GLP-1 slows solid gastric emptying and inhibits insulin, glucagon, and PYY release in humans

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GLP-1 slows solid gastric emptying and inhibits insulin, glucagon, and PYY release in humans. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R910–R916, 1999.—The aim of the present study was to assess the effect of glucagon-like peptide-1 (GLP-1) on solid gastric emptying and the subsequent release of pancreatic and intestinal hormones. In eight men [age 33.6 ± 2.5 yr, body mass index 24.1 ± 0.9 (means ± SE)], scintigraphic solid gastric emptying during infusion of GLP-1 (0.75 pmol·kg⁻¹·min⁻¹) or saline was studied for 180 min. Concomitantly, plasma concentrations of C- and N-terminal GLP-1, glucose, insulin, C-peptide, glucagon, and peptide YY (PYY) were assessed. Infusion of GLP-1 resulted in a profound inhibition of both the lag phase (GLP-1: 91.5, range 73.3–103.6 min vs. saline: 19.5, range 10.2–43.4 min) and emptying rate (GLP-1: 0.34, range 0.06–0.56 %min⁻¹ vs. saline: 0.84, range 0.54–1.33 %min⁻¹; P < 0.01 for both) of solid gastric emptying. Concentrations of both intact and total GLP-1 were elevated to supraphysiological levels. Plasma glucose and glucagon concentrations were below baseline during infusion of GLP-1 in contrast to saline infusion, where concentrations were elevated above baseline (both P < 0.001). The insulin and C-peptide responses were lower during infusion with GLP-1 than with saline (P < 0.004 and P < 0.001, respectively). Plasma PYY concentrations decreased below baseline during GLP-1 infusion in contrast to saline, where concentrations were elevated above baseline (P = 0.04). Infusion of GLP-1 inhibits solid gastric emptying with secondary effects on the release of insulin, C-peptide, and glucagon, resulting in lower plasma glucagon concentrations. In addition, the release of PYY into the circulation is inhibited by GLP-1 infusion, suggesting a negative feedback of GLP-1 on the function of the L-cell.

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SUBJECTS AND METHODS

Subjects. Eight healthy men [age 33.6 ± 2.5 yr, body mass index 24.1 ± 0.9 (means ± SE)] were recruited for this study. The Ethics and Radiation Protection Committees of the Karolinska Hospital approved the study, and informed consent was obtained from each subject.

Study protocol. The study was performed in a randomized crossover fashion on two occasions, 1 wk apart. The subjects were studied after an overnight fast at 8:00 in the morning, and an indwelling catheter was placed in each antecubital vein. Simultaneously with the intake of a ⁹⁹ᵐTc-labeled omelet, either GLP-1 (0.75 pmol·kg⁻¹·min⁻¹; Bachem AG, Bubendorf, Switzerland) dissolved in 0.9% saline containing 1% albumin (Albumin Kabi, 200 mg/ml) subjected to sterile filtration, and stored at −20°C until use or saline was started.
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in one of the intravenous catheters and continued for 180 min.

Scintigraphic gastric emptying. The scintigraphic gastric emptying test of a solid meal has been described in detail elsewhere (10), and the present technique differs only in that water and not fruit punch was served with the meal. In short, subjects fasted overnight were studied after ingesting a 310-kcal omelet with 12–15 MBq 99mTc-labeled macroaggregated albumin (Pulmonate; Amersham International, Little Chalfont, UK). Anterior and posterior 1-min acquisitions were performed with the subject in sitting position. Acquisitions were obtained every 5 min during the first 50 min and thereafter every 10 min during 70 min and finally at 180 min.

The following parameters were calculated: 1) lag phase, defined as the time period from termination of meal until 90% of radioactivity remained in the stomach; 2) gastric emptying rate, defined as percentage of radioactivity per minute during the linear slope after termination of the lag phase; and 3) half-emptying time ($T_{50}$), defined as the time for 50% emptying of gastric radioactivity after termination of the meal.

During infusion with saline, there was a biphasic release of GLP-1. C-terminal GLP-1 data for each individual were assessed by two independent observers, and the time of the first and second elevation of plasma GLP-1 concentrations was identified. The anatomical location for the leading edge of the meal was then assessed in the scintigraphic frame corresponding to the exact time of the elevation of plasma C-terminal GLP-1 in each individual.

Blood samples and RIAs. The blood samples were collected in prechilled heparinized tubes for the analysis of glucose, insulin, C-peptide, C- and N-terminal GLP-1, PYY, and glucagon 20 and 10 min before intake of the 99mTc-labeled omelet and then at the same time intervals as the scintigraphic acquisitions. The samples were centrifuged at 4°C for 10 min at 2,000 g. Plasma was collected and stored at −20°C for analysis in series.

