Rapid Tachyphylaxis of the Glucagon-Like Peptide-1–Induced Deceleration of Gastric Emptying in Humans

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OBJECTIVE—Glucagon-like peptide (GLP)-1 lowers postprandial glycemia primarily through inhibition of gastric emptying. We addressed whether the GLP-1–induced deceleration of gastric emptying is subject to rapid tachyphylaxis and if so, how this would alter postprandial glucose control.

RESEARCH DESIGN AND METHODS—Nine healthy volunteers (25 ± 4 years old, BMI: 24.6 ± 4.7 kg/m²) were examined with intravenous infusion of GLP-1 (0.8 pmol · kg⁻¹ · min⁻¹) or placebo over 8.5 h. Two liquid mixed meals were administered at a 4-h interval. Gastric emptying was determined, and blood samples were drawn frequently.

RESULTS—GLP-1 decelerated gastric emptying significantly more after the first meal compared with the second meal (P = 0.01). This was associated with reductions in pancreatic polypeptide levels (marker of vagal activation) after the first but not the second meal (P < 0.05). With GLP-1, glucose concentrations declined after the first meal but increased after the second meal (P < 0.05). The GLP-1–induced reductions in postprandial insulin and C-peptide levels were stronger during the first meal course (P < 0.05). Likewise, glucagon levels were lowered by GLP-1 after the first meal but increased after the second test meal (P < 0.05).

CONCLUSIONS—The GLP-1–induced delay in gastric emptying is subject to rapid tachyphylaxis at the level of vagal nervous activation. As a consequence, postprandial glucose control by GLP-1 is attenuated after its chronic administration.
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 GLP-1/placebo administration).

After drawing basal blood specimens, at ~30 min an intravenous infusion of GLP-1 [7–36 amide], 0.8 pmol · kg⁻¹ · min⁻¹, or placebo (0.9% NaCl containing 1% human serum albumin) was started and continued for 510 min. This infusion rate was based on previous studies (5,14) and was selected to increase plasma GLP-1 concentrations into the pharmacologic range (approximately three- to fourfold higher concentrations in comparison with those measured after oral glucose) (15). The infusion was begun at ~30 min to ensure elevated GLP-1 [7–36 amide] plasma concentrations already at the time point of administration of the first liquid meal. Blood was drawn at the time points indicated in Fig. 1, and plasma glucose was determined immediately.

Gastric emptying. Before the study, a nasogastric tube (Freka-Ernährungssonde, 120 cm, CH12, Fresenius AG) was placed and tape-fixed with the tip approximately 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 a near-complete aspiration of instilled fluid. The subjects were lying on their back 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

![Graphs and Figures]

FIG. 1. Plasma concentrations of GLP-1 (A), glucose (C), insulin (D), glucagon (E), C-peptide (F), PP (G), and GIP (H), and gastric emptying (B) after instilling two liquid test meals (amino acids and sucrose) via a nasogastric tube into the stomach at 0 and 240 min in nine healthy young volunteers during the intravenous infusion of GLP-1 (0.8 pmol · kg⁻¹ · min⁻¹) or placebo. Mean ± SEM. P values were calculated by repeated-measures ANOVA. Asterisks (*) indicate significant (P < 0.05) differences at specific time points between experiments with placebo and GLP-1. †Significant difference from the corresponding time point after the first meal during the exogenous administration of GLP-1.
meal was instilled into their stomach. The liquid test meal was composed of 50 g sucrose dissolved in 400 mL of mixed amino acids.

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 (314 kcal = 40%) and 50 g sucrose (196 kcal = 60%); total energy content was 327 kcal (energy density: 0.82 kcal/mL).

Gastric emptying was measured as described (10) using a double-sampling dye dilution technique using phenol red (Merck AG, Darmstadt, Germany) according to Gerov (16), with modifications introduced to reduce measure-ment error by Hurwitz (17). According to the expected rate of gastric em-ptying, gastric contents were determined at intervals shown in Fig. 1 over 480 min.

