Erythromycin Antagonizes the Deceleration of Gastric Emptying by Glucagon-Like Peptide 1 and Unmasks Its Insulinotropic Effect in Healthy Subjects

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Glucagon-like peptide 1 (GLP-1) has been proposed to act as an incretin hormone due to its ability to enhance glucose-stimulated insulin secretion. Because GLP-1 also decelerates gastric emptying, it physiologically reduces rather than augments postprandial insulin secretory responses. Therefore, we aimed to antagonize the deceleration of gastric emptying by GLP-1 to study its effects on insulin secretion after a meal. Nine healthy male volunteers (age 25 ± 4 years, BMI 25.0 ± 4.9 kg/m^2) were studied with an infusion of GLP-1 (0.8 pmol · kg^{-1} · min^{-1} from −30 to 240 min) or placebo. On separate occasions, the prokinetic drugs metoclopramide (10 mg), domperidone (10 mg), cisapride (10 mg, all at −30 min per oral), or erythromycin (200 mg intravenously from −30 to −15 min) were administered in addition to GLP-1. A liquid test meal (50 g sucrose and 8% mixed amino acids in 400 ml) was administered at 0 min. Capillary and venous blood samples were drawn for the determination of glucose (glucose oxidase), insulin, C-peptide, GLP-1, glucagon, gastric inhibitory polypeptide (GIP), and pancreatic polypeptide (specific immunoassays). Gastric emptying was assessed by the phenol red dilution technique. Statistical analyses were performed using repeated-measures ANOVA and Duncan's post hoc test. GLP-1 significantly decelerated the velocity of gastric emptying (P < 0.001). This was completely counterbalanced by erythromycin, whereas the other prokinetic drugs used had no effect. Postprandial glucose concentrations were lowered by GLP-1 (P < 0.001 vs. placebo), but this effect was partially reversed by erythromycin (P < 0.05). Insulin secretory responses to the meal were lower during GLP-1 administration (P < 0.05 vs. placebo). However, when erythromycin was added to GLP-1, insulin concentrations were similar to those in placebo experiments. The suppression of meal-related increments in glucagon secretion by GLP-1 was reversed by erythromycin (P < 0.001). The time course of GIP secretion was delayed during GLP-1 administration (P < 0.05), but when erythromycin was added, the pattern was similar to placebo experiments. GLP-1 administration led to a reduction in pancreatic polypeptide plasma concentrations (P < 0.05). In contrast, pancreatic polypeptide levels were markedly increased by erythromycin (P < 0.001). Intravenous erythromycin counteracts the deceleration of gastric emptying caused by GLP-1, probably by interacting with the parasympathetic nervous system (pancreatic polypeptide responses). Despite augmented rises in insulin secretion, the glucose-lowering effect of GLP-1 is markedly reduced when the deceleration of gastric emptying is antagonized, illustrating the importance of this facet of the multiple antidiabetic actions of GLP-1. *Diabetes* 54: 2212–2218, 2005

Insulin secretion after meal ingestion is stimulated not only by the rise in glycemia, but also by the secretion of peptide hormones ("incretins") from the gut (1,2). The postprandial enhancement of insulin secretion by gut-derived factors is called the incretin effect (2,3). The first incretin hormone identified was gastric inhibitory polypeptide (GIP), which is also referred to as glucose-dependent insulinotropic polypeptide (4,5). An incretin role has also been proposed for the proglucagon-derived peptide glucagon-like peptide 1 (GLP-1) (6). This was based on its ability to enhance insulin secretion and to suppress glucagon release during an intravenous glucose infusion in healthy human volunteers (6). Moreover, the GLP-1 receptor antagonist exendin [9–39] blocked the insulin secretory response after intraduodenal administration of glucose in rats (7). Later, Edwards et al. (8) demonstrated a marked deterioration of oral glucose tolerance in healthy subjects during the administration of exendin [9–39]. However, when GLP-1 was administered during meal ingestion, a dose-dependent deceleration of gastric emptying as well as a reduction in postprandial insulin secretion was found in healthy volunteers as well as in patients with type 2 diabetes (9,10). Because the velocity of gastric emptying is a major determinant of postprandial glycemia and insulin secretion (11,12), it is difficult to ascertain an insulinotropic effect of GLP-1 after meal ingestion. One way to counterbalance the GLP-1 effects on gastric emptying would be to administer prokinetic drugs before meal ingestion. Therefore, it was the aim of the present experiments to antagonize the decelerating effects of GLP-1 on gastric emptying using a panel of prokinetic drugs with different modes of action (metoclo-

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GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1.

pramide, domperidone, cisapride, and erythromycin) to unmask its insulinotropic effects in the postprandial state.

