0021-972X/96/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 81, No. 1
Printed in U.S.A.

Gastric Emptying, Glucose Responses, and Insulin Secretion after a Liquid Test Meal: Effects of Exogenous Glucagon-Like Peptide-1 (GLP-1)-(7–36) Amide in Type 2 (Noninsulin-Dependent) Diabetic Patients*

BEREND WILLMS, JENS WERNER, JENS JUUL HOLST, CATHRINE ØRSKOV, WERNER CREUTZFELDT, AND MICHAEL A. NAUCK

Fachklinik für Diabetes und Stoffwechselkrankheiten (B.W., J.W.), Bad Lauterberg, Germany; the Departments of Anatomy and Physiology, Panum Institute, University of Copenhagen (J.J.H., C.Ø.), Copenhagen, Denmark; the Division of Gastroenterology and Endocrinology, Department of Medicine, Georg-August-University (W.C.), Göttingen, Germany; and the Department of Medicine, Ruhr-University (M.A.N.), Knappschafts-Krankenhaus, Bochum, Germany

ABSTRACT

The aim of the study was to investigate whether inhibition of gastric emptying of meals plays a role in the mechanism of the blood glucose-lowering action of glucagon-like peptide-1-(7–36) amide [GLP-1-(7–36) amide] in type 2 diabetes. Eight poorly controlled type 2 diabetic patients (age, 58 ± 6 yr; body mass index, 30.0 ± 5.2 kg/m^2; hemoglobin A_{1c}, 10.5 ± 1.2%) were studied in the fasting state (plasma glucose, 11.1 ± 1.1 mmol/L). A liquid meal of 400 mL containing 8% amino acids and 50 g sucrose (327 Kcal) was administered at time zero by a nasogastric tube. Gastric volume was determined by a dye dilution technique using phenol red. In randomized order, GLP-1-(7–36) amide (1.2 pmol/kg·min; Saxon Biochemicals) or placebo (0.9% NaCl with 1% human serum albumin) was infused between −30 and 240 min. In the control experiment, gastric emptying was completed within 120 min, and plasma glucose, insulin, C-peptide, GLP-1-(7–36) amide, and glucagon concentrations transiently increased. With exogenous GLP-1-(7–36) amide (plasma level, ~70 pmol/L), gastric volume remained constant over the period it was measured (120 min; $P < 0.0001$ vs. placebo), and plasma glucose fell to normal fasting values (5.4 ± 0.7 mmol/L) within 3–4 h, whereas insulin was stimulated in most, but not all, patients, and glucagon remained at the basal level or was slightly suppressed. In conclusion, GLP-1-(7–36) amide inhibits gastric emptying in type 2 diabetic patients. Together with the stimulation of insulin and the inhibition of glucagon secretion, this effect probably contributes to the blood glucose-lowering action of GLP-1-(7–36) amide in type 2-diabetic patients when studied after meal ingestion. At the degree observed, inhibition of gastric emptying, however, must be overcome by tachyphylaxis, reduction in dose, or pharmacological interventions so as not to interfere with the therapeutic use of GLP-1-(7–36) amide in type 2 diabetic patients. (*J Clin Endocrinol Metab* **81:** 327–332, 1996)

GLUCAGON-LIKE peptide-1-(7–36) amide [GLP-1-(7–36) amide] is an insulinotropic hormone secreted from enteroglucagon-producing L cells in the lower gut, *i.e.* the ileum and colon/rectum (1–3). Physiologically, together with gastric inhibitory polypeptide from the upper gut, it functions as an incretin hormone (2, 4, 5). Because, in contrast to gastric inhibitory polypeptide, GLP-1-(7–36) amide retains its insulinotropic activity in type 2 (noninsulin-dependent) diabetic patients (6), a therapeutic potential has been suggested, especially as it was possible to normalize fasting plasma glucose in hyperglycemic type 2 diabetic patients apparently without danger of inducing hypoglycemia (7). GLP-1-(7–36) amide also reduced meal-related insulin requirements in both type 1 (insulin-dependent) and type 2 diabetic patients (8). However, after such a mixed meal, influences on not only insulin and glucagon secretion, but also inhibition of gastric emptying (9) may have contributed to the antidiabetogenic effect (8). Therefore, the aim of the present study was to characterize the endocrine pancreatic and glucose responses to a pharmacological dose of exogenous GLP-1-(7–36) amide in type 2 diabetic patients who were fed a liquid test meal and to follow the velocity of gastric emptying using an indicator dye dilution technique (10). Preliminary results have been published in abstract form (11).

