Effect of acute administration of gliclazide on the glucose sensitivity of pancreatic B-cells in healthy subjects

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SUMMARY

1. Sulphonylurea derivatives are commonly used in the treatment of non-insulin-dependent diabetes mellitus. It is, however, unclear whether the hypoglycaemic effect of sulphonylurea derivatives is additive to the effect of glucose, or whether sulphonylurea derivatives act by increasing B-cell glucose sensitivity.

2. We assessed the effect of gliclazide on glucose-stimulated insulin secretion in eight healthy volunteers. Sixty minute hyperglycaemic glucose clamps (blood glucose levels of 8, 11 and 32 mmol/l) were performed, with and without prior administration of gliclazide (80 mg) 90 min before the glucose clamp.

3. Dose–response characteristics were assessed with a modified Michaelis–Menten equation. The $V_{max}$ (maximal B-cell responsiveness) was not significantly changed (1.5 ± 0.1 versus 1.3 ± 0.2 and 5.0 ± 0.5 versus 4.8 ± 0.5 mmol/l for the first- and second-phase insulin secretion, respectively), whereas the $ED_{50}$ (half-maximally stimulating blood glucose concentration) was significantly decreased by gliclazide for first-phase insulin secretion (7.6 ± 0.3 versus 9.1 ± 0.6 mmol/l) but not for second-phase insulin secretion (12.0 ± 0.5 versus 12.3 ± 0.5 mmol/l).

4. We conclude that gliclazide indeed leads to a shift to the left of the dose–response curve of first-phase insulin release in vivo without a change in $V_{max}$, which indicates an apparent enhancement of B-cell glucose sensitivity.

Key words: gliclazide, insulin secretion, sulphonylurea.

Abbreviations: $ED_{50}$, half-maximally stimulating blood glucose concentration; NIDDM, non-insulin-dependent diabetes mellitus; SU, sulphonylurea; $V_{max}$, maximal B-cell responsiveness.

INTRODUCTION

Sulphonylurea (SU) derivatives have often been advocated in the management of non-insulin-dependent diabetes mellitus (NIDDM). Although there is a continuing debate about their mode of action during chronic use in patients with NIDDM, notably whether the hypoglycaemic effect of the SU derivatives is mainly due to an insulinogenic effect or to an extrapancreatic effect, i.e. a stimulation of insulin action, there is little doubt about the capacity of these compounds to stimulate insulin secretion in vitro and, during acute administration, in vivo [1–3].

Studies in vitro have shown that the stimulation of insulin secretion from pancreatic B-cells by glucose involves the closure of ATP-sensitive K+ channels, leading to depolarization of the cell membrane [4–7]. This depolarization is assumed to gate voltage-dependent Ca2+ channels, leading to Ca2+ influx, a prerequisite for insulin release [8–14].

The mode of action of SU derivatives has been reported to involve the closure of the same so-called K+(ATP) channels [15–18]. If the stimulating effect of SU derivatives on insulin secretion were to involve the same mechanism as glucose (i.e. via K+ channels), the use of these drugs would result in a shift to the left in the dose–response characteristics of glucose-stimulated insulin secretion without a change in the maximum secretion rate. If the stimulating effect were to involve other mechanisms, the dose–response characteristics would presumably show an increase in the maximum secretion rate without a shift to the left. In order to investigate this question, we studied the effect of acute administration of gliclazide on insulin secretion in eight healthy volunteers.

METHODS

Subjects

Eight healthy subjects with a mean age of 22 ± 2 years and a weight of 96 ± 2.9% of ideal were studied on six
different occasions. None of the subjects took any medication.

The study was approved by the local Ethical Committee, and, after the nature of the study had been explained to each subject, informed consent was obtained.

**Determination of dose–response characteristics for glucose-stimulated insulin secretion**

The subjects were studied in the morning after an overnight fast on six different occasions. They had been instructed to maintain their usual diet.

On each occasion, an intravenous line was inserted in an ante-cubital vein of each arm and was kept patent by a slow saline [0.9% (w/v) NaCl] infusion. One line was used for blood sampling, and the other for the infusion of glucose. After 90 min, a hyperglycaemic clamp was performed [19], starting with an intravenous bolus of glucose [20% (w/v); 30 mg/kg per mmol/l intended increase in blood glucose level], followed by a 60 min variable glucose [20%, w/v] infusion aimed at maintaining a blood glucose level of 8, 11 or 32 mmol/l.

Each of these clamps was performed twice, once with and once without administration of gliclazide (80 mg) 90 min before the start of the hyperglycaemic clamp.

