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Short Communication

Gastric inhibitory polypeptide (GIP) dose-dependently stimulates glucagon secretion in healthy human subjects at euglycaemia

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Abstract

Aims/hypothesis. In the isolated perfused pancreas, gastric inhibitory polypeptide (GIP) has been shown to enhance glucagon secretion at basal glucose concentrations, but in healthy humans no glucagonotropic effect of GIP has yet been reported. Therefore, we studied the effect of GIP on glucagon secretion under normoglycaemic conditions.
Methods. Ten healthy subjects (9 men, 1 woman; age 33±11; BMI 26.8±2.2 kg/m^2) received three different doses of intravenous GIP (7, 20, and 60 pmol/kg body weight) and placebo. Venous blood samples were drawn over 30 min for glucagon and GIP concentrations (specific radioimmunoassays). In addition, 31 healthy subjects (16 men, 15 women; 42±11 years; BMI 24.4±2.7 kg/m^2) were studied with 20 pmol GIP/kg. Statistics were done with RM-ANOVA and Duncan's post hoc tests.
Results. Gastric inhibitory polypeptide dose-dependently stimulated glucagon secretion (p=0.019) with a maximal increment after 10 min. Incremental glucagon concentrations ($\Delta_{10–0\ min}$) were 0.1±0.7, 1.4±0.5, 2.4±0.5, and 3.4±0.8 pmol/l (for placebo and for 7, 20, and 60 pmol GIP/kg, respectively; p=0.017). After the injection of 20 pmol GIP/kg b.w. in 31 healthy subjects, glucagon concentrations increased over the baseline from 7.5±0.5 to 9.3±0.7 pmol/l (p=0.0082).
Conclusions/interpretation. Glucagon secretion is dose-dependently stimulated by GIP at basal glucose concentrations. The absence of a glucagonotropic GIP effect in previous studies could be due to the hyperglycaemic conditions used in these experiments. Our results underline differences between GIP and the glucagonostatic incretin GLP-1. [Diabetologia (2003) 46:798–801]

Keywords Gastric inhibitory polypeptide, GIP, glucagon, incretin hormones, glucose homeostasis.

Insulin secretion after an oral glucose load is stimulated not only by the rise of glucose concentrations, but also by the secretion of incretin hormones, namely gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) [1]. Both hormones stimulate insulin secretion in healthy subjects, but in contrast to GLP-1, GIP has almost lost its insulinotropic effect in patients with Type 2 diabetes [2, 3]. Studies in the isolated perfused rat pancreas indicated that GIP stimulates glucagon secretion, but only at glucose concentrations below 5.5 mmol/l [4]. In studies with healthy volunteers and in patients with Type 2 diabetes, however, no effects of GIP on glucagon secretion have been observed [1]. This could be due to the hyperglycaemic conditions used in these studies. Therefore, our aim was to evaluate the effects of intravenous GIP on glucagon secretion at basal glucose concentrations.

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Abbreviations: GIP, Gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; IR, immunoreactive.

Subjects and methods

Study protocol. The study protocol was approved by the ethics committee of the medical faculty of the Ruhr-University, Bochum, on December 12th, 1999 (registration number 1420)