RESEARCH DESIGN AND METHODS

The study protocol was approved by the ethics committee of the medical faculty of Ruhr University (Bochum, Germany) before the study. Written informed consent was obtained from all participants.

Nine healthy male volunteers were studied. They were 25 ± 4 years old, 181 ± 4 cm tall, and weighed 82 ± 17 kg. Their BMI was 25.0 ± 4.9 kg/m^2. All had a normal oral glucose tolerance according to World Health Organization criteria (fasting glucose 5.1 ± 0.4 mmol/l, 120-min value 5.0 ± 1.1 mmol/l). None had a family history of diabetes or a personal history of gastrointestinal disorders. Blood cell counts, serum transaminases, creatinine values, triglyceride, cholesterol, and HDL cholesterol concentrations were in the normal range.

The study was performed in a single-blinded fashion with all participants being studied in random order on six occasions.

1) A liquid mixed meal (50 g sucrose plus amino acids, 400 ml Aminosteril Hepa 8%; Fresenius AG, Bad Homburg, Germany) was instilled intragastrically at time 0. Placebo (0.9% NaCl with 1% human serum albumin (Behring AG, Marburg, Germany) was infused intravenously from −30 to 240 min.

2) A liquid test meal was administered as described in 1. In addition, a continuous intravenous administration of GLP-1 at a dose of 0.8 pmol · kg^{-1} · min^{-1} was started 30 min before the meal (at −30 min) and continued until 240 min.

3) In addition to the administration of the meal and GLP-1 (as described in 1 and 2), metoclopramide (Paspertin; 10 mg/10 ml) was administered orally at −30 min.

4) In addition to the administration of the meal and GLP-1 (as described in 1 and 2), domperidone (Motilium Saft; 10 mg/10 ml) was administered orally at −30 min.

5) In addition to the administration of the meal and GLP-1 (as described in 1 and 2), cisapride (Propulsin Saft; 10 mg/10 ml) was administered orally at −30 min.

6) In addition to the administration of the meal and GLP-1 (as described in 1 and 2), erythromycin (Erycinum; 200 mg/100 ml) was administered intravenously between −30 and −15 min.

An interval of at least 10 days was kept between the experiments to exclude carryover effects.

Peptides. Synthetic GLP-1 [7–36 amide] was purchased from Saxon Biochemicals (Hannover, Germnay). The lot number of GLP-1 [7–36 amide] (pharmaceutical grade) was PGAS 242, FGLP7369301 A, net peptide content 88%. The peptide was dissolved in 0.9% NaCl and 1% human serum albumin (HSA Behring, Marburg, Germany), filtered through 0.2 μm nitrocellulose filters (Sartorius, Göttingen, Germany), and stored frozen at −30°C as previously described (9). High-performance liquid chromatography profiles (provided by the manufacturer) showed that the preparation was >99% pure (single peak with appropriate standards). Samples were analyzed for bacterial growth (standard culture techniques) and for pyrogens (limulus amebocyte lysate endo-LAL; Chromogenix AB, Mölndal, Sweden). No bacterial contamination was detected. Endotoxin concentrations in the GLP-1 stock solutions were <0.03 EU/ml.

Experimental procedures. The tests were performed in the morning after an overnight fast. Two forearm veins were punctured with a Teflon cannula (Moskito 123, 18 gauge; Vygon, Aachen, Germany) and kept patent using 0.9% NaCl (for blood sampling and for GLP-1/placebo administration).

After drawing basal blood specimens, at −30 min an intravenous infusion of GLP-1 [7–36 amide] or placebo (0.9% NaCl containing 1% human serum albumin) was started and continued for 270 min. Blood was drawn at the time points −45, −30, −15, 0, 15, 30, 45, 60, 120, 180, and 240 min, and plasma glucose was determined immediately.

Before the study, a nasogastric tube (Freka-Ernährungssonde, 120 cm, CH12; Fresenius AG, Bad Homburg, Germany) was placed and tape-fixed with the tip ~55 cm from the nostrils. Gastric juice was aspirated and an acidic pH was ascertained using pH-sensitive Lackmus paper. The gastric lumen was washed with 100 ml water (37°C). The position of the tube was, if necessary, adjusted to allow near-complete aspiration of instilled fluid. The subjects were in a semirecumbent position with the upper half of the body 45 degrees upright. At 0 min, 400 ml (total volume) of the liquid test meal was instilled into the stomach. It was composed of 50 g sucrose dissolved in 400 ml Aminosteril Hepa 8%. This composition of the meal was chosen because the solution had to be clear for the photometric measurement of phenol red (measurement of gastric emptying, see below) and should be similar in caloric and nutrient content to a normal mixed meal. The meal contained 32 g mixed amino acids (131 kcal = 40%) and 50 g sucrose (196 kcal = 60%), with a total energy content of 327 kcal (energy density 0.82 kcal/ml).