Blood specimens. Blood was drawn into channeled tubes containing EDTA and aprotinin (Trasylol; 20,000 KIU/mL, 200 μL per 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.

Laboratory determinations. Glucose was measured using a glucose oxidase method, as described (10). Insulin was measured using an insulin microparticle enzyme immunoassay (DxI Insulin, Abbott Laboratories, Wiesbaden, Germany), as described (10). C-peptide was measured using C-peptide-antibody-coated microtiter wells (C-peptide MTPL EIA) from DRG Instruments GmbH, Marburg, Germany, as described (10). Plasma concentrations of total GLP-1 were determined in ethanol-extracted plasma as previously described (15,18), using antisemur 89 390 for the measurement of intact GLP-1 [7] determined in ethanol-extracted plasma as previously described (15,18), using antiserum 89 390 for the measurement of intact GLP-1 [7]

RESULTS

GLP-1 plasma concentrations. During the placebo experiments, meal ingestion led to an increment in plasma GLP-1 concentrations from basal levels of 5 ± 1 pmol/L to peak levels of 11 ± 2 pmol/L and 11 ± 2 pmol/L after the first and second test meals, respectively (P < 0.05; Fig. 1A). Exogenous GLP-1 administration rapidly increased GLP-1 concentrations to steady-state levels of 50–55 pmol/L (P < 0.0001 vs. placebo), with superimposed small increments after the meal administrations. No major adverse outcome was reported during the experiments.

Gastric emptying. The velocity of gastric emptying was significantly retarded by exogenous GLP-1 compared with placebo, both after the first and second meals (P < 0.0001 for the comparison of GLP-1 vs. placebo over the time course; Fig. 1B). When gastric emptying was compared between the two test meals (period 1 vs. 2) during GLP-1 infusion, a significant interaction between period and time course was found (P = 0.010), and subsequent post hoc tests revealed significant differences in gastric volume 120 min after meal administration (Fig. 1B). On the basis of these analyses, GLP-1 significantly slowed gastric emptying after both test meals (periods 1 and 2), but the degree by which gastric emptying was slowed was less prominent 120 min after meal ingestion with the second meal. When only the experiments with placebo administration were analyzed, no significant differences in gastric emptying were found between the experiments (periods) over the time course (P = 0.38; Fig. 1B).

Postprandial concentrations of glucose, insulin, and C-peptide. During placebo administration, there was a small increment in glycemia after the instillation of both meals (from 4.9 ± 0.1 mmol/L at t = −30 min to 6.4 ± 0.2 mmol/L at t = 30 min, and from 4.6 ± 0.1 mmol/L to 6.1 ± 0.3 mmol/L after the second meal, P < 0.05, respectively; Fig. 1C). Postprandial plasma glucose levels were lower with concomitant GLP-1 infusions over 60 min after administering the meals (P < 0.05; Fig. 1C).

However, although postprandial glucose concentrations were lowered by GLP-1 during the first hour after the first meal (from 5.0 ± 0.1 mmol/L at t = −30 min to 4.4 ± 0.2 mmol/L at t = 30 min), there was even a small increment in glycemia after the second meal (from 4.5 ± 0.1 mmol/L to 5.2 ± 0.2 mmol/L, respectively). The differences in postprandial glucose concentrations during GLP-1 infusion between the first meal and the second meal were statistically significant (P < 0.05).

Plasma insulin and C-peptide concentrations increased significantly after instillation of both the first and second test meals during the placebo experiments (P < 0.001, respectively; Fig. 1D and F). GLP-1 administration led to an increase in fasting insulin and C-peptide concentrations (P < 0.05 vs. placebo). However, the postprandial increases in insulin and C-peptide levels were ~80 and ~55% lower, respectively, during GLP-1 infusion compared with placebo (P < 0.001; Fig. 1D and F). The GLP-1–induced reduction in postprandial insulin and C-peptide concentrations was less pronounced during the second test meal (P < 0.05 vs. the first meal).