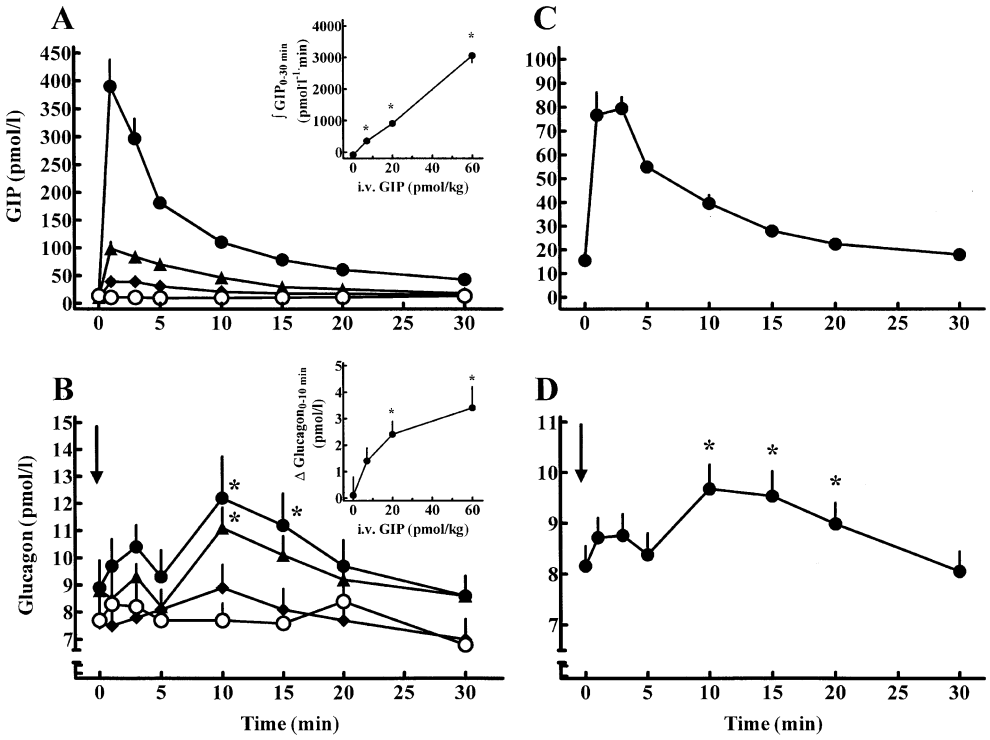

Fig. 1A–D. Plasma concentrations of total GIP (1–42 plus split products) (**A** and **C**) and glucagon (**B** and **D**) after the intravenous bolus administration of placebo (*open circles*) or GIP at 7 (*filled diamonds*), 20 (*filled triangles*), or 60 (*filled circles*) pmol per kg body weight injected at t=0 min (*arrows*) in healthy subjects. Left panels (**A** and **B**): Dose-response relation studied in ten healthy subjects. Right panels (**C** and **D**): Single-dose study in 31 healthy subjects. GIP increased significantly ($p<0.0001$) with all doses employed (Repeated-measures ANOVA). Glucagon increased in a dose-dependent fashion (**B**, $p=0.019$; **D**, $p=0.008$). Inserts in the left panels indicate dose-response relations for integrated incremental GIP (**A**, $p<0.0001$) and increments (10 min vs baseline) in glucagon plotted against the amount of GIP administered (**B**, $p=0.017$). Data are reported as means ± SEM. Asterisks indicate significant differences ($p<0.05$) to placebo at specific time points (**A**, **B**; ANOVA, Duncan's post-hoc test) or relative to baseline values (**C**, **D**)

prior to the study. Written informed consent was obtained from all participants.

Study design. A screening visit included an OGTT (75 g) and measurements of standard haematological and clinical chemistry parameters. Having first-degree or second-degree relatives with diabetes mellitus, anaemia (haemoglobin <12 g/dl), or an increase in liver enzymes (ALAT, ASAT, AP, γ-GT) to higher than double the respective normal value, or increased creatinine concentrations (>1.5 mg/dl), were the exclusion criteria.

Dose-response study. Ten healthy volunteers participated in four experiments with the intravenous administration of 7, 20, and 60 pmol GIP/kg body weight, and placebo. At least one day had to pass between the experiments.

Single-dose study. Furthermore, 31 subjects were studied with the intravenous administration of 20 pmol GIP/kg.

Subject characteristics: dose-response study. We studied ten healthy subjects with the following characteristics (means ± SD): 9 men, 1 woman; age 33±11 years; HbA_{1c} 4.9±1.2% (normal range 4.8–6.0%); BMI 26.8±2.2 kg/m2; OGTT fasting glucose concentrations 5.6±0.3 mmol/l; OGTT 120 min glucose concentrations 5.7±1.5 mmol/l; total cholesterol 4.94±1.92 mmol/l; HDL-cholesterol 1.04±0.65 mmol/l; LDL-cholesterol 3.41±1.35 mmol/l; triglycerides1.40±0.70 mmol/l. No subject had a history of arterial hypertension or hyperlipidaemia.

Subject characteristics: single-dose study. The 31 healthy subjects with the following characteristics were studied: 16 men, 15 women; age 42±11 years; HbA_{1c} 5.5±0.4%; BMI 24.4±2.7 kg/m^2; OGTT fasting glucose concentrations 5.7±0.6 mmol/l; OGTT 120 min glucose concentrations 6.9±1.4 mmol/l; total cholesterol 5.41±1.12 mmol/l; HDL-cholesterol 1.46±0.57 mmol/l; LDL-cholesterol 3.28±1.33 mmol/l; triglycerides 1.23±0.63 mmol/l. Six subjects had a history of arterial hypertension and four subjects had hyperlipidaemia.

Experimental procedures. The tests were carried out in the morning with the subjects in a sitting position and after an overnight fast. Forearm veins were punctured with a teflon cannula (Moskito 123, 18 gauge, Vygon, Aachen, Germany), and kept patent using 0.9% NaCl. After drawing two basal blood specimens at 0 min, a bolus of synthetic human GIP or placebo (0.9% NaCl/1% human serum albumin) was administered intravenously. Venous blood samples were obtained at 1, 3, 5, 10, 15, 20, and 30 min and processed as described [3].

Peptides. Synthetic GIP was purchased from PolyPeptide Laboratories, Wolfenbüttel, Germany (lot number E-0517) and was prepared for intravenous administration as described previously [3]. No bacterial contamination was detected. Endotoxin concentrations in samples from the GIP stock solution were 0.08 IU/ml.

Laboratory determinations. Total and intact IR-GIP were determined as described previously [5]. IR-glucagon was

measured using antibody 4305 in ethanol-extracted plasma, as described [6].

Calculations. Integrated incremental responses were calculated using the trapezoidal rule. Increments (Δ) in glucagon concentrations were calculated as differences between values determined 10 min after the administration of GIP or placebo and basal concentrations (0 min).