Blood specimens. Blood was drawn into chilled tubes containing EDTA and aprotinin (Trasylol; 20,000 KIU/ml, 200 μl/10 ml blood; Bayer AG, Leverkusen, Germany) and kept on ice. A sample (~100 μl) was stored in NaF (Microvette CB 300; Sarstedt, Nümbrecht, Germany) for the measurement of glucose. After centrifugation at 4°C, plasma for hormone analyses was kept frozen at −30°C.

Gastric emptying. The velocity of gastric emptying was measured as described (9) by a double-sampling dye dilution technique using phenol red (Merck AG, Darmstadt, Germany) according to George (13), with modifications introduced to reduce measurement error by Hurwitz (14). This technique allows the repeated determination of gastric emptying under different experimental conditions. Briefly, at all time points chosen to measure gastric volume, a known amount of the nonabsorbable dye phenol red was added to the translucent liquid test meal in a volume of 5–15 ml. After thorough mixing with gastric contents for ~2 min, a gastric sample was drawn and the resulting step-up in phenol red concentrations was determined photometrically. The volume of gastric contents was determined from the volume of distribution of phenol red. As the experiments proceeded, increasing amounts of phenol red were used to ensure measurability of optical density increments in the presence of previously instilled phenol red. Gastric contents were determined at 0, 30, 60, 90, 120, 180, and 240 min and were expressed as the percentage of the initial volume of 400 ml at 0 min.

Laboratory determinations. Glucose was measured 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 C-peptide-antibody–coated microtiter wells (C-peptide MTPL EIA; DRG Instruments, Marburg, Germany). Intra-assay coefficients of variation were ~6%. Human insulin and C-peptide were used as standards.

Immunoreactive GLP-1 was determined in ethanol-extracted plasma as previously described (15), using antiserum 89390 (final dilution 1:150 000) for the measurement of GLP-1 [7–36 amide] and synthetic GLP-1 [7–36 amide] for tracer preparation and as standard. The experimental detection limit (2 SD over samples not containing GLP-1 [7–36 amide]) was <5 pmol/l. Antiserum 89390 binds to the amidated carboxyl terminus of GLP-1 [7–36 amide] and therefore measures the sum of intact and degraded GLP-1 [9–36 amide] in plasma. Intra-assay coefficients of variation were ~8%.

Pancreatic glucagon was assayed in ethanol-extracted plasma using antibody 4305 as previously described (16). The detection limit was ~1 pmol/l and the intra-assay coefficient of variation was <6% in the working range.

Plasma gastric inhibitory polypeptide (GIP) was determined by radioimmunoassay using antiserum R65 as previously described (17). This assay measures the sum of both intact [1–42] and degraded GIP [3–42] in plasma.

Plasma pancreatic polypeptide was determined by radioimmunoassay. A detailed description of the assay is given by Wettergren et al. (18).

Phenol red in gastric contents was assayed photometrically after filtration through filter paper (100 μl in 2 ml Na_2HPO_4/NaH_2PO_4 buffer, 0.6 mol/l/l, pH 8.0) at a wavelength of 546 nm and read against a standard curve (phenol red in phosphate buffer) as previously described (9). Each patient's set of plasma samples was assayed at the same time to avoid errors due to interassay variation.

Statistical analysis. Results are reported as means ± SE. All statistical calculations were carried out by paired repeated-measures ANOVA using Statistica (Statsoft Europe, Hamburg, Germany). If a significant interaction of treatment and time was documented ($P < 0.05$), values at single time points were compared by one-way ANOVA and Duncan's post hoc test (paired analyses). A two-sided P value <0.05 was taken to indicate significant differences.

RESULTS

GLP-1 plasma concentrations. After the liquid test meal, GLP-1 plasma concentrations increased from 5.3 ± 0.8 pmol/l to peak concentrations of 10.3 ± 1.6 pmol/l at 15 min during placebo infusion (Fig. 1; $P = 0.0009$). GLP-1 plasma concentrations were similar in all experiments with GLP-1 infusion, reaching steady-state levels at 0 min (Fig. 1). When erythromycin was administered before the test meal, significantly higher GLP-1 plasma concentrations were observed after 30 min, compared with the