Glucose was analyzed by an enzyme assay (mutarotase and glucose dehydrogenase; Boeringer-Mannheim, Mannheim, Germany) with a Hitachi 917 automatic analyzer.

Insulin was analyzed with an enzyme immunoassay (DAKO Insulin Kit K6219, Copenhagen, Denmark). The assay cross-reacts to 0.3% with proinsulin but not with C-peptide. The detection limit of the assay was 21 pmol/l, and the coefficient of variation was 8%.

C-peptide concentrations in plasma were determined by a RIA (Euro-Diagnostica, Malmö, Sweden). The assay cross-reacts to 41% with proinsulin but not with insulin. The detection limit was 50 nmol/l, and the coefficient of variation was 5%.

GLP-1-like immunoreactivity (GLP-LI) in plasma was studied with RIAs specific for each terminus of the molecule. N-terminal immunoreactivity was measured with a newly described antiserum (12) raised in rabbits against synthetic proglucagon-(78–87) with a C-terminal cysteine coupled to keyhole limpet hemocyanin by means of the cystein thiol method. The selected antiserum (code 93242) was used in a final dilution of 1:15,000, endowing the assay with a detection limit of 50 nmol/l, and the coefficient of variation was 5%.

GLP-1-(7–36) immunoreactivity was determined using antiserum 89390 (26), which has an absolute requirement for the intact amidated C-terminus of GLP-1 (7–36) amide and cross-reacts to <0.01% with truncated fragments and to 83% with GLP-1-(9–36)amide. For both assays the coefficient of variation was <6%. GLP-1-(7–36) amide was used as standard, and 125I-labeled GLP-1-(7–36) amide was used as tracer. Before analysis, plasma was extracted with 70% ethanol (vol/vol, final concentration) before assay, giving recoveries of 75%. Separation was achieved using plasma-coated charcoal.

PYY-LI was analyzed by means of antiserum code no. 8412–211 (a gift from R. Håkanson, Dept. of Pharmacology, University of Lund, Lund, Sweden) raised in rabbits against synthetic porcine PYY-(1–36) (Peninsula Europe, Merseyside, UK) as previously described (15) but without conjugation to carrier protein (7). The antiserum cross-reacts to 100% with human PYY. The detection limit of the assay was 1 pmol/l, and the coefficient of variation was 5%.

Glucagon-LI was assayed by means of a previously described RIA technique. The glucagon assay is directed against the C-terminus of the glucagon molecule (antibody code no. 4305) and therefore measures glucagon of mainly pancreatic origin (13). The detection limit of the assay was 1 pmol/l, and the coefficient of variation was 5%.

Statistics and calculations. All values are means ± SE or median (range), as appropriate, and $P < 0.05$ was considered statistically significant. The gastric lag phase, linear emptying rate, and $T_{50}$ were statistically evaluated by means of Wilcoxon’s signed rank test for matched pairs. The gastric emptying curve and plasma concentrations of C- and N-terminal GLP-1 were analyzed by employing an ANOVA for repeated, paired measures, with time and treatment as factors. For glucose, insulin, C-peptide, PYY, and glucagon the changes from baseline were calculated by using the mean fasting value (−20, −10, and 0 min), and then the results were analyzed with an ANOVA for repeated, paired measures, with time and treatment as factors. Baseline concentrations during saline and GLP-1 infusions were compared by means of an ANOVA.

RESULTS

Gastric emptying. Infusion of GLP-1 resulted in a profound inhibition of the lag phase, emptying rate, and $T_{50}$ of solid gastric emptying compared with infusion with saline (Figs. 1 and 2; Table 1; $P < 0.001$ for

![Fig. 1. Means ± SE of scintigraphic gastric emptying in 8 normal male volunteers during intravenous infusion of glucagon-like peptide-1 (GLP-1, 0.75 pmol·kg$^{-1}$·min$^{-1}$) or saline for 180 min. $P < 0.001$ for time effect, treatment effect, and time × treatment interaction effect, respectively (ANOVA with repeated measures).](http://ajpregu.physiology.org/content/120/3/247)
time effect, treatment effect, and time × treatment interaction effect, respectively). At the end of the study, 180 min after intake of the omelet, 64.8 ± 6.4 and 6.1 ± 3.5% of the activity remained in the stomach during infusion of GLP-1 and saline, respectively.

Anatomical location of meal and release of GLP-1. There was a bimodal peak of C-terminal GLP-1 secretion after the meal (Fig. 3). In individual subjects, the first and second elevation of plasma C-terminal GLP-1 occurred 15–45 and 50–100 min, respectively, after meal ingestion. The location of the leading edge of the meal was proximal jejunum at the first elevation of plasma C-terminal GLP-1 and ileum at the second elevation of plasma GLP-1. Data for individual subjects are presented in Table 2, and the scintigraphic pictures for one representative subject are presented in Fig. 4.