**Blood sampling**

Blood samples for measurement of plasma C-peptide concentration (Novo, Copenhagen, Denmark, modified by using Sac Cell from Wellcome, Dartford, Kent, U.K., as precipitating second antibody) were obtained at the time points −90, −60, −30, 0, 3, 4, 5 and 10 min and every 10 min thereafter.

The blood glucose concentration (YSI Glucose Analyzer; YSI, Yellow Springs, OH, U.S.A.) was measured at −90, −60, −30, 0, 3, 4 and 5 min and subsequently at 2.5 min intervals during the remainder of the experiment.

**Calculations**

The increments in plasma C-peptide levels at 5 min and at 60 min during the hyperglycaemic clamps were used as an index of first- and second-phase glucose-induced insulin secretion, respectively.

In order to assess B-cell sensitivity to glucose, increments in plasma C-peptide levels at these time points were fitted to the modified Michaelis–Menten equation of Grodsky [a logistic function] [20], with a non-linear least-squares regression computer program [21]. The equation was:

\[
C\text{-}\text{pep} = \frac{V_{\text{max}} \times \text{Gluc}^N}{ED_{50} + \text{Gluc}^N}
\]

where C-pep is the measured increment in plasma C-peptide level, \(V_{\text{max}}\) is the calculated maximal B-cell responsiveness, \(ED_{50}\) is the half-maximally stimulating blood glucose concentration, and \(N\) is the exponent added by Grodsky [20] because it improved the fit of the data.

**STATISTICS**

Data are given as means ± SEM. Student’s \(t\)-test for paired data was used for statistical analysis of the data.

**RESULTS**

**Hyperglycaemic clamps**

In these hyperglycaemic clamp experiments, the blood glucose concentration increased to the intended values (8, 11 and 32 mmol/l) within 5 min, and was maintained at mean values of 8.1 ± 0.05 and 8.1 ± 0.06, 11.1 ± 0.8 and 11.1 ± 0.04, and 31.3 ± 0.23 and 31.4 ± 0.17 mmol/l with coefficients of variation of 4.0 ± 0.8 and 4.3 ± 0.85, 5.1 ± 1.0 and 5.0 ± 0.8 and 4.8 ± 0.6 and 4.5 ± 0.6%, respectively, during gliclazide and control study days.

Plasma C-peptide levels increased rapidly during the first 5 min from levels of around 0.5 ± 0.04 nmol/l to levels of 1.39 ± 0.11 and 1.29 ± 0.13 nmol/l during the hyperglycaemic clamp at 8 mmol/l glucose with and without gliclazide, respectively, to levels of 1.53 ± 0.16 and 1.51 ± 0.12 mmol/l during the clamp at 11 mmol/l glucose, and to levels of 1.77 ± 0.15 and 1.96 ± 0.16 mmol/l during the clamp at 32 mmol/l glucose. This was followed by a slower increase during the remainder of the clamps to levels of 1.62 ± 0.18 and 1.56 ± 0.16, 2.4 ± 0.25 and 2.3 ± 0.2, and 5.4 ± 0.6 and 5.6 ± 0.6 nmol/l for clamps at 8, 11 and 32 mmol/l glucose with and without gliclazide, respectively (Fig. 1).

**Dose–response characteristics for glucose-induced insulin secretion**

First- and second-phase insulin secretion increased in a dose-dependent manner (Figs. 2 and 3). The \(V_{\text{max}}\) of first-phase insulin secretion, assessed from the increments in the plasma C-peptide level, was significantly lower on the gliclazide study day than on the control study day with and without gliclazide, respectively, to levels of 1.53 ± 0.16 and 1.51 ± 0.12 mmol/l during the hyperglycaemic clamp at 11 mmol/l glucose, and to levels of 1.77 ± 0.15 and 1.96 ± 0.16 mmol/l during the clamp at 32 mmol/l glucose. This was followed by a slower increase during the remainder of the clamps to levels of 1.62 ± 0.18 and 1.56 ± 0.16, 2.4 ± 0.25 and 2.3 ± 0.2, and 5.4 ± 0.6 and 5.6 ± 0.6 nmol/l for clamps at 8, 11 and 32 mmol/l glucose with and without gliclazide, respectively (Fig. 1).

\[V_{\text{max}}\] of first-phase insulin release showed a trend towards a decrease on the gliclazide study day \((P < 0.02)\). The \(V_{\text{max}}\) of first-phase insulin release showed a trend towards a decrease on the gliclazide study day \((P = 0.10)\), whereas no significant change in \(V_{\text{max}}\) was seen for second-phase insulin release \((P > 0.2)\).

