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Effects of subcutaneous glucagon-like peptide 1 (GLP-1 [7–36 amide]) in patients with NIDDM

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Summary Intravenous glucagon-like peptide (GLP)-1 [7–36 amide] can normalize plasma glucose in non-insulin-dependent diabetic (NIDDM) patients. Since this is no form for routine therapeutic administration, effects of subcutaneous GLP-1 at a high dose (1.5 nmol/kg body weight) were examined. Three groups of 8, 9 and 7 patients (61 ± 7, 61 ± 9, 50 ± 11 years; BMI 29.5 ± 2.5, 26.1 ± 2.3, 28.0 ± 4.2 kg/m^2; HbA_{1c} 11.3 ± 1.5, 9.9 ± 1.0, 10.6 ± 0.7 %) were examined: after a single subcutaneous injection of 1.5 nmol/kg GLP [7–36 amide]; after repeated subcutaneous injections (0 and 120 min) in fasting patients; after a single, subcutaneous injection 30 min before a liquid test meal (amino acids 8 %, and sucrose 50 g in 400 ml), all compared with a placebo. Glucose (glucose oxidase), insulin, C-peptide, GLP-1 and glucagon (specific immunoassays) were measured. Gastric emptying was assessed with the indicator-dilution method and phenol red. Repeated measures ANOVA was used for statistical analysis. GLP-1 injection led to a short-lived increment in GLP-1 concentrations (peak at 30–60 min, then return to basal levels after 90–120 min). Each GLP-1 injection stimulated insulin (insulin, C-peptide, $p < 0.0001$, respectively) and inhibited glucagon secretion ($p < 0.0001$). In fasting patients the repeated administration of GLP-1 normalized plasma glucose (5.8 ± 0.4 mmol/l after 240 min vs 8.2 ± 0.7 mmol/l after a single dose, $p = 0.0065$). With the meal, subcutaneous GLP-1 led to a complete cessation of gastric emptying for 30–45 min ($p < 0.0001$ statistically different from placebo) followed by emptying at a normal rate. As a consequence, integrated incremental glucose responses were reduced by 40 % ($p = 0.051$). In conclusion, subcutaneous GLP-1 [7–36 amide] has similar effects in NIDDM patients as an intravenous infusion. Preparations with retarded release of GLP-1 would appear more suitable for therapeutic purposes because elevation of GLP-1 concentrations for 4 rather than 2 h (repeated doses) normalized fasting plasma glucose better. In the short term, there appears to be no tachyphylaxis, since insulin stimulation and glucagon suppression were similar upon repeated administrations of GLP-1 [7–36 amide]. It may be easier to influence fasting hyperglycaemia by GLP-1 than to reduce meal-related increments in glycaemia. [Diabetologia (1996) 39: 1546–1553]

Keywords GLP-1 [7–36 amide], incretin, insulin, glucagon, pharmacokinetics.

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Abbreviations: BMI, Body mass index; EDTA, ethylene diamine tetra-acetic acid; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; RM-ANOVA, repeated measures analysis of variance; NIDDM, non-insulin-dependent diabetes mellitus; IR, immunoreactive.

Glucagon-like peptide 1 (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, 2]. GLP-1 [7–36 amide], together with gastric inhibitory polypeptide (GIP) from the upper gut, acts as a physiological incretin hormone [3, 4]. In pharmacological concentrations, exogenous GLP-1 [7–36 amide or 7–37] also raised insulin and lowered glucagon concentrations in

Table 1. Characteristics of NIDDM patients studied

Parameter GLP-1 [7–36 amide] s.c.:	Study A Fasting patients Single dose	Study B Fasting patients Repeated dose	Study C Liquid meal Single dose	Significance ANOVA (p-value)
Sex (male/female)	5/3	4/5	3/4	0.69
Age (years)	61 ± 7	61 ± 9	50 ± 11	0.03
Body mass index (kg/m^2)	29.5 ± 2.5	26.1 ± 2.3	28.0 ± 4.2	0.096
Diabetes duration (years)	9 ± 3	11 ± 7	10 ± 4	0.59
HbA_{1c} (%)	11.3 ± 1.5	9.9 ± 1.0	10.6 ± 0.7	0.054
Diet (kCal/day)	1429 ± 262	1538 ± 348	1416 ± 253	0.66
Glibenclamide (mg/day)	9.6 ± 2.5	10.5 ± 0	10.5 ± 0	0.39
Metformin (yes/no)	1/7	2/7	6/1	0.007
Acarbose (yes/no)	1/7	0/9	0/7	0.35
$RR_{systolic}$ (mm Hg)	162 ± 28	138 ± 19	136 ± 12	0.043
$RR_{diastolic}$ (mm Hg)	94 ± 16	72 ± 13	82 ± 11	0.012

