Stimulation of Insulin Secretion by Intravenous Bolus Injection and Continuous Infusion of Gastric Inhibitory Polypeptide in Patients With Type 2 Diabetes and Healthy Control Subjects

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A reduced insulinotropic effect of gastric inhibitory polypeptide (GIP) is a characteristic of patients with type 2 diabetes. It was the aim of this study to determine the response of insulin secretion to different GIP doses administered by intravenous bolus injection and via continuous infusion in both healthy subjects and patients with type 2 diabetes. Eight patients with type 2 diabetes and eight healthy subjects participated in a 240-min hyperglycemic clamp (140 mg/dl) with intravenous infusion of placebo, GIP at a low dose, and GIP at a high dose, each administered continuously over 60 min. Boluses of placebo, 20 pmol GIP/kg, and 80 pmol GIP/kg were injected intravenously at 0, 60, and 120 min, respectively. Capillary and venous blood was drawn for glucose, insulin, C-peptide, and GIP. Plasma insulin and C-peptide concentrations were lower in patients than in control subjects during all infusion periods. GIP bolus administration evoked a significant increase in plasma insulin levels in both patients with type 2 diabetes and healthy subjects. In contrast, the continuous GIP infusion led to a weak increase in insulin secretion in both healthy subjects and type 2 diabetic patients. The dose-response relationship for the increase in insulin secretion after GIP bolus administration was similar in both groups, although at different degrees of β-cell function. The stimulation of insulin secretion by GIP is stronger after its bolus administration than during continuous infusion. Even though the insulin secretory capacity is generally impaired in patients with type 2 diabetes, the relative sensitivity of insulin secretion to a bolus administration of GIP is almost preserved. Therefore, the existence of a specific GIP receptor defect in type 2 diabetes appears unlikely. Diabetes 53 (Suppl. 3):S220–S224, 2004

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GIP, gastric inhibitory polypeptide.

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RESEARCH DESIGN AND METHODS

The study protocol was approved by the ethics committee of the medical faculty of the Ruhr-University, Bochum, Germany. Eight patients with type 2 diabetes and eight healthy control subjects without a family history of diabetes were studied. Patient/subject characteristics are presented in Table 1. Written informed consent was obtained from all participants.

Subjects with anemia (hemoglobin <11 g/dl), elevation in liver enzymes, or elevated creatinine concentrations were excluded. Among the patients, two were treated with metformin, whereas the other six were on diet only. All antidiabetic treatment was withdrawn at least 48 h before the experiments.
Glucose was measured with a Glucose Analyzer (Sarstedt, Nümbrecht, Germany) for immediate measurement of glucose. Samples collected from the ear lobe were stored in NaF (Microvette CB 300; Sarstedt, Nümbrecht, Germany). From these samples, plasma was obtained by centrifugation at 12,000 × g for 20 min at room temperature. Samples were kept at −80°C until analysis.

**Diabetes** was induced using 0.9% NaCl (for blood sampling and for glucose and peptide administration) and 40% glucose as a bolus at 0 min. The continuous intravenous infusion of 40% glucose was started by injecting 20% glucose (20% in water, wt/vol) until 210 min, as appropriate, based on the clamp aiming at a steady capillary plasma glucose of 7.8 mmol/l (140 mg/dl). A nasogastric tube (total length 144 cm) was inserted and maintained until 180 min. At 0 min, an intravenous infusion of placebo (1% human serum albumin in 0.9% NaCl) was started by injecting 40% glucose as a bolus at 0 min and maintained by infusing glucose (20% in water, wt/vol) until 210 min, as appropriate, based on glucose concentrations determined every 5 min and until 210 min. After an intravenous infusion of placebo (1% human serum albumin in 0.9% NaCl) was started and maintained until 110 min, 60 min, GIP infusion was started at the rate of 1.0 pmol · kg⁻¹ · min⁻¹. At 120 min, GIP infusion was increased to 2.0 pmol · kg⁻¹ · min⁻¹ and maintained until 190 min. At 0 min, an intravenous infusion of placebo (1% human serum albumin in 0.9% NaCl) was started and maintained until 60 min. At 60 min, GIP infusion was started at the rate of 1.0 pmol · kg⁻¹ · min⁻¹. At 120 min, GIP infusion was increased to 2.0 pmol · kg⁻¹ · min⁻¹ and maintained until 190 min. At 0 min, an intravenous infusion of placebo (1% human serum albumin in 0.9% NaCl) was started and maintained until 60 min. At 60 min, GIP infusion was started at the rate of 1.0 pmol · kg⁻¹ · min⁻¹. At 120 min, GIP infusion was increased to 2.0 pmol · kg⁻¹ · min⁻¹ and maintained until 190 min.