Received July 29, 1995. Revision received June 14, 1995. Accepted August 7, 1995.

Address all correspondence and requests for reprints to: Dr. Michael Nauck, Department of Medicine, Ruhr-University Bochum, Knappschafts-Krankenhaus, In der Schornau 23–25, 44892 Bochum, Germany.

* This work was supported by the Deutsche Forschungsgemeinschaft, Bonn 2 (Bad Godesberg), Grant Na 203/2–2, by the Danish Medical Research Council, and by the Novo Nordisk Foundation.

Subjects and Methods

Study protocol

The study protocol was approved by the ethics committee of the medical faculty of the Georg-August-University (Göttingen, Germany) on October 22, 1991 (registration no. 4/10/91) before the study. Written informed consent was obtained from all participants.

Patients

Eight type 2 diabetic patients were studied (Table 1). They were all being treated with diet and sulfonylurea compounds; two were also receiving metformin and one patient acarbose as a treatment. The mean multiple fasting plasma glucose concentration (from hospital charts) was 11.1 ± 1.1 mmol/L (199 ± 18 mg/dL), and the mean postprandial

TABLE 1. Patient characteristics

Patient no.	Sex	Age (yr)	Ht (cm)	Wt (kg)	Body mass index (kg/m^2)	Duration of diabetes (yr)	Therapy	Metabolic control, hemoglobin A_{1c} (%)[a]
1	f	53	167	64.5	23.1	8	D,S,B	12.9
2	m	54	187	80.5	23.1	15	D,S,A	10.7
3	f	67	156	86	35.3	23	D,S	9.2
4	m	64	177	96	30.6	8	D,S	9.6
5	f	58	165	90	33.4	8	D,S	9.7
6	m	49	179	87	27.3	4	D,S	10.0
7	f	60	165	99	36.6	13	D,S	11.6
8	f	57	153	72	30.8	13	D,S,B	10.3
Mean	3 m/5 f	58	168	84	30.0	12		10.5
SD		6	12	12	5.2	6		3.7

D, Diet; S, sulfonylurea treatment (glibenclamide, 10.5 mg/day, in all patients); B, biguanide treatment (metformin, 1700 or 2550 mg/day), A, acarbose treatment (300 mg/day).

[a] Normal value, 4.2–6.3%.

glucose concentration was 14.4 ± 1.3 mmol/L (259 ± 23 mg/dL). At the hospital, the patients consumed a diet with 1485 ± 519 Kcal/day (carbohydrate, 43 ± 2%; protein, 22 ± 3%; fat, 35 ± 2% of the total calories). Hypertension was diagnosed in five and treated in four patients (with nifedipine in three, with metoprolol in addition in one, and with captopril/hydrochlorothiazide in one patient). Blood pressure (Riva-Rocci) was 140 ± 10 mm Hg (systolic) and 79 ± 10 mm Hg (diastolic) on the occasion of the study. Albumin excretion was 21 ± 11 μg/min (range, 7.3–43 μg/min), and there was no gross proteinuria in any patient. In addition, there was evidence of coronary disease in one patient (treated by isosorbitol dinitrate), peripheral vascular disease (stage I) in another, background retinopathy in four patients, and mild peripheral neuropathy in six patients.

Patients were studied on 2 days. All antidiabetic medication was continued until the evening before the studies. A regular meal and drug schedule was allowed for 1 day between the experiments with GLP-1-(7–36) amide and placebo, which were performed in random order. On the study days, all medication was withheld until the end of the experiments.

For comparison, five young healthy volunteers (26 ± 3 yr old; 186 ± 6 cm; 79 ± 6 kg; body mass index, 23.0 ± 1.8 kg/m^2; hemoglobin A_{1c}, 5.0 ± 0.2%; oral glucose tolerance, by WHO criteria, normal) were studied using the same protocol.