Data are mean ± SD

non-insulin-dependent diabetic (NIDDM) patients [5, 6]. By these mechanisms, plasma glucose was normalized within 3–4 h by i.v. GLP-1 [7–36 amide] in NIDDM patients with secondary failure of sulphonylurea treatment [7]. Therefore, GLP-1 [7–36 amide] or GLP-1 [7–37] (which has an identical action profile in rats [8], normal humans [9] and NIDDM patients [10]) has been suggested for use in the therapy of NIDDM patients [5–7, 10, 11]. However, a mode of administration has to be sought that both makes use of the potential to normalize glycaemia in NIDDM patients [7, 10] and that will be acceptable when compared with other current therapy. One obvious way is the s.c. administration of this peptide hormone. Limited experience using small doses of GLP-1 [7–36 amide] have been reported by Gutniak et al. [12]. However, they only used it to reduce postprandial glycaemic excursions. GLP-1 [7–36 amide] was injected shortly before the ingestion of a meal, and the effects noted were smaller in comparison to the normalization of fasting hyperglycaemia that can be achieved by i.v. GLP-1 [7–36 amide] in NIDDM patients [6, 10]. Since i.v. GLP-1 [7–36 amide] profoundly influences fasting hyperglycaemia in NIDDM patients [7, 10], it was the aim to also examine the effect of s.c. GLP-1 in fasting patients, and to compare it to a preprandial administration. The amount administered in the present study was the maximum dose that was free of severe side-effects in young, healthy volunteers [13]. Preliminary results have been communicated in abstract form [14].

Subjects, materials and methods

Study protocol. The study protocols were approved by the ethics committee of the medical faculty of the Georg-August University, Göttingen, prior to the study. Written, informed consent was obtained from all participants.

Subjects. Three groups of NIDDM patients were studied, having been admitted to a specialized diabetes clinic because of unsatisfactory metabolic control. Their characteristics are shown in Table 1. All were treated with diet and oral agents (sulphonylurea in all, plus acarbose or metformin in some). Diabetes, on average, had been diagnosed approximately 10 years earlier. Metabolic control at the time of the study, as indicated by HbA_{1c} values of 10–11 % (normal: 4.3–6.1 %), was unsatisfactory. Most patients were slightly obese.

All patients were studied on two occasions; in randomized order, in a single-blind fashion, placebo (0.9 % NaCl with 1 % human serum albumin) or GLP-1 [7–36 amide] in a dose of 1.5 nmol/kg body weight was administered in the morning after an overnight fast as a single or repeated dose. Anti-diabetic medication (Table 1) was given until the night before the first experiment. Between the experiments, 1 day with a regular eating and treatment schedule (including all drugs) was allowed.

Three protocols were compared: *Study A* examined the effect of a single s.c. injection of GLP-1 [7–36 amide] (administered at time 0 min) in fasting NIDDM patients followed for 240 min. *Study B* examined the effect of a repeated s.c. injection of GLP-1 [7–36 amide] (administered at time 0 and 120 min) in fasting NIDDM patients followed for 240 min. *Study C* examined effects of a preprandial single s.c. injection of GLP-1 [7–36 amide] (administered at time –30 min) in NIDDM patients. At time 0 min, a liquid mixed meal made up of a commercially available amino acid solution (Aminosteril N-Hepa 8 %; Fresenius AG, Bad Homburg, Germany) and sucrose (50 g per 400 ml) was administered via a nasogastric tube. Results were observed over 240 min.

Peptides. Synthetic GLP-1 [7–36 amide] was purchased from Saxon Biochemicals GmbH, Hannover, Germany (PGAS 242). The peptide was dissolved in 0.9 % NaCl containing 1 % human serum albumin (Merieux, Norderstedt, Germany), filtered through 0.2 μm nitrocellulose filters (Millipore, Bedford, Mass., USA) and stored frozen at –30 °C as previously described [4, 5, 7]. Net peptide content rather than 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 analysed 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 [7–36 amide] stem solutions were always less than 0.03 endotoxin units (EU)/ml.

Experimental procedures. One forearm vein was punctured with a teflon cannula (Moskito 123, 18 gauge; Vygon, Aachen, Germany), and kept patent using 0.9 % NaCl.

In experiments with fasting NIDDM patients, after drawing basal blood specimens, at 0 min, GLP-1 [7–36 amide] was administered s.c. into the periumbilical region. The injected volume was 1 ml per 85 kg body weight. In study B, at 120 min the subcutaneous injection of GLP-1 [7–36 amide] was repeated.

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 ethylene diamine tetra-acetic acid (EDTA) and aprotinin (Trasylol; 20000 kallikrein inhibitor units/ml, 200 μl per 10 ml blood; Bayer AG, Leverkusen, Germany). After centrifugation, plasma for hormone analyses was kept frozen at –30 °C.