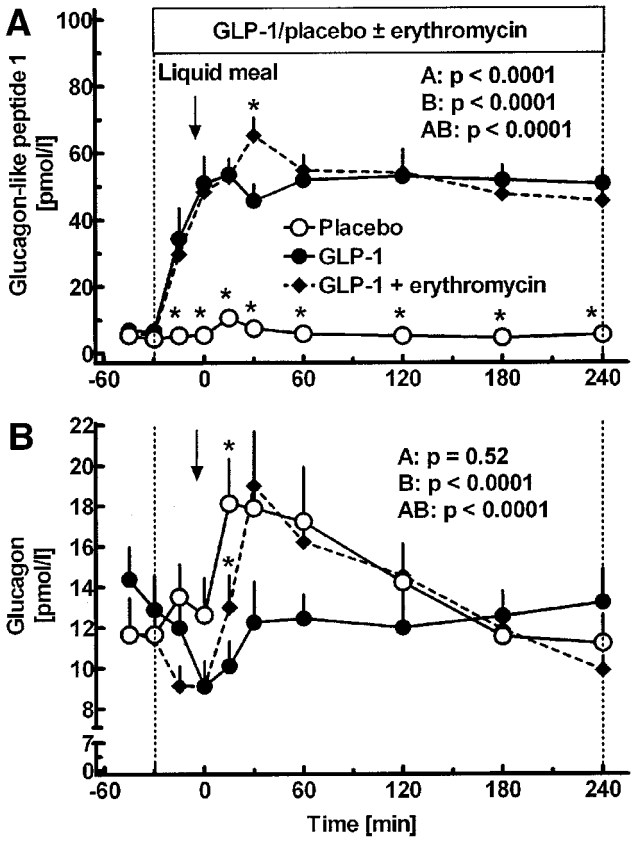

FIG. 1. Plasma concentrations of GLP-1 (*A*) and glucagon (*B*) during the intravenous administration of placebo (○), GLP-1 alone (0.8 pmol · kg^{-1} · min^{-1}) (●), or GLP-1 in combination with erythromycin (200 mg i.v.) (♦) in nine healthy volunteers. Arrows indicate the instillation of a liquid meal at 0 min. Data are means ± SE. *P* values were calculated using repeated-measures ANOVA: A, differences between the different experiments; B, differences over time; and AB, differences due to the interaction of experiment and time. *Significant differences ($P < 0.05$) versus the experiment with the administration of GLP-1 alone at individual time points (ANOVA and Duncan's post hoc test).

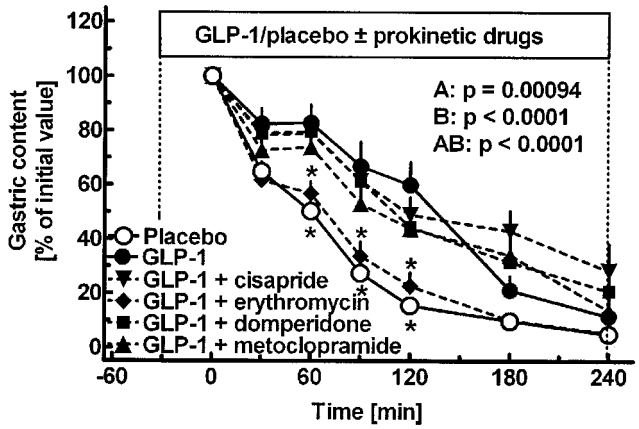

FIG. 2. Gastric content, determined using a phenol red dilution technique and expressed as the percentage of the initial content (400 ml), during the intravenous administration of placebo (○), GLP-1 alone (0.8 pmol · kg^{-1} · min^{-1}) (●), or GLP-1 in combination with erythromycin (200 mg i.v.), cisapride (10 mg), metoclopramide (10 mg), or domperidone (10 mg, all orally) in nine healthy volunteers. Data are means ± SE. *P* values were calculated using repeated-measures ANOVA: A, differences between the different experiments; B, differences over time; and AB, differences due to the interaction of experiment and time. *Significant differences ($P < 0.05$) versus the experiment with the administration of GLP-1 alone at individual time points (ANOVA and Duncan's post hoc test).

experiments with the administration of GLP-1 alone ($P = 0.0035$) (Fig. 1).

Gastric emptying. After 240 min, the stomach had almost completely emptied in all groups (Fig. 2). During the infusion of GLP-1 alone, the velocity of gastric emptying was significantly decelerated compared with placebo administration (Fig. 2) ($P < 0.0001$). With erythromycin, the decelerating effect of GLP-1 was completely counterbalanced. Accordingly, no differences occurred in the pattern of gastric emptying between the experiments with the administration of placebo and with the combined administration of GLP-1 and erythromycin (Fig. 2). In contrast, metoclopramide, domperidone, and cisapride had no significant effects on gastric emptying during GLP-1 infusion (Fig. 2).

Plasma glucose. After the liquid test meal, plasma glucose concentrations increased significantly in the placebo group ($P < 0.0001$). Intravenous infusion of GLP-1 lowered fasting glucose concentrations even before instillation of the test meal ($P < 0.05$) and the postprandial rise in glycemia was less pronounced than with placebo. The patterns of glucose concentration were not different in the experiments with the administration of GLP-1 alone or in combination with metoclopramide, domperidone, or cisapride (details not shown). When erythromycin was administered in addition to GLP-1, plasma glucose concentrations at 30 min were higher that with GLP-1 alone but lower than during placebo administration ($P < 0.05$) (Fig. 3). There were no significant differences in glycemia between the experiments with GLP-1 alone and in combination with erythromycin at any other time point.