Pancreatic glucagon. Instillation of the test meals resulted in a clear increase in plasma glucagon concentrations during the placebo experiments (Fig. 1E). GLP-1 administration led to a reduction in both fasting and postprandial glucagon levels (P = 0.0009). However, with GLP-1, glucagon concentrations were higher after the second test meal than after the first test meal (P < 0.05), whereas no differences in postprandial glucagon levels were observed in the placebo experiments.

GIP. GIP plasma concentrations increased by approximately fourfold after the test meals during placebo administra-tion (Fig. 1F). With exogenous GLP-1, the increments in GIP plasma levels were blunted and retarded after the first meal, whereas a rapid increment was observed after the second meal. The peak plasma levels of GIP were signifi-cantly lower after the first test meal compared with the second test meal during GLP-1 infusion (P < 0.05), whereas postprandial GIP levels were similar after both meals during placebo administration (Fig. 1F).

PP. Meal ingestion elicited an increase in PP concentrations during placebo administration (Fig. 1G). GLP-1 infusion led to a significant reduction in postprandial PP concentrations after the first test meal (P < 0.05; Fig. 1G). However, PP levels after the second test meal were higher than after the first meal in the GLP-1 experiments, although
there was still a significant difference in postprandial PP concentrations compared with the placebo experiments ($P < 0.05$).

**Relationship between gastric emptying and postprandial glycemia.** To determine the impact of gastric emptying on postprandial glycemia, linear regression analyses between the parameters of gastric emptying and the respective glucose increments after the liquid meal were performed. Across all experiments (with GLP-1 and placebo, analyzing both the first and the second periods), the percentage of initial gastric volume that had emptied within 60 min correlated significantly with the increase in glucose concentrations (peak minus basal concentrations; $r^2 = 0.194$, $P = 0.0044$). Likewise, 60-min emptying correlated significantly with peak glucose concentrations ($r^2 = 0.327$, $P < 0.0001$) and the time of the glucose peak after the meal ($r^2 = 0.265$, $P = 0.0007$), but not with the integrated incremental glucose area (above baseline; $r^2 = 0.022$, $P = 0.37$). Qualitatively similar (highly significant) results were obtained when the gastric emptying half-time, the lag time, or the 30-min emptying were used instead to characterize the velocity of gastric emptying (details not shown).

**DISCUSSION**

The current study was designed to examine whether the deceleration of gastric emptying by GLP-1 is subject to rapid tachyphylaxis and how this would affect postprandial glycemia. We report that the GLP-1–induced deceleration of gastric emptying is significantly diminished already after 5 h of continuous infusion compared with its initial effects. This attenuation of GLP-1 efficacy leads to increased postprandial concentrations of glucose, insulin, and glucagon, as well as changes in the concentration time pattern of GIP.

A deceleration of gastric emptying by GLP-1 has been demonstrated in patients with type 2 diabetes and healthy individuals (5,8,14). In line with these studies, the GLP-1–induced delay in gastric emptying not only prevented the postprandial increase in glycemia but also led to a marked reduction in insulin and C-peptide levels. The GLP-1–induced reduction in postprandial glycemia and insulin secretion was less pronounced during the second meal course, when the delay in gastric emptying was largely attenuated. This finding is consistent with previous studies showing that the reduction in postprandial glycemia and insulin secretion is largely diminished, when the effects of the incretin on the stomach are antagonized (10). Taken together, these studies underline the importance of delayed gastric emptying as the primary factor driving the reduction in postprandial glycemia during acute GLP-1 administration.

What are the mechanisms conferring the induction of tolerance against the GLP-1 effects on gastric emptying? One possibility would be a downregulation or desensitization of the GLP-1 receptor in response to chronically elevated GLP-1 levels. Indeed, receptor desensitization has been described for GIP (22), which shares many intracellular signaling steps with GLP-1. For GLP-1, chronic exposure to a receptor antagonist in mice in vivo did not result in a significant receptor downregulation or attenuation of its glucoregulatory effects (23), thereby rendering this explanation less likely.