Gastric emptying. In study C, before the start of the experiments, a nasogastric tube (Freka-Ernährungssonde, 120 cm, CH 12, Fresenius AG) was placed and tape-fixed with the tip approximately 55 cm from the nostrils. Gastric juice was aspirated and an acid pH was ascertained using pH-sensitive lackmus paper. The gastric lumen was washed with 100 ml tap water warmed to 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 patients were in a semi-recumbent position, with the upper body 45 ° upright. At –30 min, GLP-1 [7–36 amide] was administered s.c., and at 0 min, the liquid meal (warmed to 37 °C) was instilled into the stomach. Previous studies had indicated peak plasma concentrations around 30 min after s.c. injection of GLP-1 [7–36 amide] [13]. The meal consisted of 32 g mixed amino acids (131 kCal = 40 % of total caloric content) and 50 g sucrose (196 kCal = 60 %). Total energy content was 327 kCal (energy density 0.82 kCal/ml) [15, 16].

Gastric emptying was determined exactly as in a previous study testing intravenously infused GLP-1 [7–36 amide] in a similar group of NIDDM patients [15], by a double-sampling dye dilution technique using phenol red (Merck AG, Darmstadt, Germany), according to George [17], with modifications introduced to reduce the measurement error by Hurwitz et al. [18]. In principle, at all time-points chosen to measure gastric volume, a known amount of the non-absorbable phenol red dye was added to the translucent test meal in a volume of 5 to 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. Increasing amounts of phenol red were used as the experiments proceeded to obtain clearly measurable increments in optical density also in the presence of previously instilled phenol red [18]. In vitro, this method measured gastric volume with an accuracy of less than 6 % (coefficient of variation).

Laboratory determinations. Glucose was measured using a glucose oxidase method with a Glucose Analyser 2 (Beckman Instruments, Munich, Germany). Plasma IR-insulin and C-peptide were determined using commercial immunoassay kits. Insulin was measured using an insulin microparticle enzyme immunoassay (MEIA) IMx Insulin, Abbott Laboratories, Wiesbaden, Germany, which shows a correlation coefficient 0.982 vs RIA 100; Pharmacia, Freiburg, Germany (the assay used in our previous study [15]). Intra-assay coefficients of variation were less than 4.0 %. C-peptide was measured using C-peptide antibody-coated microtitre wells (C-peptide MTPL EIA) from DRG Instruments GmbH, (Marburg, Germany). Human insulin and C-peptide were used as standard.

IR-GLP-1 was determined in ethanol-extracted plasma as previously described [19], using antiserum 89 390 (final dilution 1:150 000) and synthetic GLP-1 [7–36 amide] for tracer preparation and as standard. Recovery of GLP-1 [7–36 amide] standards after alcohol extraction was 75 ± 8 %. The experimental detection limit (2 standard deviations over samples not containing GLP-1 [7–36 amide]) was less than 5 pmol/l. Antiserum 89 390 binds proglucagon-derived peptides containing the amidated carboxy-terminus of GLP-1 [7–36 amide], thereby being relatively specific for GLP-1 [7–36 amide] [20]. Plasma samples with expected high concentrations were diluted 1:10 with assay buffer before analysis.

Pancreatic glucagon was assayed in ethanol-extracted plasma using antibody 4305 [21]. GIP was determined using antiserum R 65 and synthetic human GIP for the preparation of standards and ^{125}GIP tracer (purified by HPLC) as described by Krarup et al. [22]. GIP was not measured in the experiments with fasting patients, since GLP-1 [7–36 amide] does not stimulate GIP release in humans [4, 5].

Each patient's set of plasma samples was assayed at the same time to avoid errors due to inter-assay variation.

Symptoms. During the experiments, the volunteers were observed and frequently asked about their state of well-being. Their answers were recorded using an open questionnaire.

Statistical analysis

Subject characteristics are reported as mean ± SD, experimental results are reported as mean ± SEM. Integration was carried out according to the trapezoidal rule, separately calculating increments over and decrements below mean baseline values. Metabolic clearance rates were calculated as the dose of s.c. GLP-1 [7–36 amide], divided by the integrated incremental response. Significances of differences were tested using repeated measurement analysis of variance (RM-ANOVA; NCSS Version 5.01, Kaysville, Utah, USA). 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; GLP-1 [7–36 amide] vs placebo). For the analysis of time courses one-way ANOVA was used, followed by *t*-tests (vs mean basal values), if indicated. For contingency table analysis, a chi square-test was used. A two-sided p-value less than 0.05 was taken to indicate significant differences.

Results

All patients were hyperglycaemic at the start of the experiments (mean plasma glucose concentrations over 11 mmol/l; Figs. 1 and 2).