Insulin secretory response. In the fasting state, insulin secretion was not different between the experiments with the administration of placebo and of GLP-1, alone or in combination with erythromycin (Fig. 3). After the liquid test meal, insulin secretory responses were significantly higher during placebo administration than during the infusion of GLP-1 ($P < 0.05$) (Fig. 3). When erythromycin was administered in addition to GLP-1, the overall pattern of insulin secretion and the integrated incremental plasma concentrations of insulin and C-peptide were more similar to placebo experiments (Fig. 3). However, there were still differences in insulin and C-peptide levels at time point 15 min after the test meal between the experiments with the combined administration of GLP-1 and erythromycin and with placebo. In contrast to the effect of erythromycin, none of the other prokinetic drugs used had an influence on postprandial insulin release during GLP-1 infusion (details not shown).

When the plasma insulin levels immediately after meal ingestion (0–30 min) were expressed in relation to the corresponding glucose concentrations, a shift of the glucose threshold required for the stimulation of insulin secretion became apparent in the experiments with GLP-1 infusion (Fig. 4). However, the glucose responsiveness of insulin secretion was not affected by erythromycin administration (Fig. 4).

Glucagon secretion. Plasma glucagon concentrations before the test meal were lowered during GLP-1 infusion (Fig. 1). After the instillation of the liquid meal, plasma glucagon concentrations significantly increased during all experiments ($P < 0.0001$), reaching higher levels during

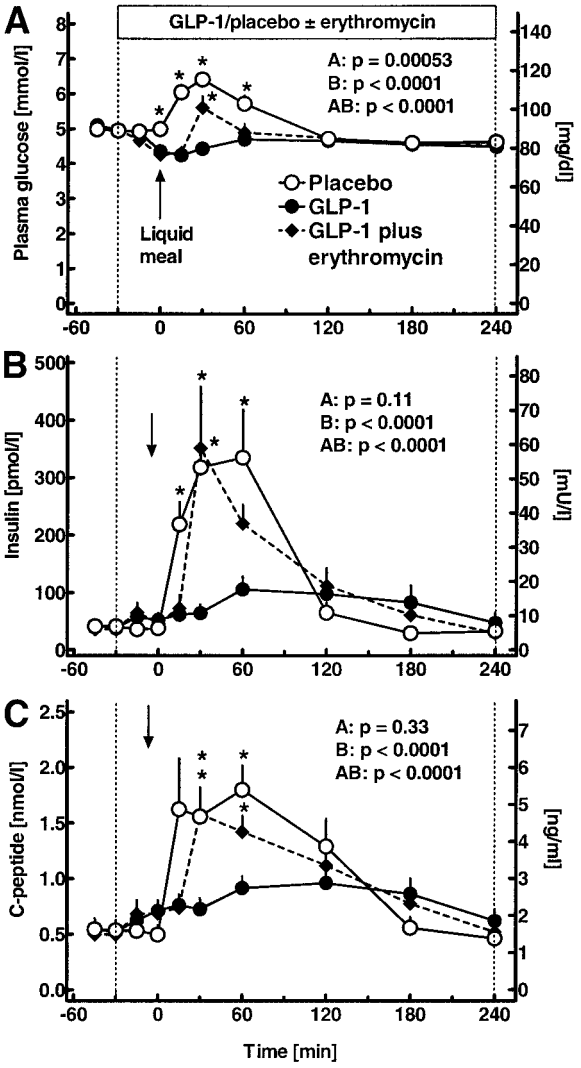

FIG. 3. Plasma concentrations of glucose (*A*), insulin (*B*), and C-peptide (*C*) during the intravenous administration of placebo (○), GLP-1 alone (0.8 pmol · kg^{-1} · min^{-1}) (●), or GLP-1 in combination with erythromycin (200 mg i.v.) (♦) in nine healthy volunteers. Arrows indicate the instillation of a liquid meal at 0 min. Data are means ± SE. *P* values were calculated using repeated-measures ANOVA: A, differences between the different experiments; B, differences over time; and AB, differences due to the interaction of experiment and time. *Significant differences ($P < 0.05$) versus the experiment with the administration of GLP-1 alone (ANOVA and Duncan's post hoc test).