The \(ED_{50}\) was significantly decreased on the gliclazide study days for first-phase insulin release \((7.56 ± 0.27 versus 9.14 ± 0.12 \text{ mmol/l}}, \ P < 0.05)\), whereas no difference was observed for second-phase release. The exponent \(N\) was not significantly different between gliclazide and control study days, neither for first- nor second-phase insulin release (Table 1).

**DISCUSSION**

The SU derivative gliclazide stimulates insulin secretion in vitro and in vivo [2-4]. The present studies were undertaken to examine the influence of acute administration of gliclazide on B-cell glucose sensitivity in both phases of
Gliclazide and glucose sensitivity in man

Fig. 1. Blood glucose concentrations (a) and plasma C-peptide concentrations (b) obtained during hyperglycaemic clamps in eight healthy volunteers with (filled symbols) and without (open symbols) administration of 80 mg of gliclazide (arrow) 90 min before the clamp. Values are means ± SEM. Glucose clamps: ○, ●, 8 mmol/l; □, ■, 11 mmol/l; △, ▲, 32 mmol/l.

Fig. 2. Dose–response curves for increases in the plasma C-peptide concentrations obtained at 5 min (first-phase insulin release) during the hyperglycaemic clamps in Fig. 1 with (▲) and without (△) gliclazide. Values are means ± SEM (n = 8).

Fig. 3. Dose–response curves for increases in the plasma C-peptide concentrations obtained at 60 min (second-phase insulin release) during the hyperglycaemic clamps in Fig. 1 with (▲) and without (△) gliclazide. Values are means ± SEM (n = 8).

Insulin release in healthy subjects. Both phases were studied in order to assess whether this SU derivative may have different effects in these phases. We used plasma C-peptide levels instead of plasma insulin levels as an assessment of insulin secretion, since insulin is known to have non-linear pharmacokinetics (with a non-linear clearance) [22–27].

Previous studies from our laboratory indicated a difference in dose–response characteristics for glucose-induced insulin release when assessed using plasma C-peptide levels or plasma insulin levels, with a significantly higher ED₅₀ for the assessment using plasma insulin levels [28].

The ED₅₀ values of 9.1 and 12.3 mmol/l obtained in the present study on the control day agree with our previous studies in that the ED₅₀ for first-phase secretion is somewhat lower than the ED₅₀ for second-phase secretion. These values of ED₅₀ are comparable with the ED₅₀ of around 10 mmol/l which has been found in a study in vitro [29]. Since glucose stimulation of insulin secretion involves glucose metabolism [30–33], it has been postulated that the degrading enzyme that has dose–response characteristics comparable with overall degradation (pace-setting enzyme) acts as the glucose ‘sensor’ of the B-cell [33]. It is now assumed that glucokinase fulfills this role [29, 33].

In the present study, we assessed the influence of oral administration of the SU derivative, gliclazide, on glucose-induced insulin release in healthy men at moderately elevated and at high glucose levels. During the hyperglycaemic clamps at 8 mmol/l glucose, gliclazide induced 15% higher increases in the plasma C-peptide level for first-phase insulin secretion than on the control study day, whereas no differences were found for the
Table 1. Influence of acute administration of gliclazide (80 mg) on the dose–response characteristics of glucose-induced insulin release in eight healthy volunteers

<table>
<thead>
<tr>
<th></th>
<th>( V_{\text{max}} ) (nmol/l)</th>
<th>( ED_{50} ) (mmol/l)</th>
<th>Exponent ( N )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First-phase insulin release</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gliclazide</td>
<td>1.30 ± 0.15</td>
<td>7.56 ± 0.27*</td>
<td>7.4 ± 1.13</td>
</tr>
<tr>
<td>Control</td>
<td>1.51 ± 0.10</td>
<td>9.14 ± 0.62</td>
<td>5.7 ± 0.75</td>
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<tr>
<td><strong>Second-phase insulin release</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gliclazide</td>
<td>4.8 ± 0.51</td>
<td>12.0 ± 0.53</td>
<td>3.6 ± 0.26</td>
</tr>
<tr>
<td>Control</td>
<td>5.0 ± 0.47</td>
<td>12.3 ± 0.52</td>
<td>3.8 ± 0.38</td>
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</table>

Values are means ± SEM. Statistical significance: *P < 0.05 compared with control.
This strongly suggests that lowered plasma concentrations of gliclazide are not the cause of our finding that the ED₉₀ of second-phase glucose-induced insulin secretion remained unchanged. However, the studies in vitro of Lebrun et al. [22] of the influence of gliclazide on insulin output in perfused rat islets indicate a marked increase in both first- and second-phase insulin output.

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