**Blood specimens.** Venous blood was drawn into chilled tubes containing EDTA and aprotinin (TrasyloL; 20,000 KIU/ml, 200 μl per 10 ml blood; Bayer AG, Leverkusen, Germany) and kept on ice. After centrifugation at 4°C, plasma for hormone analyses was kept frozen at −28°C. Capillary blood samples collected from the ear lobe were stored in NaF (Microvette CB 300; Sarstedt, Nümbrecht, Germany) for immediate measurement of glucose.

**Laboratory determinations.** Glucose was measured with a Glucose Analyzer (Beckman Instruments, Munich, Germany). Insulin and C-peptide were measured using enzyme-linked immunosassays (ELISAs) (7). GIP immunoreactivity was determined using two different radioimmunoassays specific for either the COOH-terminus or the NH₂-terminus of the peptide (12). Calculations. Increments (Δ) in insulin, C-peptide, and GIP concentrations after the bolus administrations were calculated as differences between peak concentrations reached within 5 min and concentrations at the beginning of each infusion period (t = 0, 60, and 120 min, respectively). To calculate the respective increments during the continuous infusion periods, mean steady-state plasma concentrations (during the last 40 min of each infusion period) were subtracted from the baseline concentrations at the beginning of the infusion periods in a similar way. Relative increases in plasma levels were calculated as percentage values of these baseline levels. For dose-response relationships, B_max (the maximum response) and k_s (the GIP concentration eliciting half-maximal responses) were estimated by nonlinear regression analysis (GraphPAD Prism, version 4.0) using the equation $y = B_{\text{max}} \times (k_s + x)$. Statistical analysis. Results are reported as means ± SE. Statistical calculations were carried out using repeated-measures ANOVA (RM-ANOVA), using Statistica Version 5.0 (Statsoft Europe, Hamburg, Germany). This analysis provides P values for differences between groups (A), differences over time (B), and for the interaction of groups with time (AB). If a significant interaction of treatment and time was documented (P < 0.05), values at single time points were compared by one-way ANOVA. A P value < 0.05 was taken to indicate significant differences. Regression analyses were carried out using GraphPad Prism 4 (San Diego, CA).

**RESULTS**

Plasma concentrations of total and intact GIP were not different between type 2 diabetic patients and control subjects during the experiments (Fig. 1). Fasting glucose levels were significantly higher in patients (P < 0.001). During the hyperglycemic clamp, glucose concentrations were similar in all groups (Fig. 1). Glucose infusion rates were higher in control subjects than in patients (P < 0.05). GIP infusion led to a stepwise increase in the amount of glucose required to maintain the clamp conditions in control subjects, whereas glucose infusion rates only slightly increased in patients (Fig. 1).

Fasting insulin concentrations were not different between patients and control subjects (Fig. 1). Hyperglycemia led to an increase in insulin secretion in control subjects (P = 0.019 and P = 0.001), whereas it remained almost unchanged in the patients (P = 0.59 and P = 0.98, for insulin and C-peptide, respectively). Constant GIP infusion only slightly increased insulin secretion in healthy control subjects (1.7 ± 0.1-fold and 1.8 ± 0.1-fold during the low-dose GIP infusion, P = 0.15 and P = 0.029, respectively; and 1.2 ± 0.1-fold and 1.2 ± 0.1-fold during the high-dose GIP infusion, P = 0.64 and P = 0.45 for insulin and C-peptide, respectively). The stimulation of insulin secretion by constant GIP infusion was even weaker in patients (1.3 ± 0.1-fold and 1.2 ± 0.04-fold during the low-dose GIP infusion, P = 0.13 and P = 0.38, respectively; and 1.05 ± 0.04-fold and 1.2 ± 0.04-fold during the high-dose GIP infusion, P = 0.67 and P = 0.77, respectively).

The bolus administration of GIP elicited a more pronounced insulin secretory response (Fig. 1). In the control subjects, the low-dose GIP bolus raised insulin secretion 2.9 ± 0.2-fold and 1.7 ± 0.1-fold (P = 0.035 and P = 0.041, for insulin and C-peptide, respectively), the high-dose GIP bolus further increased insulin secretion 2.8 ± 0.3-fold and 1.6 ± 0.1-fold (P = 0.071 and P = 0.22, respectively). In the patients, insulin secretion increased 2.2 ± 0.2-fold and 1.4 ± 0.1-fold after the low-dose GIP bolus (P = 0.004 and P = 0.21 for insulin and C-peptide, respectively) and 2.5 ± 0.2-fold and 1.6 ± 0.1-fold after the high-dose GIP bolus (P < 0.001 and P = 0.072, respectively).

