\beta$-blockade.

In conclusion, prescribing the $\beta_1$-selective blocker metoprol to IDDM patients appears safe with regard to hypoglycemia. The hypoglycemic symptoms are not masked and the epinephrine/growth hormone responses are more pronounced. The recognition of low blood glucose may even come earlier in some patients.

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