Study A. A single s.c. administration of GLP-1 [7–36 amide] reduced plasma glucose concentrations by approximately 2–3 mmol/l (Fig. 1A), mainly during the initial 120 min, but the effect was maintained throughout the 240-min period. Insulin (Fig. 1B) and C-peptide (Fig. 1C) were stimulated (peak 30 min after GLP-1 [7–36 amide] administration) significantly,

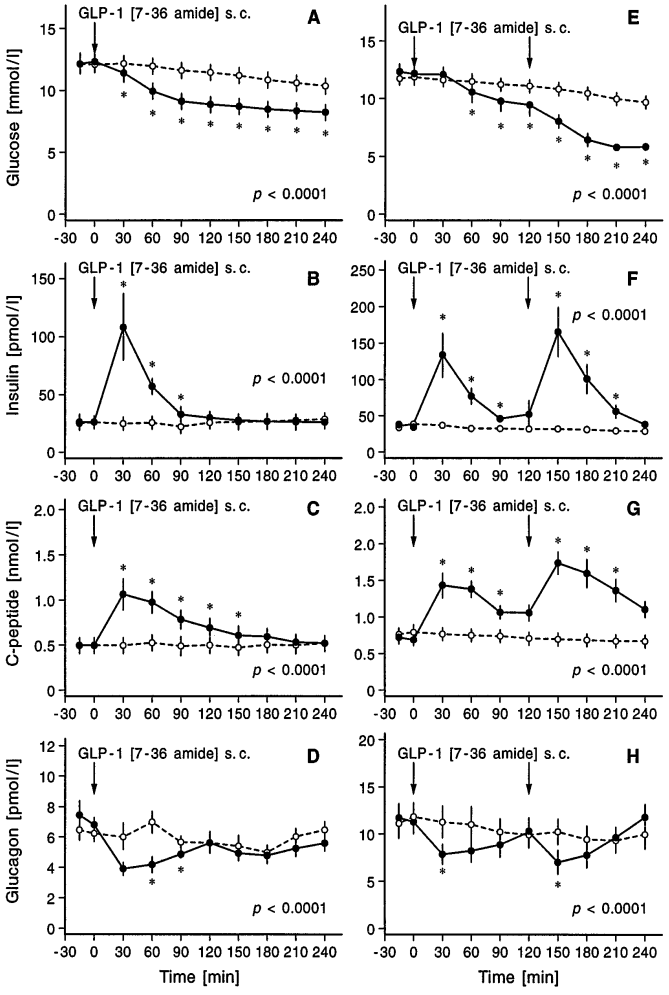

Fig. 1. A–H. Effects of single (*left panels*, **A–D**) and repeated (right panels, E-H) s.c administrations of GLP-1 [7–36 amide] (1.5 nmol/kg body weight) on plasma glucose (**A, E**), insulin (**B, F**) C-peptide (**C, G**), and glucagon (**D, H**) concentrations in NIDDM patients. *Arrows* indicate time point of GLP-1 [7–36 amide] administration. Experiments with GLP-1 [7–36 amide] (●) or placebo (○). *P*-values indicate significance of interaction of experiment (GLP-1 [7–36 amide] vs placebo) and time. *: Differences at specific time points (*t*-test, $p < 0.05$)

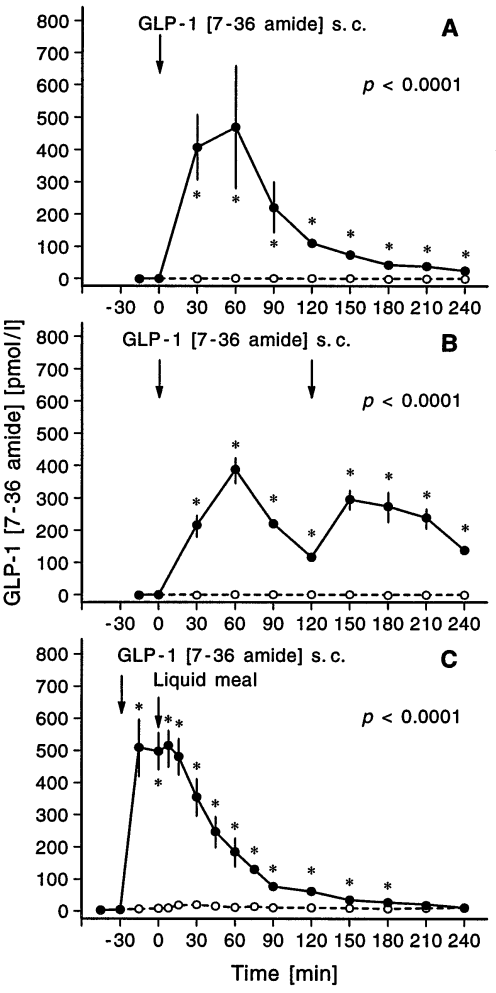

Fig. 2. A–C. Effects of single (**A**) and repeated (**B**) s.c. administrations of GLP-1 [7–36 amide] (1.5 nmol/kg body weight) in the fasting state, and of single (**C**) s.c. administrations of GLP-1 [7–36 amide] administered before a liquid test meal on plasma GLP-1 [7–36 amide] concentrations in NIDDM patients. Arrows indicate time point of GLP-1 [7–36 amide] administration and of the intragastric installation of the meal. Experiments with GLP-1 [7–36 amide] (●) or placebo (○). *P*-values indicate significance for the interaction of experiment (GLP-1 [7–36 amide] vs placebo) and time. *: Differences at specific time points (*t*-test, $p < 0.05$)

but transiently, and returned to baseline values after 90–120 (insulin) and 150–180 (C-peptide) min.