Expressing increments in insulin concentrations after GIP bolus injection in relation to the respective increments in GIP plasma concentrations, a dose-dependent relationship between insulin secretion and the increase in GIP levels was obvious in both groups (Fig. 2). Interestingly, although the overall amount of insulin secreted in
response to GIP was lower in the patients, the pattern of
the dose-response curves was almost identical in patients
with type 2 diabetes and control subjects (Fig. 2). Accord-
ingly, the maximal responses of insulin secretion after
bolus administration were significantly lower in patients
with type 2 diabetes compared with control subjects (\(B_{\text{max}}\) 18.3 ± 0.62 and 74.4 ± 0.9 mU/l, respectively; \(P < 0.05\)),
but the GIP concentrations eliciting half-maximal re-
responses were similar in both groups (\(k_D\) 55.2 ± 7.14 and
45.9 ± 1.96 pmol/l, respectively, NS).

**DISCUSSION**

A reduced insulinotropic effect of GIP in patients with type
2 diabetes has uniformly been described in different stud-
ies (5–8,13), but the underlying reasons are rather ob-
Scure. Most notably, the preserved effectiveness of the
other incretin hormone, GLP-1, gave rise to the assump-
tion that a specific defect in GIP action may be of
importance for the development of type 2 diabetes (9).
Therefore, various groups have attempted to determine
structural changes of the GIP receptor in type 2 diabetic
patients (14,15) as well as phenotypic alterations of the
GIP responsiveness in patients at high risk (7,16). How-
ever, no conclusive evidence was gathered for a primary
defect in GIP action in type 2 diabetes.

The present data demonstrate that insulin secretion in
type 2 diabetic patients can still be stimulated by GIP if the
peptide is administered as a bolus. Interestingly, although
the absolute amount of insulin released in response to the
GIP boluses was substantially lower in the patients, the
pattern of the dose-response curve was almost identical in
both groups, indicating similar relative β-cell sensitivity
toward GIP. In contrast, the continuous infusion of two
different GIP doses had only a minor impact on insulin
secretion in the control subjects and almost completely failed to increase insulin levels in type 2 diabetic patients.

Vilsbøll et al. (8) chose another approach to investigate the capability of GIP to augment insulin secretion in type 2 diabetic patients and healthy control subjects. In their experiments, GIP and GLP-1 were administered as a bolus or by constant infusion and the insulin secretory response to GIP was assessed in comparison to the response to GLP-1 administration. The authors conclude that the late phase of insulin secretion after stimulation with GIP is impaired in type 2 diabetes, whereas the response in the early phase is almost preserved (8,17). This is different from GLP-1, which is capable of augmenting insulin secretion in both patients and healthy subjects during the early and the late phases (8,18,19).

The fundamental question arising from these and our studies is why the β-cells in type 2 diabetes respond differently if exposed to GIP acutely or constantly. Also, it is unclear why GLP-1 is capable of stimulating insulin release in type 2 diabetic patients, even during its continuous administration over prolonged periods of time (18,20,21). The explanation may be found in the intracellular actions of both peptides. In a kinetic model for the trafficking of secretory granules within single β-cells, different functional vesicle pools have been assumed to constitute insulin secretion (22). A readily releasable pool of granules is associated with first-phase insulin secretion, whereas reserve pools of vesicles are thought to sustain second-phase secretion (22). According to this model, it is conceivable that while GIP only stimulates the exocytosis of insulin granules from the readily releasable pool, GLP-1 also activates insulin biosynthesis and induces the intracellular trafficking of vesicles from the reserve pool via additional pathways (23).

In isolated β-cells from healthy human subjects, the mechanisms by which GIP and GLP-1 stimulate insulin release have been studied in detail: both peptides 1) inhibit KATP channel activity, 2) increase the influx of Ca2+ through voltage-dependent calcium channels, and 3) augment calcium-dependent exocytosis from the readily releasable pool (24,25). It would be interesting to study the signaling pathways and the trafficking of insulin secretory vesicles in isolated islets or single β-cells from patients with type 2 diabetes as well in order to elucidate the diverging actions of both peptides in such patients.

While there is little doubt about the therapeutic potential of GLP-1 in type 2 diabetes (26,27), the case for using GIP as an antidiabetic drug appears less convincing (28). Indeed, normalization of glucose concentrations has been observed during intravenous administration of GLP-1 or its derivatives in type 2 diabetic patients (18,19,29). With GIP and its analogs, however, glucose concentrations were lowered minimally (30). Based on the dose-response relationships for GIP in the present study as well as in previous studies using even higher GIP doses (8), it appears unlikely that further increased GIP doses would be sufficient to normalize glycemia in type 2 diabetic patients in a similar way as for GLP-1 (18,19).

In conclusion, the stimulation of insulin secretion by GIP is more pronounced after its bolus administration than during continuous infusion. The relative sensitivity of insulin secretion to a GIP bolus is preserved in patients with type 2 diabetes, even though at a lower level of β-cell function. Therefore, the existence of a GIP receptor defect in type 2 diabetes appears unlikely.

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