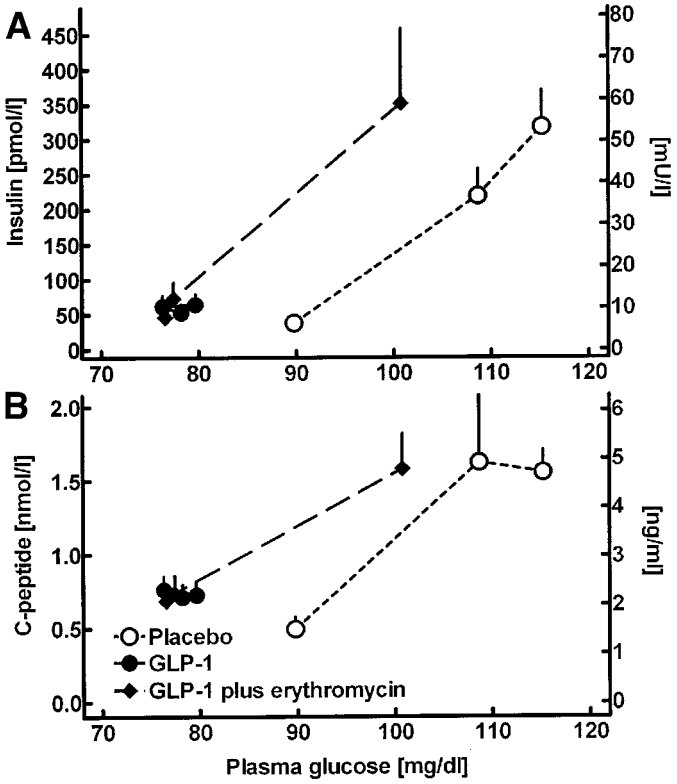

FIG. 4. Plasma concentrations of insulin (*A*) and C-peptide (*B*) in relation to the concomitant plasma glucose concentrations for the immediate postprandial period (0–30 min), representing the ascending part of the glycemic rise (see Fig. 3) during the intravenous administration of placebo (○), GLP-1 alone (0.8 pmol · kg^{-1} · min^{-1}) (●), or GLP-1 in combination with erythromycin (200 mg i.v.) (♦) in nine healthy volunteers. Data are means ± SE.

placebo administration than with GLP-1 (Fig. 1). Again, erythromycin almost completely reversed the changes in glucagon secretion by GLP-1, resulting in a similar pattern of glucagon concentrations as during the placebo experiments (Fig. 1). No changes in glucagon secretion were found after the administration of metoclopramide, domperidone, or cisapride (details not shown).

GIP secretion. GIP plasma concentrations significantly increased after the liquid test meal during all experiments (Fig. 5) ($P < 0.0001$). The total amount of GIP secreted after the meal was similar in all experiments ($P = 0.38$), but the time course of GIP secretion was delayed during GLP-1 infusion ($P < 0.05$ vs. placebo). These changes were almost completely reversed by erythromycin administration (Fig. 5). Accordingly, peak concentrations of GIP were reached after 60 min during placebo administration, after 180 min during GLP-1 infusion, and after 30 min during the combined administration of erythromycin and GLP-1 (Fig. 5). GIP secretion was not affected by the other prokinetic drugs used (details not shown).

Pancreatic polypeptide secretion. The secretion of pancreatic polypeptide was significantly suppressed by GLP-1 infusion (Fig. 5). Erythromycin administration led to a marked increase in postprandial pancreatic polypeptide concentrations compared with the placebo experiments ($P < 0.0001$). The secretion of pancreatic polypeptide was not influenced by metoclopramide, domperidone, or cisapride (details not shown).

DISCUSSION

Because GLP-1 reduces postprandial glycemia not only via endocrine pancreatic secretion (6) but also by decelerating the velocity of gastric emptying (9,10,19,20), it was the aim of this study to assess GLP-1–induced insulin secretion after a meal, independent of changes in gastric emptying. Although insulin secretion is typically enhanced by GLP-1 under fasting conditions, a dose-dependent reduction rather than stimulation of postprandial insulin secretion has previously been observed during the administration of GLP-1 in healthy subjects and in patients with type 2 diabetes (9,10). Similar to those studies, a considerably smaller amount of insulin was secreted after the meal during GLP-1 administration in the present experiments. However, when changes in gastric emptying were reversed by erythromycin, insulin secretion was increased