Plasma GLP-1 concentrations. Infusion of GLP-1 at 0.75 pmol·kg⁻¹·min⁻¹ resulted in significantly elevated plasma concentrations of both C- and N-terminal GLP-1 (Fig. 5; P < 0.003 for time effect, treatment effect, and time × treatment interaction effect, respectively, for C- and N-terminal GLP-1 concentrations).

Plasma glucose, insulin, C-peptide, and glucagon concentrations. Baseline plasma glucose concentrations were 5.2 ± 1.0 and 5.2 ± 1.5 mmol/l before GLP-1 and saline infusion, respectively. Postprandial glucose concentrations were significantly lower during GLP-1 infusion than during saline infusion (P < 0.001 for time effect, P = 0.007 for treatment effect, and P < 0.001 for time × treatment interaction effect; Fig. 6).

Baseline plasma insulin concentrations were 7.2 ± 1.8 and 7.3 ± 1.3 mU/l before GLP-1 and saline infusion.

Table 1. Gastric emptying during infusion of GLP-1 or saline for 180 min

<table>
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<th>Saline (n = 8)</th>
<th>GLP-1 (n = 8)</th>
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<td>Lag phase (90%), min</td>
<td>19.5 (10.2–43.4)</td>
<td>91.5 (73.3–103.6)</td>
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<tr>
<td>Half-emptying time, min</td>
<td>71.4 (43.6–107.2)</td>
<td>226.1 (144.8–900.8)</td>
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<td>Linear emptying rate, %/min</td>
<td>0.84 (0.54–1.33)</td>
<td>0.34 (0.06–0.56)</td>
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Data shown as median, range in parentheses; n = no. of healthy male subjects. GLP-1, glucagon-like peptide-1. All P = 0.01, Wilcoxon’s signed rank test for matched pairs.

Fig. 2. Scintigraphic frames at 0, 60, 120, and 180 min during infusion of saline (A) and GLP-1 (B) in one study subject during gastric emptying of solid meal.

Fig. 3. Means ± SE of plasma (P) C-terminal GLP-1 concentrations after 310-kcal solid meal during intravenous infusion of saline (■) for 180 min in 8 normal male volunteers.
infusion, respectively. Postprandial insulin levels were significantly lower during GLP-1 infusion than during saline infusion (P < 0.03 for time effect, P < 0.01 for treatment effect, and P < 0.004 for time x treatment interaction effect; Fig. 6).

Baseline plasma C-peptide concentrations were 0.74 ± 0.14 and 0.76 ± 0.13 nmol/l before GLP-1 and saline infusion, respectively. Postprandial C-peptide concentrations were lower during GLP-1 infusion than during saline infusion (P < 0.001 for time effect, P = 0.004 for treatment effect, and P < 0.001 for time x treatment interaction effect; Fig. 6).

Baseline plasma glucagon concentrations were 27.7 ± 1.0 and 26.0 ± 1.5 pmol/l before GLP-1 and saline infusion, respectively. Postprandial glucagon concentrations were lower during GLP-1 infusion than during saline infusion and were reduced to under baseline values during GLP-1 infusion (P = 0.03 for time effect, P = 0.006 for treatment effect, and P < 0.001 for time x treatment interaction effect; Fig. 6).

Plasma PYY. Baseline PYY concentrations were not significantly different before GLP-1 and saline infusion (7.3 ± 1.9 and 4.8 ± 1.9 pmol/l, respectively). Treatment with GLP-1 resulted in decreased plasma PYY concentrations to levels below baseline and different

<table>
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<th>Second Elevation of GLP-1</th>
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DISCUSSION

This study demonstrates that GLP-1 has a powerful inhibitory effect on solid gastric emptying in man. Previous studies show that GLP-1 administered intravenously, subcutaneously, and as buccal tablets inhibits gastric emptying of a liquid meal (11, 22, 29, 37, 38) and that subcutaneous GLP-1 administered as one dose 20 min after a meal inhibits the lag phase of solid gastric emptying (30). Our present study extends these observations to the emptying of a standard solid meal during continuous intravenous administration of GLP-1 and demonstrates that both phases of solid gastric emptying are inhibited by GLP-1 during intravenous administration. Both the gastric lag phase and the linear emptying rate were retarded, resulting in a markedly prolonged $T_{50}$ with GLP-1 compared with saline infusion. This slowing of gastric emptying seems to be in effect during the whole infusion period up to 3 h, as indicated by the constant reduction of emptying rate.