Peptides

Synthetic GLP-1-(7–36) amide was purchased from Saxon Biochemicals (Hannover, Germany). The same lot number was used as in previous studies [GLP-1-(7–36) amide: PGAS 242, lot ZE 865; net peptide content, 79.3%] (5–7). The peptide was dissolved, filtered through 0.2-μm nitrocellulose filters (Millipore Corp., Bedford, MA), and stored frozen at −30 C as previously described. The net peptide content rather than the gross weight was used for dose calculations. High performance liquid chromatography profiles (provided by the manufacturer) showed that the preparation was more than 99% pure (single peak coeluting with appropriate standards). Samples were analyzed for bacterial growth (standard culture techniques) and pyrogens (Limulus amebocyte lysate endo-LAL, Chromogenix, Mölndal, Sweden). No bacterial contamination was detected. Endotoxin concentrations in the GLP-1-(7–36) amide stem solutions were always less than 0.03 endotoxin units/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 GLP-1-(7–36) amide/placebo administration].

After drawing basal blood specimens, an iv infusion of GLP-1-(7–36) amide or placebo (0.9% NaCl containing 1% human serum albumin; Merieux, Norderstedt, Germany) was started at −30 min at an infusion rate of 1.2 pmol/kg·min and continued for 270 min. This infusion rate has been used in previous studies (6, 7) as a pharmacological dose that raised plasma GLP-1-(7–36) amide to approximately 2- to 3-fold higher concentrations than those produced by oral nutrients (1, 2, 5). The infusion was begun at −30 min to assure elevated GLP-1-(7–36) amide plasma concentrations at the time of administration of the liquid meal. Blood was drawn at the time points indicated in Figs. 2 and 3, and plasma glucose was determined immediately.

Gastric emptying

Before the study, a nasogastric tube (Freka-Ernährungssonde, 120 cm, CH12, Fresenius, 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). If instilled water could not be completely aspirated, the position of the tube was adjusted to allow a near-complete aspiration of instilled fluid. The subjects were in a semirecumbent position, with the upper half of the body 45° upright, lying on their back. At 0 min, 400 mL of a liquid test meal made from a commercial amino acid solution (8% Aminosteril N-Hepa, Fresenius, Bad Homburg, Germany) with the addition of 50 g saccharose/400 mL were instilled into the stomach. This composition 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 to a normal mixed meal. Amino acids were added to stimulate the release of cholecystokinin (12), a physiological regulator of gastric emptying in humans (13). The meal contained 32 g mixed amino acids (131 Kcal = 40%) and 50 g saccharose (196 Kcal = 60%) (14); the total energy content was 327 Kcal (energy density, 0.82 Kcal/mL). Gastric emptying was measured by a double sampling dye dilution technique using phenol red (Merck, Darmstadt, Germany) according to the method of George (10) with modifications (15) introduced to reduce measurement error. In principle, 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 approximately 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 as the volume of distribution of phenol red. Increasing amounts of phenol red were used as the experiments proceeded to obtain measurable increments in optical density in the presence of previously instilled phenol red. *In vitro*, this method measured gastric volume with an accuracy of less than 6% (coefficient of variation). According to the expected rate of gastric emptying (9, 10, 13, 15), gastric contents were determined at the intervals shown in Fig. 1 over 120 min.

Blood specimens

Blood was drawn into heparinized tubes [immunoreactive (IR) insulin and C-peptide measurements]. A sample was stored in NaF (Microvette CB 300, Sarstedt, Nümbrecht, Germany) for the measurement of glucose. For glucagon and GLP-1-(7–36) amide measurements, blood was drawn into tubes containing ethylenediamine tetraacetate and aprotinin (20,000 kallikrein inhibitor units/mL, 200 μL/10 mL

blood; Trasylol, Bayer, Leverkusen, Germany). After centrifugation, plasma for hormone analyses was kept frozen at −30 C.

