Gastric inhibitory polypeptide does not inhibit gastric emptying in humans

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Gastric inhibitory polypeptide (GIP) is a 42-amino acid peptide secreted from K cells in the gut in response to nutrient ingestion (5, 12). Early studies in dogs demonstrated an inhibition of gastric acid secretion during GIP administration (2, 3), leading to the assumption of GIP being an “enterogastrone.” Later, inhibition of gastrin release by GIP was described as the mechanism underlying the reduction in gastric acid output (33). However, when more physiological doses of GIP were used, other investigators failed to demonstrate GIP effects on gastric acid secretion (34). Although the suppression of gastric acid secretion does not appear to be the major physiological role of GIP, glucose-dependent stimulation of insulin secretion was consistently observed in response to GIP administration under different experimental conditions (1, 7–10). Therefore, in 1976 Brown and Pederson suggested that the peptide be renamed as a “glucose-dependent insulinotropic polypeptide” (4).

Similarly to GIP, a second incretin, glucagon-like peptide (GLP-1), stimulates insulin secretion during hyperglycemia (13, 21). In addition, GLP-1 profoundly decelerates gastric emptying (16, 23, 30), thereby slowing the entry of nutrients into the circulation. In the postprandial state, the deceleration of gastric emptying by GLP-1 has a major influence on glucose homeostasis (16, 23). Because GIP and GLP-1 are released under similar physiological conditions (20), act via similar types of receptors and downstream signaling pathways (16, 23), and exhibit parallel effects on insulin secretion (20), it is necessary to define those actions that are specific to one of the two incretins. For example, GLP-1 suppresses glucagon secretion (22), whereas GIP, under certain conditions, is stimulatory (18). Moreover, GLP-1 stimulates insulin secretion even in patients with type 2 diabetes, whereas the insulinotropic effect of GIP is lost in these patients (21). Therefore, it was the aim of the present study to assess gastric emptying during GIP administration in healthy human volunteers.

PATIENTS AND METHODS

Study protocol. The study protocol was approved before the study by the ethics committee of the Ruhr-University of Bochum on January 29, 2002 (registration number 1835). Written informed consent was obtained from all participants.

Patients. Fifteen healthy male volunteers participated in the study. The age was 23.9 ± 3.3 (SD) yr, the body mass index was 23.7 ± 2.3 kg/m², and the waist-to-hip ratio was 0.96 ± 0.04. Mean HbA1c was 5.5 ± 0.1% (normal range: 4.8–6.0%), total cholesterol concentrations were 4.42 ± 1.07 mmol/l (normal: <5.2 mmol/l), triglyceride concentrations were 1.17 ± 0.40 mmol/l (normal: <2.3 mmol/l), and fasting glucose concentrations were 5.36 ± 0.94 mmol/l (normal fasting range: <6.0 mmol/l). One subject presented with a plasma
glucose concentration of 6.1 mmol/l, but on a second occasion glucose values were in the normal range (5.1 mmol/l). All other subjects showed normal fasting glucose concentrations. None of the patients had a history of gastrointestinal disorders, had previously undergone abdominal surgery, or was taking any medication with a known modulating effect on gastrointestinal motility. One patient was a current smoker; the other subjects were nonsmokers.

From all participants, blood was drawn in the fasting state for measurements of standard hematological and clinical chemistry parameters. Subjects with anemia (hemoglobin <12 g/dl), an elevation in liver enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, y-glutamyltransferase) to higher activities than double the respective normal value, or elevated creatinine concentrations (>1.5 mg/dl) were excluded.

**Study design.** All participants were studied on three occasions. At a screening visit, blood was drawn in the fasting state for laboratory parameters, and a clinical examination was performed. If subjects met the inclusion criteria, they were recruited for the following tests. On separate occasions, gastric emptying was determined over 360 min during the intravenous administration of GIP (2 pmol/kg⁻¹·min⁻¹) or placebo (0.9% NaCl with 1% human serum albumin) from 30 to 360 min. The tests were carried out in random order. At least 1 wk had to pass between the tests to avoid carryover effects.

**Peptides.** Synthetic GIP was purchased from PolyPeptide Laboratories (Wolfenbüttel, Germany) and processed for infusion as described (19).

**Experimental procedures.** The tests were performed in the morning after an overnight fast with subjects in a supine position throughout the experiments, with the upper body lifted by 30°. Two forearm veins were punctured with a Tellon cannula (Moskito 123, 18 gauge; Vygon, Aachen, Germany) and kept patent using 0.9% NaCl (for blood sampling and for GIP/placebo administration, respectively).