GLP-1 exhibits incretin properties (17, 18, 21, 34) and has been shown to stimulate insulin secretion and inhibit glucagon secretion (8, 25). In the present study, infusion of GLP-1 at supraphysiological concentrations resulted in an early reduction of postprandial plasma glucose along with decreased plasma glucagon concentrations. In addition, there is a marked inhibition of gastric emptying. Thus the observed reduced postprandial plasma insulin and C-peptide concentrations during GLP-1 infusion are most likely the result of falling plasma glucose concentrations and a decreased stimulation of insulin release caused by an inhibited delivery of nutrients into the duodenum and jejunum with reduced preabsorptive bioavailability. Further studies with a GLP-1 antagonist, such as exendin (9—39)amide, are needed to fully elucidate the effect of GLP-1 on glucose disposal and metabolic control in the postprandial state.

This study demonstrates that there is a continuous recruitment of GLP-1-releasing cells after a meal, with a first elevation of plasma GLP-1 corresponding to the point when the leading edge of the meal reaches the proximal jejunum. The GLP-1 secreting L-cells are most abundantly found in the lower gut (ileum to rectum) (6), which is consistent with our finding of a second elevation of plasma GLP-1 as the meal reaches...
the ileum. Administration of nutrients into the lower gut has been shown to inhibit upper gastrointestinal functions, the so-called ileal brake mechanism (31). Thus the main physiological role for GLP-1 may be to contribute to the ileal physiological role on gastrointestinal motility, which in turn results in decreased plasma glucose concentrations as a result of a slower and more sustained delivery of nutrients into the upper gut from the stomach. This effect seems to be mediated via vagal afferent-mediated central mechanisms as shown in the rat (16).

At least a subpopulation of the GLP-1-secreting L-cells also contains and releases PYY (2). PYY and GLP-1 have been shown to exert similar effects on upper gastrointestinal function in several species, including man (1, 35), and have an additive inhibitory effect on gastric acid secretion (3). Like GLP-1, PYY secretion may be stimulated by the presence of nutrients in the gut lumen. The lower plasma concentrations observed in the present study may thus be secondary to the decreased exposure of the gut to nutrients during the GLP-1 infusion. However, the fact that plasma PYY concentrations actually decreased below basal concentrations during GLP-1 infusion suggests that GLP-1 may have a direct inhibitory effect on the release of PYY. Thus our data may reflect a negative feedback of GLP-1 on the function of the L-cells.

GLP-1 has been tried as a novel therapeutic for the treatment of non-insulin-dependent diabetes mellitus (NIDDM) (32). There has, however, been concern that the inhibitory effect of the peptide on gastric functions may result in gastrointestinal side effects and nutritional problems when the peptide is given at high doses (1.2 pmol·kg⁻¹·min⁻¹). When GLP-1 is infused at a rate of 0.8 pmol·kg⁻¹·min⁻¹, gastric emptying of a liquid meal is nearly complete after 240 min (22). For comparison, the present study demonstrates that only 40% of a solid meal is emptied after 180 min during infusion of GLP-1 at 0.75 pmol·kg⁻¹·min⁻¹. Thus the inhibitory effect of GLP-1 on gastric emptying seems more profound for a solid than for a liquid meal. The plasma concentrations of C-terminal GLP-1 [comprising both intact GLP-1 and its primary metabolite, GLP-1-(9—36) amide (4)] are 40–80% higher than those seen under physiological conditions (27), indicating that the infusion rate slightly exceeded the postprandial secretory rate. The plasma concentrations of N-terminal GLP-1 (comprising exclusively the intact, biologically active peptide) were also slightly above postprandial levels (4) but similar to the plasma concentrations required to normalize plasma glucose concentrations in patients with NIDDM (5). The long-term effect of a profound inhibition of solid gastric emptying as seen with GLP-1 is unknown. In terms of nausea, infusion of GLP-1 during 8 h at the same rate as in the present study did not result in nausea (23), and a continuous infusion at the rate of 1.2 pmol·kg⁻¹ · min⁻¹ for 1 wk did not result in any gastrointestinal side effects or diminished biological response (19).

In conclusion, our study demonstrates that intravenous administration of GLP-1 is a powerful inhibitor of gastric emptying in man, with secondary effects on the release of insulin, C-peptide, and glucagon, most likely through the inhibitory action of GLP-1 on gastric emptying resulting in lower plasma glucose concentrations. In addition, GLP-1 also seems to act on more distal parts of the gut as the release of PYY, which is colocalized with GLP-1 in the L-cells of the intestinal mucosa, is inhibited, suggesting a negative feedback mechanism.

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