Laboratory determinations

Glucose was measured using a glucose oxidase method with a Glucose Analyzer 2 (Beckman Instruments, Munich, Germany). Plasma IR-insulin and C peptide were determined using commercial RIA kits (Insulin RIA 100, Pharmacia, Freiburg, Germany; RIA-mat C-peptide II, Byk-Sangtec Diagnostika, Dietzenbach, Germany) with human insulin and C peptide as standards. IR-GLP-1 was determined in ethanol-extracted plasma as previously described (16, 17), using antiserum 89 390 (final dilution, 1:150,000) and synthetic GLP-1-(7–36) amide for tracer preparation and as standard. The recovery of GLP-1-(7–36) amide standards after alcohol extraction was 75 ± 8%. The experimental detection limit [2 SD over samples not containing GLP-1-(7–36) amide] was less than 5 pmol/L. Antiserum 89390 binds to the amidated carboxy-terminus of GLP-1-(7–36) amide (17). With this assay methodology, basal plasma GLP-1-(7–36) amide concentrations in normal and diabetic subjects are approximately 5–10 pmol/L, with postprandial peak concentrations of 20–50 pmol/L (1, 2, 5).

Pancreatic glucagon was assayed in ethanol-extracted plasma using antibody 4305 (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; pH 8.0) at a wavelength of 546 nm and read against a standard curve (phenol red in phosphate buffer).

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 the mean ± SEM. Integrations were carried out according to the trapezoidal rule, separately calculating increments above and decrements below baseline. The significances of differences were tested using repeated measurement ANOVA (RM-ANOVA; version 5.01, Number Cruncher Statistical Software, Kaysville, UT). If a significant interaction of treatment and time was documented ($P < 0.05$), values at single time points were compared by Student's t test (paired analyses). P values were corrected for the number of comparisons made according to the method of Bonferroni-Holm. A corrected two-sided $P < 0.05$ was taken to indicate significant differences.

Results

Gastric emptying

With placebo, gastric volume in type 2 diabetic patients declined over 120 min to a residual value of 58 ± 19 mL, whereas under the influence of exogenous GLP-1-(7–36) amide, gastric content remained around 400 mL, the starting value ($P < 0.0001$; Fig. 1A). Similar results were found in normal glucose-tolerant healthy volunteers (Fig. 1B).

Plasma glucose

Although the liquid test meal caused an increment in plasma glucose with placebo, glucose concentrations started to decline when the exogenous (iv) administration of GLP-1-(7–36) amide was initiated (Fig. 2). After 180 min, normal fasting plasma glucose concentrations were reached (5.4 ± 0.7 mmol/L), whereas starting values were reached again at the end of placebo studies ($P < 0.0001$).

Insulin secretion

Insulin and C peptide increased during the infusion of GLP-1-(7–36) amide (starting at −30 min) or, in the placebo experiment, after instilling the liquid meal (0 min; Fig. 2).

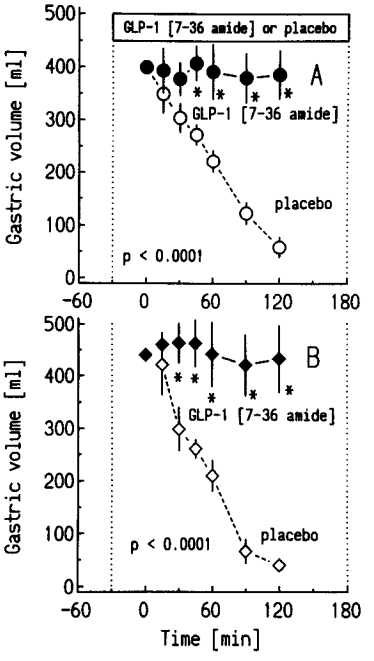

FIG. 1. Gastric volume after intragastric instillation of a mixed liquid meal, as determined by a double sampling dye dilution technique using phenol red in eight type 2 diabetic patients (*upper panel*, A) and in five normal volunteers (*lower panel*, B) in response to the iv infusion of GLP-1-(7–36) amide (1.2 pmol/kg·min; duration of infusion indicated by a *bar*) or placebo. Values are the mean ± SEM. The P value was determined by RM-ANOVA (interaction of experiment and time); *asterisks* indicate a significant difference at the respective time point (by paired comparisons using Student's t test, $P < 0.05$).

Plasma GLP-1 levels

Intravenous infusions of GLP-1-(7–36) amide at a rate of 1.2 pmol/kg·min raised plasma concentrations of GLP-1-(7–36) amide to approximately 70 pmol/L throughout the experiment. With placebo, there was a transient increment from basal values of approximately 9 pmol/L to a peak of 21 ± 4 pmol/L 30 min after instillation of the liquid meal (Fig. 3).