GLP-1 [7–36 amide] concentrations reached peak values of around 400–550 pmol/l 30–60 min after s.c. administrations of 1.5 nmol/kg body weight GLP-1 [7–36 amide] (Fig. 2A) and returned to baseline values within 120–180 min after injection.

Glucagon concentrations were transiently, but significantly ($p < 0.0001$) suppressed during 90 min after GLP-1 [7–36 amide] administration (Fig. 1D).

Study B. In contrast to the single s.c. administration of GLP-1 [7–36 amide], the repeated administration (two doses administered at 0 and 120 min) resulted in a step-wise normalization of fasting glycaemia in NIDDM patients (Fig. 1E; $p < 0.0001$ vs placebo). The plasma glucose reached at the end of the experiment was significantly lower (5.8 ± 0.4 mmol/l) than after the single dose (8.2 ± 0.7 mmol/l; $p = 0.0065$, *t*-test), while there was no significant difference in the integrated decremental response.

Insulin and C-peptide showed a second increment after the administration of GLP-1 [7–36 amide] at 120 min (Fig. 1F and G), which made the overall insulin and C-peptide responses greater ($p < 0.05$ for both) than in the experiment with single doses of GLP-1 [7–36 amide] (Fig. 1B and C). The responses of insulin and C-peptide to the second injection of GLP-1 [7–36 amide] were similar in magnitude to those of the first or single administration (Fig. 1F and

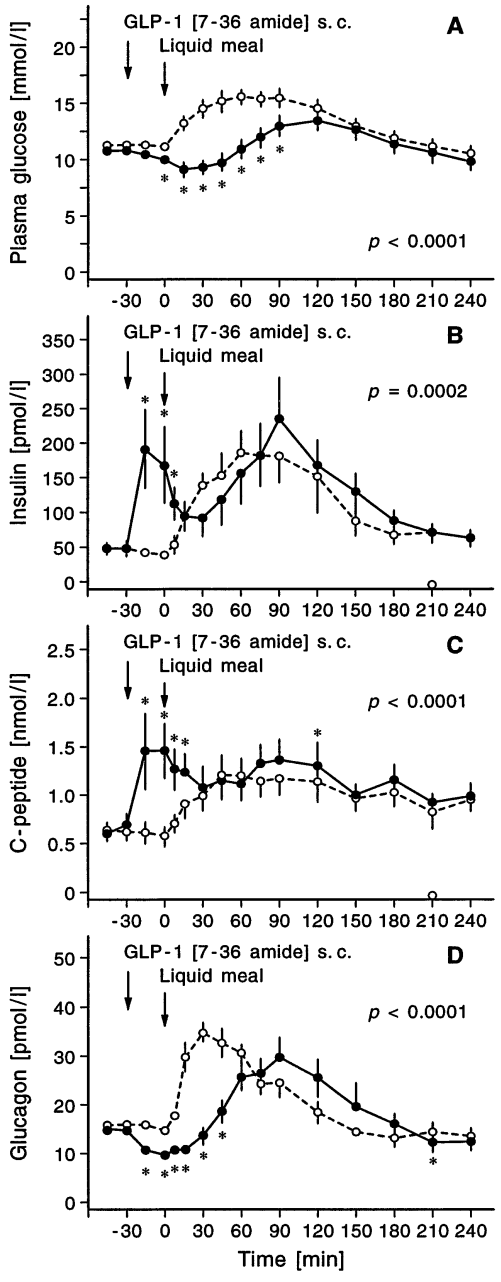

Fig. 3. A–D. Effects of a single s.c. administration of GLP-1 [7–36 amide] (1.5 nmol/kg body weight) on plasma glucose (**A**), insulin (**B**), C-peptide (**C**), and glucagon (**D**) concentrations in NIDDM patients before and after feeding a liquid test meal. *Arrows* indicate the time point of GLP-1 [7–36 amide] administration and of the intragastric instillation of the meal. Experiments with GLP-1 [7–36 amide] (•) or placebo (○). *P*-values indicate significance for the interaction of experiment (GLP-1 [7–36 amide] vs placebo) and time. *: Differences at specific time points (*t*-test, $p < 0.05$)