As blood samples had been drawn (−45 and −30 min), the experiments were started with the infusion of GIP or placebo at −30 min. After 0 min, a standard test meal (one egg, two slices of bread, 5 g of margarine, 150 ml of water; 250 kcal) containing 100 mg of [13C]sodium octanoate was served, and breath samples were collected in 15-min intervals during the first 180 min and in 20-min intervals during the last 180 min. Venous blood samples were collected in 30-min intervals. All participants were contacted within 48 h after the experiments for a report of any adverse effects.

**Blood specimen.** Venous blood was drawn into chilled tubes containing EDTA and aprotinin (TrasyloI; 20,000 kallikrein inhibitor units/ml; Bayer, Leverkusen, Germany) and kept on ice. After centrifugation at 4°C, plasma for hormone analyses was kept frozen at −28°C. This procedure has previously been shown to prevent in vitro degradation of incretin hormones in human plasma samples (6).

**Determination of gastric emptying.** Gastric emptying was determined as described (16). Briefly, [13C]sodium octanoate (100 mg; Euriso-top, Saint-Aubin, France) was mixed into scrambled eggs to label the solid component of the test meal. At intervals of 15 or 20 min, breath specimens were sampled into gas-tight plastic bags, and the 13CO2 content was determined within 24 h by nondispersive infrared spectrometry (Wagner Analysetechnik, Bremen, Germany).

To measure the proportion of the substrate given by mouth that is metabolized, the results were expressed as a percentage dose of 13C recovered (PDR) over time for each time interval from which the cumulative PDR (cPDR) for each time interval was calculated, according to Ghoos et al. (9).

The evaluation of the octanoate breath test for gastric emptying was done by nonlinear regression analysis (GraphPad Prism, version 2; San Diego, CA) of the 13CO2 excretion curves (PDR) to the formula PDR(t) = a·e⁻^kt, which has been derived from the χ² distribution in statistics. The expression ln a, as gastric emptying coefficient (GEC), is a reliable parameter to describe the rate at which the stomach empties (9).

The percentage of 13CO2 cumulative values was fit with a model given by cPDR(t) = M(1 - e⁻^-kt), where M, k, and B are regression-estimated constants, with M the total amount of 13CO2 expired when time is infinite. Gastric half-emptying time (t50) was calculated by taking PDR(t) as equal to M/2 in the PDR equation, which is expressed as t50 = (−1/k)ln(1 − 2−^0.69) (1).

Gastric emptying was expressed as a percentage of the initial gastric contents (M = 100%) by computing the difference from this initial value at each time point according to the formula in which gastric content (t) = [M − cPDR(t)]/M·100 (in %).

**Laboratory determinations.** Glucose was measured as described (19), using a glucose oxidase method with a Glucose Analyzer 2 (Beckman Instruments, Munich, Germany).

Insulin was measured using an insulin microparticle enzyme immunoassay, IMX Insulin (Abbott Laboratories, Wieshagen, Germany). Intra-assay coefficients of variation were ∼4%.

C-peptide was measured using an ELISA from DAKO (Cambridge, UK). Intra-assay coefficients of variation were 3.3–5.7%; interassay variation was 4.5–5.7%. Human insulin and C-peptide were used as standards.

**GIP immunoreactivity was determined by using two different assays specific for either the COOH terminus or the NH2 terminus of the peptide, as described (7). The COOH-terminal assay measures the sum of intact GIP-(1–42) and the truncated metabolite GIP-(3–42) by use of antisera R65. The assay has a detection limit of <2 pmol/l and an intra-assay variation of ∼6%. The NH2-terminal assay measures the concentration of intact GIP-(1–42) by use of antisera 98171. The cross-reactivity with GIP-(3–42) was <0.1%. The lower detection limit of the assay is ∼5 pmol/l. Intra-assay variation was <6%, and interassay variations were ∼8% and 12% for 20 and 80 pmol/l standards, respectively. For both assays, human GIP (Peninsula Laboratories) was used as standard, and radiolabeled GIP was obtained from Amersham Pharmacia Biotech (Aylesbury, UK). Valine- pyrrolidide (0.01 mmol/l, final concentration) was added to the assay buffers to prevent NH2-terminal degradation of GIP during the assay incubation.

**Statistical analysis.** Results are reported as means ± SE. All statistical calculations were carried out using repeated-measures analysis of variance (ANOVA) with Statistica version 5.0 (Statsoft Europe, Hamburg, Germany). This analysis provides P values for differences between the experiments, differences over time, and for the interaction of experiment with time. 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 two-sided P value < 0.05 was taken to indicate significant differences.