Pancreatic glucagon

In the placebo study, there was a clear meal-related response of glucagon ($P = 0.0001$, by paired t test for the comparison of mean basal and peak stimulated values; Fig. 3), which was not seen during the infusion of GLP-1-(7–36) amide. Rather, glucagon concentrations tended to decline below baseline levels with exogenous GLP-1-(7–36) amide ($P = 0.0039$ for comparison of mean basal and mean suppressed values at 0, 7.5, and 15 min of the time scale in Fig. 3).

Integrated responses

Integrated responses summarizing the behavior of glucose, insulin, C-peptide, and glucagon are shown in Table 2.

Symptoms

There were no side-effects of GLP-1-(7–36) amide; especially, the patients did not report discomfort or any gastro-

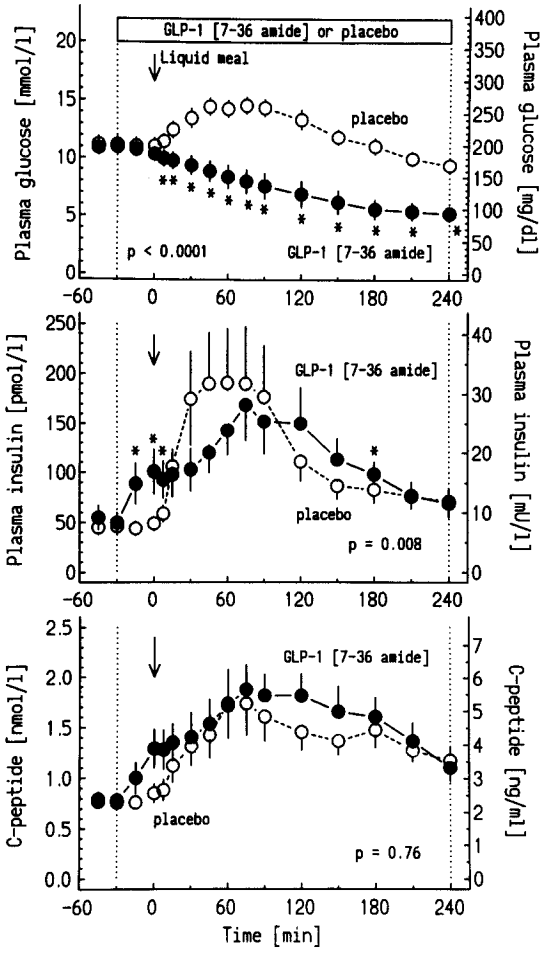

FIG. 2. Plasma glucose (*upper panel*), insulin (*middle panel*), and C peptide (*lower panel*) responses after intragastric instillation of a mixed liquid meal in eight type 2 diabetic patients in response to the iv infusion of GLP-1-(7–36) amide (1.2 pmol/kg·min; duration of infusion indicated by a *bar*) or placebo. Values are the mean ± SEM. The *P* value was determined by RM-ANOVA (interaction of experiment and time); *asterisks* indicate a significant difference at the respective time point (by paired comparisons using Student's *t* test, $P < 0.05$).

intestinal symptoms that could be causally related to a reduced velocity of gastric emptying.

Discussion

The present study extend previous observations on the normalization of fasting hyperglycemia in type 2 diabetic patients using a pharmacological infusion of GLP-1-(7–36) amide (7), however, with its focus on changes introduced by a liquid test meal. In this postprandial situation, the predominant action of exogenous GLP-1-(7–36) amide was a profound inhibition of gastric emptying. As a consequence, under the influence of exogenous GLP-1-(7–36) amide, type 2 diabetic patients showed plasma glucose, insulin, C peptide, and glucagon responses very similar to those of fasting patients studied previously (7). With total gastric volumes fluctuating around starting values, it is likely that some gastric content had emptied even with the administration of GLP-1-(7–36) amide, as some gastric juice probably had been

