Gastric inhibitory polypeptide does not inhibit gastric emptying in humans

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GASTRIC HINHIBITORY 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, 18), 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 Hb A₁c was 5.5 ± 0.1% (normal range: 4.8–6.0%), total cholesterol concentration was 6.0 mmol/l. One subject presented with a plasma glucose concentration of 9.9 mmol/l. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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, γ-glutamyltranspeptidase) to Ghoos et al. (9), or patients who smoked were excluded from further analysis.

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·min) or placebo (0.9% NaCl with 1% human serum albumin) from 0–30 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.

Pepitides. 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.

Steady-state plasma concentrations of GIP were calculated according to Ghoos et al. (9) 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, Wiesbaden, 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 antiserum 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 antiserum 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.

Gastric emptying was expressed as a percentage of the initial gastric content (9). This procedure has previously been shown to be 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 regressionestimated constants, with M the total amount of 13CO2 expired when time is infinite. Gastric half-emptying time (t1/2) was calculated by taking PDR(t) as equal to M/2 in the PDR equation, which is expressed as PDR(t) = (t1/2/k)(1 - e^{-kt}) (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 %).

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Statistics. 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
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 in 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 \( \text{NH}_2 \) terminus of the GIP molecule provided evidence that a considerable proportion \((\sim 35\%)\) 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 \[^{13}\text{C}\]octanoid breath test employed for the determination of gastric emptying has proven...
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 flatulence 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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