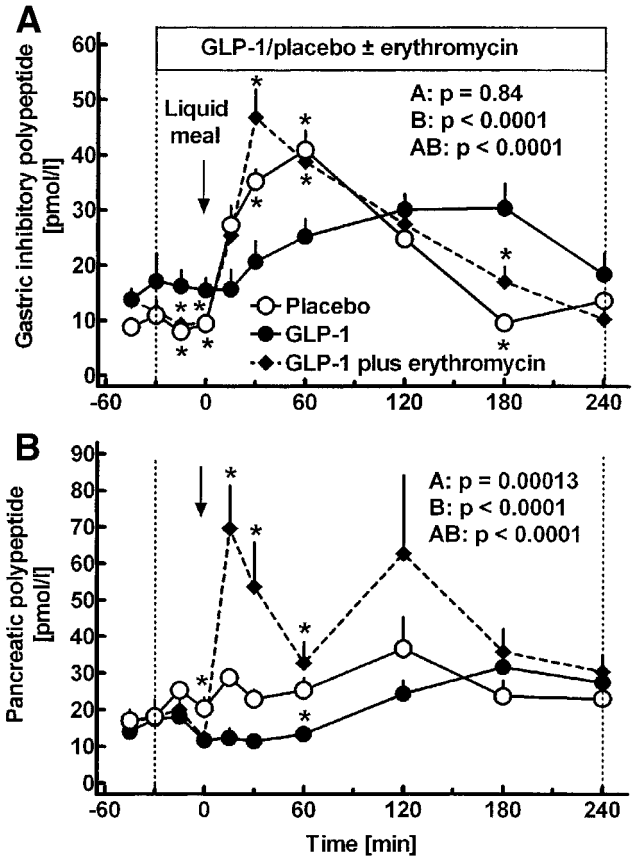

FIG. 5. Plasma concentrations of GIP (*A*) and pancreatic polypeptide (*B*) during the intravenous administration of placebo (○), GLP-1 alone (0.8 pmol · kg^{-1} · min^{-1}) (●), or GLP-1 in combination with erythromycin (200 mg i.v.) (♦) in nine healthy volunteers. Arrows indicate the instillation of a liquid meal at 0 min. Data are means ± SE. *P* values were calculated using repeated-measures ANOVA: A, differences between the different experiments; B, differences over time; and AB, differences due to the interaction of experiment and time. *Significant differences ($P < 0.05$) versus the experiment with the administration of GLP-1 alone (ANOVA and Duncan's post hoc test).

to similar levels as in the placebo experiments, although at lower levels of glycemia (Fig. 3). This indicates that the glucose concentration threshold required to stimulate insulin secretion was shifted downward due to the administration of GLP-1. Indeed, when plasma insulin concentrations are expressed in relation to the corresponding glucose levels (Fig. 4), it becomes clear that during GLP-1 administration insulin secretion was increased at lower levels of glycemia compared with placebo. Therefore, in the postprandial state GLP-1 possesses insulinotropic properties independent of changes in the rate of gastric emptying in healthy individuals.

Interestingly, despite the similar patterns of insulin, C-peptide, and glucagon concentrations, postprandial plasma glucose levels were lower in the GLP-1 plus erythromycin experiments than in the placebo experiments. Most likely, this was based on the glucose-lowering effect of GLP-1 before instillation of the test meal. In support of this, plasma glucose concentrations were already lowered by ~13 mg/dl at the time of meal administration in the GLP-1 experiments. If these initial differences in glycemia are taken into consideration, the glycemic profiles appear rather similar between the time points of 30 and 60 min in the experiments with GLP-1 plus erythromycin and with placebo. However, there is still a discrepancy regarding the plasma levels of glucose, insulin, C-peptide, and glucagon at the 15-min time point after meal administration between these experiments. Because GIP plasma levels were already increased at that time point in the experiments with GLP-1 and erythromycin, differences in the rate of gastric emptying (which was not determined at this time point) or intestinal nutrient absorption are unlikely to explain this discrepancy. Thus, it is possible that erythromycin had an independent effect on glucose tolerance beyond its effects on gastric emptying.

The present data give rise to a reconsideration of the role of GLP-1 as an incretin hormone. By definition, incretin hormones are released in response to nutrient ingestion and stimulate pancreatic β-cells at their typical postprandial concentrations, especially in the presence of elevated plasma glucose concentrations (2,21). Such properties have been ascribed to GIP (22–24) and cholecystokinin (shown only in dogs and rodents) (25,26). According to this strict definition, GLP-1 would apparently not fulfill the criteria for an incretin hormone in the setting of the present experiments because it reduced rather than stimulated postprandial insulin secretion. In contrast, when the GLP-1 effects on gastric emptying were counterbalanced, the peptide augmented postprandial insulin secretory responses.

However, a word of caution has to be mentioned regarding the GLP-1 doses used for the present experiments. In fact, GLP-1 plasma levels reached with the infusion of 0.8 pmol · kg^{-1} · min^{-1} exceeded those typically reached after meal ingestion by ~100%. Therefore, even though the present data seem to support the notion that, regardless of its gastric emptying effects, GLP-1 possesses typical incretin properties, they do not allow conclusions about the quantitative importance of endogenous GLP-1 for postprandial gastric emptying and glucose tolerance.