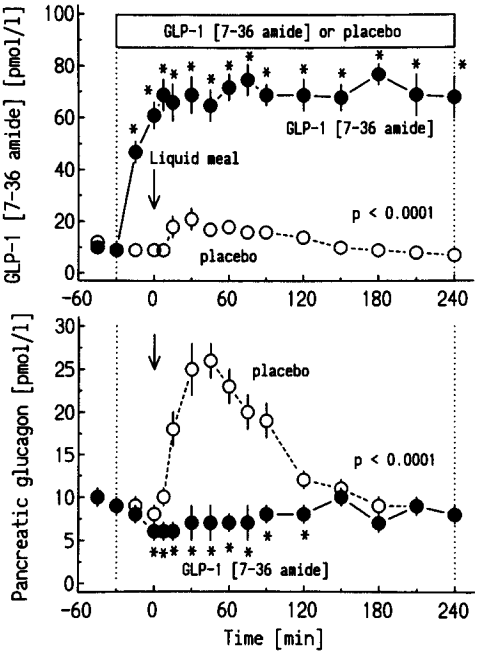

FIG. 3. Plasma GLP-1-(7–36) amide (antibody 89390; *upper panel*) and pancreatic glucagon (antibody 4305; *lower panel*) concentrations after intragastric instillation of a mixed liquid meal in eight type 2 diabetic patients in response to the iv infusion of GLP-1-(7–36) amide (1.2 pmol/kg·min; duration of infusion indicated by a *bar*) or placebo. Values are the mean ± SEM. The *P* values were determined by RM-ANOVA (interaction of experiment and time); *asterisks* indicate a significant difference at the respective time point (by paired comparisons using Student's *t* test, $P < 0.05$).

TABLE 2. Integrated incremental responses of glucose, insulin, C peptide, and glucagon after a liquid intragastric meal in type 2 diabetic patients in response to GLP-1-(7–36)amide

Parameter	Placebo	GLP-1-(7–36)amide	*P* value
Glucose (mmol/L^{-1} · min)			
Positive	388.3 ± 55.5	5.2 ± 5.0	0.0078
Negative	−110.0 ± 60.2	−932.0 ± 111.5	0.0031
Insulin (nmol/L^{-1} · min)			
Positive	17.9 ± 4.6	17.0 ± 3.6	0.14
C Peptide (nmol/L^{-1} · min)			
Positive	152.7 ± 29.7	197.1 ± 33.9	0.64
Glucagon (pmol/L^{-1} · min)			
Positive	1365.0 ± 175.2	150.4 ± 61.0	0.0001
Negative	−85.9 ± 26.3	−542.0 ± 99.6	0.0009

Values are the mean ± SEM. Integration was carried out starting at baseline (−45 and −30 min). Positive responses are increments over this baseline, negative responses are decrements below this baseline.

secreted during the study (19). However, the contribution of acid secretion to the intraluminal gastric volume was probably small, especially as GLP-1-(7–36) amide is considered an inhibitor of gastric acid secretion in humans (20, 21). The near-complete inhibition of gastric emptying is confirmed by the lack of an increment in glucose and glucagon concentrations under the influence of exogenous GLP-1-(7–36) amide. The fact that the degree of inhibition of gastric emp-

tying by GLP-1-(7–36) amide was different from that observed previously in young healthy volunteers (9) may be related to differences in the subject/patient characteristics, the composition of the meals (which were liquid in both cases), or the method of determination [szintigraphic (9) *vs.* dye dilution technique (this study)]. The most obvious difference between the groups studied was their plasma glucose concentrations (9). As insulinotropic (4, 5, 22) and glucagonostatic (6, 23, 24) actions of GLP-1-(7–36) amide are characterized by a delicate interaction with ambient glucose concentrations, the question arose of whether the inhibition of gastric emptying could in any way be related to plasma glucose concentrations, especially as hyperglycemia itself has profound influences on gastric and intestinal motility (25, 26). However, using identical meals and the same methodology to study gastric emptying, almost identical emptying rates with and without GLP-1-(7–36) amide were found in hyperglycemic type 2 diabetic patients and normoglycemic healthy volunteers, therefore excluding profound influences of the difference in glycemia. In the light of the present results, it will be possible to interpret two studies dealing with effects on plasma glucose and insulin levels in type 2 diabetic patients after the ingestion of meals (8, 27). The results of Nathan *et al.* (27) are compatible with a transient inhibition of gastric emptying during a 30-min infusion of GLP-1-(7–37) [which has similar biological actions as GLP-1-(7–36) amide (27, 28)]. Apparently, due to the short half-life of GLP-1-(7–36) amide or GLP-1-(7–37) in the circulation (4–6, 27), soon after stopping the infusion, gastric emptying will proceed (indicated by a rise in plasma glucose and insulin concentrations) (27). This has not been studied in our protocol, where, based on glucose and glucagon responses, gastric emptying probably occurred at very low rates throughout the 270-min GLP-1-(7–36) amide infusion period.