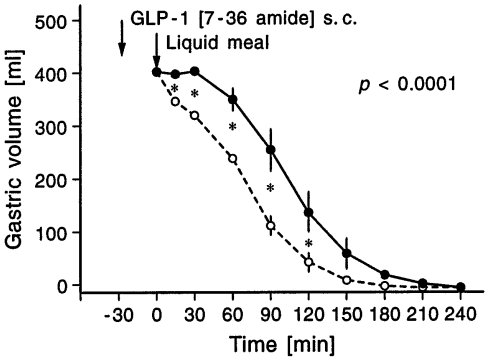

Fig. 4. Effects of single s.c. administrations of GLP-1 [7–36 amide] (1.5 nmol/kg body weight) on gastric emptying rates after the intragastric instillation of a liquid test meal in NIDDM patients. *Arrow* indicates time point of GLP-1 [7–36 amide] administration. Experiments with GLP-1 [7–36 amide] (•) or placebo (○). *P*-values indicate the significance for the interaction of experiment (GLP-1 [7–36 amide] vs placebo) and time. *: Differences at specific time points (*t*-test, $p < 0.05$)

G; $p = 0.99$ and 0.48 for integrated incremental insulin and C-peptide responses, respectively), although plasma glucose concentrations had already been lowered in response to the first injection (Fig. 1E).

GLP-1 [7–36 amide] plasma values showed a second peak of similar magnitude (Fig. 2B), and glucagon concentrations were reduced significantly ($p < 0.0001$) 30 min after each administration (Fig. 1H). The reduction in glucagon was similar after the first and second injection ($p = 0.51$). However, the overall reduction in glucagon (integrated decremental responses) was not significantly different from experiments with a single dose of GLP-1 [7–36 amide].

Study C. Intragastric instillation of a liquid mixed meal containing 50 g sucrose, together with the administration of placebo, raised mean plasma glucose concentrations to over 15 mmol/l (Fig. 3A). However, with GLP-1 [7–36 amide] administered s.c. 30 min before the meal, plasma glucose values were lowered rather than elevated during the initial 30–45 min after the meal, with a later rise that was attenuated in comparison with placebo studies (Fig. 3A). The rise in glycaemia over basal values (integrated incremental responses), however, was only reduced from 529.2 ± 54.3 mmol · l^{-1} · min (placebo) to 318.6 ± 106.7 (GLP-1 [7–36 amide], which was only of borderline significance ($p = 0.051$). There was a short increment in insulin (Fig. 3B) and C-peptide (Fig. 3C) plasma concentrations during the initial 30 min after GLP-1 [7–36 amide] administration (i.e. before the meal was given), but later insulin and C-peptide curves were similar to those with the placebo. Due to the initial peak in insulin and C-peptide (Fig. 3B and C), the overall integrated incremental insulin (21.6 ± 4.7 vs 15.9 ± 3.8 nmol · l^{-1} · min, $p = 0.0032$) and C-peptide (139.9 ± 20.6 vs 97.9 ± 20.1 nmol · l^{-1} · min, $p = 0.0054$) response was greater with s.c GLP-1 [7–36 amide].

The time course of GLP-1 [7–36 amide] plasma concentrations (Fig. 2C) after s.c injection was similar to that described in fasting patients (Fig. 2 A and B, Table 2). There was a significant increment in GLP-1 [7–36 amide] concentrations after the liquid meal

Table 2. Integrated incremental responses of GLP-1 [7–36 amide] plasma concentrations after s.c. injection (cf Fig. 2) into fasting NIDDM patients and calculated metabolic clearance rates

Study	Interval [min]	Integrated incremental GLP-1 [7–36 amide] [nmol · l^{-1} · min^{-1}]	Metabolic clearance rate [ml · kg^{-1} · min^{-1}]
Study A	0–240	39.5 ± 11.7	51 ± 7
Study B	0–120	25.6 ± 2.5	62 ± 5
	120–240[a]	25.4 ± 2.2	62 ± 5
Study C	–30–240	40.9 ± 5.0	41 ± 6[b]
ANOVA (p-value)		0.17	0.039

Data are mean ± SEM

[a] Integration was carried out over true baseline values (measured at –15 and 0 min); [b] significant difference (Student's t-test; $p < 0.05$) to both periods of study B

with placebo (ANOVA: $p = 0.0002$, from basal 7 ± 1 to 19 ± 3 pmol/l after 30 min, $p = 0.002$ by t-test, also significant vs basal at 45, 75, and 90 min).

Glucagon concentrations were elevated by the liquid meal containing amino acids (Fig. 3D) in the placebo study, but with s.c. GLP-1 [7–36 amide] there was a reduction of glucagon values to below fasting values before and shortly after the meal was instilled (Fig. 3D). Later, there was a delayed peak of glucagon concentrations (at 90 instead of 30 min). The total magnitude of the glucagon response (1347 ± 163 pmol · l^{-1} · min with GLP-1 [7–36 amide] vs 1428 ± 205 with placebo), however, was not different between GLP-1 [7–36 amide] and placebo administration ($p = 0.53$). Plasma GIP concentrations rose in response to the meal (not shown). GLP-1 [7–36 amide] administration delayed that increment by approximately 45–60 min, also without changing the overall response (3386 ± 661 vs 4075 ± 651 pmol · l^{-1} · min, $p = 0.19$).