The present experiments also underline the importance of gastric emptying for postprandial glucose control. The nutrient supply into the systemic circulation, which is predominantly regulated by the velocity of gastric emptying, has been demonstrated to be a major determinant of postprandial glucose and insulin concentrations (11,12,27). Notably, at least 35% of the variance in postprandial glucose levels can be explained by gastric emptying (11). In addition, there is evidence that the initial rate of duodenal glucose entry not only dictates the subsequent responses in glycemia and insulin secretion, but also impacts on the postprandial rise in incretin hormone secretion (28). This is consistent with the present observation that the pattern of GIP secretion was delayed during the administration of GLP-1 (Fig. 5), which was effectively reversed by erythromycin. Taken together, these findings emphasize the previous observation that the presence of nutrients in the proximal gut and contact with duodenal and ileal K cells are the major stimulus for the secretion of GIP (29). A considerable number of K cells have recently been identified in these portions of the upper gut (30). The close relationship between intestinal nutrient absorption and incretin secretion is further supported by the recent observation that inhibition of intraduodenal free fatty acid generation, using the lipase inhibitor or-

listat, attenuates the secretion of GIP and GLP-1 (31). The GLP-1 induced delay in GIP secretion observed in the present study may also be taken as a confirmation that a deceleration of gastric emptying by GLP-1 really occurred and was physiologically relevant.

Stimulation of insulin secretion after 1 week of erythromycin treatment (1,200 mg/day) has been previously reported by Ueno et al. (32) in patients with type 2 diabetes. However, these effects may have been secondary to the improvement in postprandial glycemia, as shown for other prokinetic drugs (12,33,34). In the present study, insulin secretion was not acutely influenced by the administration of 200 mg erythromycin in the fasting state (Fig. 3). However, erythromycin effects on postprandial insulin secretion were not directly addressed. Because cholinergic stimulation represents an important regulator of islet hormone secretion in humans, the increase in pancreatic polypeptide secretion observed after erythromycin administration may be interpreted as an indication of a direct insulinotropic effect of erythromycin.

One potential limitation of the study may be seen in the fact that metoclopramide, cisapride, and domperidone were administered orally, whereas erythromycin was infused intravenously. Moreover, the doses chosen for the administration of metoclopramide, cisapride, and domperidone were comparably low (35–37). In fact, to avoid overstimulation of gastric emptying and overall gut motility, we aimed for the lower limits of the respective therapeutic ranges used in the treatment of patients with gastric motility disorders (12). Therefore, it is possible that the plasma levels achieved with the oral administration of metoclopramide, cisapride, and domperidone were not sufficient to counterbalance the effects of GLP-1, although in principle these prokinetic drugs would have the potential to decelerate gastric emptying in the presence of GLP-1.

Alternatively, the discrepant efficacy of the prokinetic drugs used in this study may reflect different mechanisms of action. In this way, domperidone and metoclopramide primarily antagonize central and peripheral dompamine receptor activity, whereas cisapride mainly stimulates serotonin 5-hydroxytryptamine-4 receptors (12). In contrast, the action of erythromycin involves activation of motilin receptors as well as direct stimulation of vagal cholinergic nerves (38,39). GLP-1, on the other hand, decelerates gastric emptying by inhibiting the parasympathetic outflow (40–42). Consistent with these theoretical considerations, in the present experiments erythromycin induced a pronounced rise in pancreatic polypeptide plasma concentrations, whereas GLP-1 suppressed the release of pancreatic polypeptide (Fig. 5). Therefore, it is possible that the effectiveness of erythromycin in this study reflects a direct interaction of GLP-1 and erythromycin at the level of vagal activation.

The potent deceleration of gastric emptying induced by GLP-1 may have substantial consequences for the treatment of patients with type 2 diabetes with incretin hormones. Given the high prevalence of gastric motility disorders in patients with diabetes, further inhibition of gastric emptying in these patients could potentially induce upper gastrointestinal symptoms, such as nausea, vomiting, or reflux. In fact, nausea and vomiting have been observed in some individuals in response to GLP-1 or its derivatives/analogs (43–45). Antagonizing the GLP-1–induced deceleration of gastric emptying using erythromycin may potentially reduce these side effects.

In conclusion, intravenous erythromycin counterbalances the GLP-1–induced deceleration of gastric emptying and unmasks its insulinotropic effect in the postprandial period. The unequal effectiveness of the different prokinetic agents tested suggests an involvement of the vagal nervous system in the mediation of GLP-1 effects of the stomach. Although the consequences resulting from the delay in gastric emptying by GLP-1 under physiological conditions require further investigation, the present data demonstrate that, at pharmacological concentrations, the predominant effect of GLP-1 on postprandial glucose homeostasis is mediated by a delay in gastric emptying rather than by a modulation of endocrine pancreatic secretion.

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