An inhibition of gastric emptying (probably of a similar degree as that observed in the present study) very likely explains much of the antidiabetogenic effect observed by Gutniak *et al.* (8) in both type 1 and type 2 diabetic patients. It appears that the diminished rate of glucose absorption (leading to a reduced rate of glucose appearance in the circulation) caused by the inhibition of gastric emptying led to smaller rates of insulin infusion according to the algorithm guiding glucose control by the Biostator used in that study (8). This is the only explanation for the effects of the magnitude described in type 1 diabetic patients (who, according to the criteria of selection, were unable to secrete insulin). Especially, a potential glucagon-lowering effect of GLP-1-(7–36) amide, which might have reduced plasma glucose increments to a certain degree, can be excluded because there was a meal-related increment in plasma glucagon in that study (8). The 16% change in apparent insulin sensitivity (disregarding changes in glucagon concentrations, which were not reported but probably occurred under these conditions) observed in the hyperinsulinemic euglycemic clamp study in type 1 diabetic patients certainly was too small in magnitude to make an important contribution to the postprandial situation. It appears from glucose and insulin responses presented in the study by Gutniak *et al.* (8) that gastric emptying was possibly affected more in diabetic than in normal subjects. However, direct measurement does not support this conclusion.

In principle, the inhibition of gastric emptying leads to a slowed entry of nutrients, which can only be absorbed from the intestines. So a reduced velocity of gastric emptying may contribute to the action profile of GLP-1-(7–36) amide as a therapeutic agent in type 2 diabetic patients, similar to α-glucosidase inhibition (29). It is questionable, however, whether the degree of inhibition of gastric emptying observed in this study is compatible with normal nutrition. It is not known whether the inhibition of gastric emptying is sensitive to changes in the dose and, consequently, plasma concentrations of GLP-1-(7–36) amide, and whether it affects solid meal components to a similar degree (30). Such details should be studied, especially as the mechanism of action of GLP-1-(7–36) amide regarding the inhibition of gastric emptying has not been sufficiently characterized. Any regimen of administration of GLP-1 or potential analogs that leads to a day-long elevation of plasma concentrations into the pharmacological range might, in principle, greatly interfere with substrate entry into the circulation and, hence, with normal nutrition if the concentrations or bioactivities are similar to those achieved in the present study. Tachyphylaxis for this effect, but not for the other glucose-lowering actions of GLP-1 (1, 2, 6–8), might occur and spontaneously circumvent this problem. Otherwise, additional measures to limit this effect could be to lower the dose of GLP-1-(7–36) amide administered or to study the effects of prokinetic drugs along with GLP-1. Alternatively, the deceleration of gastric emptying should be less pronounced with regimens that raise plasma levels for a shorter duration (27), as after sc injections of the peptide (31). Still another approach could be a different timing, for example to increase plasma GLP-1 concentrations shortly after meals (to allow initial gastric emptying to occur), but this may be difficult to achieve for pharmacokinetic reasons. In conclusion, the predominant action of a pharmacological dose of exogenous GLP-1-(7–36) amide, when infused into type 2 diabetic patients who ingested a liquid meal, was a near-complete inhibition of gastric emptying with a subsequent normalization of fasting plasma glucose concentrations, as previously described in fasting type 2 diabetic patients (7). At the degree observed, the inhibitory effect on gastric emptying should be overcome by additional measures to fully exploit its therapeutic potential of GLP-1 for the normalization of glycemia in type 2 diabetic patients.

Acknowledgments

The excellent technical assistance of Ms. S. Bierkamp, A. Bernhard, L. Rabenhøj, and L. Albæk is gratefully acknowledged. We thank R. Ettler and U. Niedereichholz for performing gastric emptying studies in normal subjects.

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