Subcutaneous GLP-1 [7–36 amide] delayed gastric emptying by 30–45 min, with an initial complete cessation lasting 30 min ($p < 0.0001$). Thereafter, the time course of gastric emptying was parallel to the placebo studies with the s.c. administration of GLP-1 [7–36 amide] (Fig. 4). Emptying was near-complete after 150 and 180 min, respectively.

Side effects. In study A, one female patient experienced nausea and vertigo 20 min after the s.c. administration of GLP-1 [7–36 amide]. In study B, nausea and unproductive vomiting occurred with both the first and the second administration of GLP-1 [7–36 amide] in one male patient. Blood pressure and pulse did not change in a clinically relevant fashion during these episodes. In study C, no clinically relevant side-effects were noted; likewise the placebo caused no symptoms.

Pharmacokinetics of GLP-1 [7–36 amide]. Based on integrated incremental GLP-1 [7–36 amide] responses and the dose administered s.c., an apparent metabolic clearance rate of approximately 50 ml · kg^{-1} · min^{-1} could be calculated (Table 2).

Discussion

The present results show that, with large doses of s.c. GLP-1 [7–36 amide], elevated fasting plasma glucose concentrations in NIDDM patients can be normalized as in previous studies using continuous i.v. administrations of GLP-1 [7–36 amide] or [7–37] [7, 10], provided that plasma GLP-1 [7–36 amide] concentrations are elevated for a prolonged period of time. This was achieved by repeated s.c. injections of GLP-1 [7–36 amide] (Figs. 1 and 2), but it can be extrapolated that similar results can be obtained with continuous s.c. infusions of GLP-1 [7–36 amide] or with a preparation that has retarded absorption kinetics. Such a preparation should elevate plasma GLP-1 [7–36 amide] levels into the effective concentration range (~ 100 pmol/l according to previous studies [5–7, 10, 11]) for a minimum of 3–4 h [7, 10]. With such an agent, one could attempt the normalization of fasting glycaemia by an overnight administration. Normal fasting glucose concentrations are an important determinant of overall glycaemic control in NIDDM patients [23, 24].

It should be considered that not all the GLP-1-like material detected by current radioimmunoassay methods is biologically active GLP-1 [7–36 amide]. Recent studies have indicated the extent to which GLP-1 [7–36 amide] is subject to proteolytic attack by exopeptidases, giving rise to GLP-1 [9–36 amide], which is totally devoid of biological activity [25, 26]. This has also been shown during the present experiments (details not shown, see [27]), and is also suggested if one compares the time course of GLP-1 [7–36 amide] concentrations (Fig. 2) as measured by the C-terminal- (amidation-specific) antibody 89390 (see methods) with effects on insulin (Fig. 1B, C, F and G) and glucagon secretion (Fig. 1D and H). In any case, the duration of elevation of GLP-1 [7–36 amide] above 100 pmol/l (a concentration that in previous studies led to a constant stimulation of insulin and the suppression of glucagon secretion [7, 10]) was longer than the duration of effects on both insulin and glucagon. This is also evident when interpreting the time course of inhibition of gastric emptying by GLP-1 [7–36 amide]. Such an effect had been noted under the influence of continuous i.v. infusions of GLP-1 [7–36 amide] in normal subjects [28, 29] and in NIDDM patients [15, 28]. Furthermore, the inhibition of gastric emptying at plasma levels of 100 pmol/l was near-complete for a period of at least 2–4 h [15, 29]. In the present experiments (Fig. 4),

gastric emptying was completely stopped for approximately 30 min (i. e. until 60 min after the s. c. administration of GLP-1 [7–36 amide]), but proceeded at a normal rate (as with placebo administration) shortly thereafter, although GLP-1 [7–36 amide] plasma values remained elevated much longer (Fig. 2C). In interpreting the gastric emptying data (Fig. 4), the somewhat unphysiological composition of the liquid meal studied should be kept in mind.

In comparison to previous studies using s. c. GLP-1 [7–36 amide] in NIDDM patients, the dose administered was larger in the present examination (approximately fivefold in comparison to Gutniak et al. [12]). The effects, as expected, were larger and of longer duration. From the integrated increments in GLP-1 [7–36 amide] concentrations (Fig. 2) after s. c. administration (Table 2) and the dose administered (1.5 nmol/kg), the apparent metabolic clearance rate (approximately 50 ml · kg^{-1} · min^{-1}) can be calculated, in line with previous results in normal subjects [13]. Since, at plasma concentrations between 100 and 300 pmol/l, the metabolic clearance rate of GLP-1 [7–36 amide] infused i. v. was approximately 14 ml · kg^{-1} · min^{-1} [3–5], and because there is no reason to believe that the elimination of GLP-1 [7–36 amide] absorbed from s. c. depots should follow different kinetics compared to that of GLP-1 [7–36 amide] administered directly into the bloodstream, the higher metabolic clearance rate after s. c. administration (by approximately 3.5-fold) probably indicates a reduced bioavailability, which can be estimated to be in the order of 25–30 %. The small differences in integrated GLP-1 [7–36 amide] responses between experiments (Table 2) most likely reflect differences in the integration period.