**Calculations.** Steady-state plasma concentrations of GIP were calculated by means of the values determined between 240 and 360 min, because at these time points the contribution of endogenous GIP secretion was negligible. Integrated incremental areas under the curve for GIP plasma concentrations were calculated according to the trapezoidal rule. For the calculation of endogenous GIP secretion after meal ingestion during GIP infusion, integrated incremental plasma concentrations between 0 and 240 min were calculated over the GIP concentrations from 240 to 360 min, because at these time points steady-state plasma levels were reached.

**RESULTS**

During the exogenous infusion, GIP plasma concentrations rose to steady-state levels of 159 ± 15 and 34 ± 4 pmol/l for total and intact GIP, respectively (P < 0.0001; Fig. 1). Meal ingestion further increased GIP concentrations to peak levels of 265 ± 20 and 82 ± 9 pmol/l for total and 67 ± 7 and 31 ± 3 pmol/l for intact GIP (during the administration of GIP or
GIP AND GASTRIC EMPTYING

Fig. 1. Plasma concentrations of total gastric inhibitory polypeptide [GIP-(1–42)] plus spit products (A) and intact GIP-(1–42) (B) during administration of GIP (2 pmol·kg⁻¹·min⁻¹ iv, ♦) or placebo (human serum albumin, ◦) from −30 to 360 min in 15 healthy male subjects. At 0 min, a mixed meal (250 kcal) was served (arrows). Data are expressed as means ± SE. P values were calculated using repeated-measures ANOVA and denote differences between experiments (A), differences over time (B), and differences from interaction of experiment and time (AB).

placebo, respectively). The total amount of endogenous GIP secreted after the meal was similar during the two experiments (AUCtotal GIP 0–360 min: 8,938 ± 1,395 and 7,410 and 1,040 pmol·l⁻¹·min⁻¹; P = 0.39; AUCintact GIP 0–240 min: 2,178 ± 289 and 1,738 ± 277 pmol·l⁻¹·min⁻¹; P = 0.28, for GIP and placebo administration, respectively).

Gastric emptying was almost complete during the experimental period of 360 min, with 17.5 ± 2.3 and 16.2 ± 3.8% of the initial content retained in the stomach at the end of the experiments (for GIP and placebo, respectively; P = 0.78; Fig. 2). There were no differences in the time course of gastric emptying between the experiments (P = 0.98). Gastric half-emptying times were 120 ± 9 min and 120 ± 18 min (P = 1.0), and gastric emptying coefficients were 2.5 ± 0.2 and 2.6 ± 0.1 (for GIP and placebo, respectively; P = 0.75).

There was no correlation between the total amount of GIP secreted after meal ingestion during the placebo experiments (AUCGIP 0–360 min) and the gastric emptying half-times (r² = 0.21, P = 0.086 for total GIP and r² = 0.15, P = 0.15 for intact GIP; details not shown).

After meal ingestion, plasma glucose concentrations increased significantly in both experiments (P < 0.0001; Fig. 3). This was accompanied by a rise in insulin secretion (P < 0.0001 for insulin and C-peptide concentrations). There were no differences between the experiments with GIP or placebo administration (P = 0.22, P = 0.13, and P = 0.85 for glucose, insulin, and C-peptide, respectively; Fig. 3).

Five patients reported mild gastrointestinal side reactions within 24 h after the experiments with GIP infusion. Those were diarrhea in two cases, flatulence in four cases, and abdominal pain in one case. In contrast, no subject reported side reactions after placebo infusion (P = 0.014).

DISCUSSION

Because GIP is released from endocrine cells in the intestinal mucosa in response to meal ingestion and acts to inhibit gastric acid secretion in animals as well as humans (3, 15, 27, 32), it was proposed to be an enterogastrone (31, 32). On the basis of the original definition, this would also imply inhibitory activities on gastric motility (11, 31). However, the present data do not support a role for GIP in the regulation of gastric emptying in humans. This was rather unexpected, particularly because the other incretin hormone, GLP-1, potently decelerates emptying of the stomach in healthy subjects as well as in patients with type 2 diabetes (16, 23, 30).