Statistical analysis. Results are reported as means ± SEM. All statistical calculations were carried out using repeated-measures ANOVA using Statistica version 5.0 (Statsoft Europe, Hamburg, Germany). Values at single time points were compared by one-way ANOVA followed by a Duncan`s post hoc test. A two-sided p value of less than 0.05 was taken to indicate significant differences.

Results

Dose-response study. After the intravenous administration of placebo, and of 7, 20, and 60 pmol GIP per kg body weight, GIP plasma concentrations rose to a peak of 11±3, 39±6, 99±12, and 390±48 pmol/l, respectively for total GIP (1–42 plus split products; $p<0.0001$; Fig. 1A) and 8±1, 27±4, 68±8, and 233±19 pmol/l for intact GIP (1–42; $p<0.0001$). Glucagon concentrations dose-dependently rose after GIP administration, with maximal concentrations being reached after 10 min ($p=0.019$; Fig. 1B). Also calculating increments in glucagon concentrations after 10 min over baseline, a significant stimulation of glucagon secretion was observed with 20 and 60 pmol GIP/kg b.w. ($p=0.017$).

Single-dose study. To mimic physiological conditions after meal ingestion, the dose of 20 pmol GIP per kg b.w. was chosen for the following experiments. Gastric inhibitory polypeptide administration raised plasma concentrations to 77±9 pmol/l for total GIP (Fig. 1C), and 47±7 pmol/kg for intact GIP. Similar to the dose-response study, a significant rise of glucagon concentrations over baseline reaching a peak after 10 min was observed after GIP injection (Fig. 1D; $p=0.0082$).

Discussion

The secretion of incretin hormones has been shown to be a major stimulus of postprandial insulin release [1]. However, although GIP and GLP-1 are both secreted under similar physiological conditions, share large structural homologies and exert their actions via similar trans-membrane receptors, both hormones differ with respect to some important features. While GLP-1 stimulates insulin secretion also in patients with Type 2 diabetes, the insulinotropic effect of GIP is almost lost in these patients [2, 3]. Morerover, due to central effects on the control of appetite and food intake, GLP-1 has a potential to reduce body weight, whereas GIP has recently been shown to cause weight gain via its effects on adipose tissue [7].

A glucose-dependent suppression of glucagon secretion in response to GLP-1 has been observed in healthy subjects as well as in patients with Type 1 and 2 diabetes [2]. In contrast, during the administration of GIP, no effects on glucagon concentrations have been described in published human studies [1, 2, 3]. Only in patients with liver cirrhosis and basal hyperglucagonaemia, a glucagonostatic GIP effect has been observed [8]. When studying GIP effects on glucagon secretion, it is important to consider the prevailing glucose concentrations. In the isolated perfused rat pancreas, it has been shown that glucagon secretion is exclusively stimulated by GIP at glucose concentrations below 5.5 mmol/l [4], but due to the glucose-dependency of the GIP effects on insulin secretion, hyperglycaemic conditions have been chosen in most previous experiments. We have previously studied the effects of an intravenous infusion of GIP (2 pmol·kg^{-1}·min over 60 min) during a hyperglycaemic clamp experiment aiming at plasma glucose concentrations of 140 mg/dl [3]. In this study, there was not even a tendency towards a stimulation of glucagon secretion during the GIP infusion, compared to placebo ($p=0.99$). Therefore, an absence of GIP effect on glucagon secretion in earlier studies is most likely due to increased glucose concentrations.

Comparing the different physiological actions of GIP on endocrine pancreatic secretion, it is interesting that glucagon secretion is stimulated only under basal glucose concentrations, whereas insulin secretion is most prominently enhanced in the presence of hyperglycaemia [3], suggesting a role for GIP in the feedback control of glucose homeostasis.

Since GIP concentrations in the single-dose study correspond to those endogenously reached after the ingestion of a mixed meal (100±11 and 54±9 pmol/l for total and intact GIP, respectively [9]), the stimulation of glucagon secretion seems to be relevant at physiological conditions and could be operative, for example, after triglyceride-rich meals that release GIP but do not raise plasma glucose concentrations.

Gastric inhibitory polypeptide analogues have recently been suggested for the treatment of Type 2 diabetes [10]. However, the question arises, whether GIP possesses an action profile favorable for the treatment of Type 2 diabetes similar to that of GLP-1. It has been shown that GIP and its analogues have a glucose-lowering effect, but particularly in patients with Type 2 diabetes this effect is much weaker than that of GLP-1 [2]. In addition, the glucose-lowering effect of GLP-1 is not only based on the stimulation of insulin release, but also on the suppression of glucagon secretion. Our data show that GIP, in contrast to GLP-1, even stimulates glucagon secretion. Therefore, our study provides additional reasons to prefer GLP-1 derivatives over GIP analogues for the treatment of Type 2 diabetes.

In conclusion, we have shown that GIP dose-dependently stimulates glucagon secretion in humans at basal glucose concentrations. The glucagonotropic effect is suppressed by increased glucose concentrations. These data highlight the diversity between the incretin hormones GIP and GLP-1.

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