The alternative hypothesis would be tachyphylaxis to the effects of GLP-1 at the level of the vagal nerve. Such a mechanism would explain why the effects of GLP-1 on gastric emptying are obviously more affected by the induction of tolerance than the islet hormone responses (24). Furthermore, chronic adaptation of the autonomous nervous system is a well-recognized principle, which is also responsible for many other phenomena, such as hypoglycemia unawareness (25), tolerance to β-adrenergic agonists (26), and opioid tolerance (27). Finally, the time kinetics of tolerance induction observed in the current study (within ~5 h) are more typical for the mechanism of tachyphylaxis than for receptor downregulation, which usually requires longer periods of exposure. The hypothesis of vagal nervous tachyphylaxis is further supported by the plasma concentrations of PP, which are thought to mirror the systemic level of vagal nervous activation (10,28). In fact, PP levels were markedly suppressed by GLP-1 during the first meal course, but slightly elevated during the second test meal, suggesting a more pronounced inhibition of parasympathetic outflow during the first test meal. Thus, GLP-1 seems to inhibit gastric emptying primarily through inhibition of the vagal nerve (28), but this mechanism is subject to rapid tachyphylaxis after chronic GLP-1 exposure.

The present data give rise to consider which of the multiple GLP-1 effects are subject to rapid tachyphylaxis and which are not. Clearly, the GLP-1–induced inhibition of gastric emptying was less pronounced during the second meal course compared with the first meal. In contrast, the quantitative amount of insulin released after the second test meal was even greater than after the first test meal, thereby arguing against an attenuation of the insulinoergic effect of GLP-1 during continuous exposure. For glucagon, an opposite picture was found, with a marked suppression after the first meal and higher levels after the second meal. However, because these postprandial changes in insulin and glucagon levels were primarily driven by the changes in gastric emptying, it is impossible to draw firm conclusions regarding a rapid tachyphylaxis to the GLP-1 effects on islet hormone secretion from this study. By taking into account the results obtained from the various long-term studies with GLP-1 analogs (24,29,30), there is little reason to assume the presence of a mechanism. Thus, although the GLP-1 actions on the stomach seem to critically depend on the time kinetics of GLP-1 levels, its insulinotropic and glucagon static effects are likely less dependent on the fluctuations in GLP-1 levels.

Of note, glucagon concentrations increased after the ingestion of the test meal. This is in contrast with previous studies showing a significant decline in glucagon levels after intravenous or oral glucose ingestion (31,32). This apparent discrepancy is most likely due to the high amino acid content of the test meal, which is known to stimulate rather than suppress glucagon secretion (33).

Another interesting finding from this study is the alteration in GIP secretion by GLP-1. During the first test meal, the GLP-1–induced retardation of gastric emptying led to a significant delay in GIP secretion. Given the importance of GIP as a physiologic incretin hormone (15,34), this might theoretically impair postprandial insulin responses and thus worsen glucose control. In the present experiments, the concentration time patterns of insulin and C-peptide seemed to closely mirror the respective GIP levels, thereby emphasizing the role of GIP as a physiologic enhancer of postprandial insulin release. In addition, GIP has also been shown to augment glucagon secretion in healthy humans (35). It is therefore possible that the differences in the postprandial concentrations of glucagon between the two test meals were also partly secondary to the changes in GIP release. However, given the complexity of these
interactions among vagal nervous activation, gastrointestinal motility, gut hormone release, and ileal hormone secretion, it is difficult to fully elucidate these relationships on the basis of the present experiments.

Although the present studies in healthy individuals do not allow for firm conclusions regarding the therapeutic use of GLP-1–based drugs, it would be tempting to speculate that the development of tachyphylaxis might be involved in the unequal susceptibility of patients to develop nausea when treated with either short- or long-acting GLP-1 analogs. It would therefore be of interest to examine the induction of tachyphylaxis in patients with type 2 diabetes during the chronic treatment with different GLP-1 analogs.

In conclusion, the delay in gastric emptying by GLP-1 is markedly attenuated during continued exposure to high GLP-1 concentrations. Most likely, this is due to the induction of tachyphylaxis at the level of vagal neural activation. As a consequence, postprandial glucose control by GLP-1 is attenuated after its chronic administration.

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