Given the lack of GIP effect on gastric emptying in the present experiments, the following methodical aspects may be reconsidered. The infusion rate of GIP chosen in the present study raised GIP plasma levels into the supraphysiological range (Fig. 1). Therefore, inappropriately low dosing of the GIP infusion does not explain the absence of GIP effects. Moreover, the determination of intact, biologically active GIP-(1–42) with a highly specific immunoassay raised against the NH₂-terminus of the GIP molecule provided evidence that a considerable proportion (~35%) of the total GIP infused was still intact and thus biologically active. The GIP preparation used was from the same charge and manufacturer as in previous studies from our group and has proven its potency to stimulate insulin and glucagon secretion under different experimental conditions (17–19). Also, the [¹³C]octanoid breath test employed for the determination of gastric emptying has proven

Fig. 2. Time pattern of gastric emptying of a solid meal (250 kcal) during intravenous administration of GIP (2 pmol·kg⁻¹·min⁻¹, ♦) or placebo (human serum albumin, ◦) from −30 to 360 min in 15 healthy male subjects. Gastric emptying was determined from the measurement of ¹³CO₂ in breath samples collected after the ingestion of a test meal (arrow) labeled with 100 mg [¹³C]sodium octanoate with infrared absorptiometry and expressed as a percentage of initial gastric content. Data are expressed as means ± SE. P values were calculated using repeated-measures ANOVA and denote differences between experiments (A), differences over time (B), and differences from interaction of experiment and time (AB).
GIP secretion to correlate with the gastric emptying half-times, effects. However, in this case one would expect endogenous further raising GIP concentrations did not have additional affected gastric emptying in the placebo experiments and that the meal, it is possible that endogenous GIP had already the infusion of GIP.

Because GIP was also released endogenously in response to the meal, it is possible that endogenous GIP had already affected gastric emptying in the placebo experiments and that further raising GIP concentrations did not have additional effects. However, in this case one would expect endogenous GIP secretion to correlate with the gastric emptying half-times, which was not the case. Also, the total amount of GIP secreted after the test meal was much lower than that reported after the ingestion of larger meals in previous studies (12, 28). Therefore, it appears unlikely that endogenous GIP secretion had a major impact on gastric emptying in the present experiments.

One way to assess the effects of endogenous GIP on gastric emptying in more detail would be to antagonize the peptide by use of receptor antagonists (14, 26). However, because GIP antagonists are not yet available for administration to humans, this question can only be addressed in animal studies.

Interestingly, GIP plasma concentrations were further increased after meal ingestion despite its exogenous administration at relatively high plasma concentrations (Fig. 1). This indicates that the secretion of GIP from K cells is not suppressed by elevated plasma concentrations, as we know it is for other peptide hormones including insulin (8). The absence of such feedback mechanisms may be important for the potential therapeutic use of incretin hormones for the treatment of type 2 diabetes.

In the present study, adding exogenous GIP to endogenously secreted GIP did not affect glycemia as well as insulin secretion. This may also be explained by the fact that peak GIP concentrations after the meal were already close to the upper end of the dose-response curve, as discussed with respect to gastric emptying, but it is more likely that the absence of GIP effects on glycemia and insulin secretion was due to the low peak glucose concentrations (100 and 94 mg/dl with placebo and GIP administration, respectively), at which insulin secretion is almost not influenced by GIP (29).

Our data are in good agreement with previous work from Schirra et al. (24), who studied the interaction of gastric emptying and the endogenous release of GIP and GLP-1. In that study, as in our findings, gastric emptying was not associated with the endogenous secretion of GIP, whereas GLP-1 secretion was identified as a major determinant of gastric emptying (24). These data underline the importance of GLP-1 as a major regulator of gastric motility (23).

Although emptying of the stomach is independent of GIP action, the incretin might be involved in the regulation of distal gut function, particularly in the induction of small bowel motility. Such effects would explain the significantly higher incidence of diarrhea and flatulences after GIP infusion observed in the present experiments. In line with this observation, earlier studies in dogs indicated a stimulation of intestinal motility by GIP (25). It might be of interest to evaluate the effects of GIP on distal gut motility in more detail.

In conclusion, the present data demonstrate no effect of GIP on gastric emptying in normoglycemic human subjects. Therefore, its role as an enterogastrone may be challenged. Given the well-characterized GIP effects on insulin secretion, the term glucose-dependent insulinotropic polypeptide appears to be more appropriate to denote GIP than gastric inhibitory polypeptide.

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GRANTS

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Fig. 3. Plasma concentrations of glucose (A), insulin (B), and C-peptide (C) during iv administration of GIP (2 pmol·kg⁻¹·min⁻¹, ○) or placebo (human serum albumin; ●) from -30 to 360 min in 15 healthy male subjects. At 0 min a mixed meal (250 kcal) was served (arrows). Data are expressed as means ± SE. P values were calculated using repeated-measures ANOVA and denote differences between experiments (A), differences over time (B), and differences from interaction of experiment and time (AB).
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