Serious side-effects were not noted; however, nausea and vomiting occurred in 2 of 24 patients studied. In line with our previous study in normal subjects [13], the dose- and concentration range used in the present experiments probably comes close to the maximum to be used in future clinical trials. Hopefully with a slower release of GLP-1 [7–36 amide] from s. c. depots, peak values will be lower and side-effects should be less likely.

As in previous studies [6, 12], the fall in plasma GLP-1 [7–36 amide] or [7–37] concentrations after administration before a meal resulted in a rebound increment of plasma glucose (Fig. 3A), insulin (Fig. 3B) and C-peptide (Fig. 3C), when the stomach started to empty again (Fig. 4). Therefore, the reduction in glycaemia was seen only transiently, and the overall effect on the integrated incremental glucose concentrations was of borderline significance only ($p = 0.051$). This may be due to the fact that the absorption of meal components is only slightly postponed by the administration of GLP-1 [7–36 amide], as also suggested by the glucagon (Fig. 3D) and GIP responses, which remained similar in overall magnitude with the s. c. administration of GLP-1 [7–36 amide], although their time course was considerably different in comparison to placebo studies (Fig. 3D). If, in fact, glucose concentrations can be normalized in hyperglycaemic NIDDM patients, as has been shown with i. v. GLP-1 [7–36 amide] administrations [7, 10], the effects on gastric emptying of GLP-1 [7–36 amide] administered with meals may become the most predominant effect, since the actions on insulin and glucagon secretion are glucose-dependent [7, 10, 30] and will be of less magnitude at decreasing blood glucose levels. Along this line, the self-limited stimulation of insulin secretion during the i. v. administration of GLP-1 [7–36 amide] [7, 10] probably was, the consequence of the glucose-dependence of this effect. In study B, the second injection of GLP-1 [7–36 amide] was still able to stimulate insulin secretion, because normoglycaemia had not been reached with the first dose administered 120 min earlier. An alternative explanation is that the beta cells had been "primed" by the first injection, which may even potentiate secretory responses to a second stimulation, as has been shown in animal experiments [31].

Based on these considerations, one may speculate that better use can be made of GLP-1 [7–36 amide] in the fasting state than in association with meal ingestion, especially since it has not been completely clarified whether the deceleration of gastric emptying introduced by therapeutic plasma levels of GLP-1 [7–36 amide] can be considered beneficial [15]. Therefore, one potential therapeutic use of GLP-1 [7–36 amide] could be its overnight administration in order to normalize fasting glycaemia in the morning [23, 24], especially as the normalization of plasma glucose concentrations continued for at least another 4 h after stopping its administration [10], so that effects lasting longer than the period of administration may be anticipated with such a regimen.

It is of interest to compare the responses to repeated administrations of GLP-1 [7–36 amide], since homologous receptor desensitization has been observed when insulinoma cell lines (carrying GLP-1 receptors) were incubated with large concentrations of GLP-1 [7–37] [32]. Such effects were obvious within 10–40 min. In contrast, in the present experiments, the insulin secretory responses or glucagon suppression were no different when GLP-1 [7–36 amide] was administered a second time within 120 min (Fig. 1). This was the case although glucose concentrations had already fallen in response to the first GLP-1 [7–36 amide] administration (Fig. 1E). Based on the known glucose-dependency of insulinotropic and glucagonostatic actions of GLP-1 [7–36 amide] [2, 7, 8, 27], this reduction in glycaemia alone could have diminished the response to a second administration of GLP-1 [7–36 amide], which was not the case. Therefore, an important short-term tachyphylaxis cannot be deduced from the present study. Along

this line, GLP-1 [7–36 amide] administered s.c. 3 times daily before meals (dose as in [12]) did not lose its effectiveness over 2 weeks (Gutniak M, Efendic S, personal communication). In diabetic Zucker rats, no loss in effectivity was seen over at least 4 weeks [33]. However, more long-term studies should be performed in humans to clarify this point.

In conclusion, s.c. GLP-1 [7–36 amide] can normalize fasting glycaemia in NIDDM patients, when repeated doses are administered to maintain elevations in GLP-1 [7–36 amide] concentrations over 3–4 h. A retarded-absorption preparation would be preferable for clinical trials. There was no short-term tachyphylaxis of GLP-1 [7–36 amide] effects with repeated dosing. Administration before meals retarded gastric emptying for 30–60 min, with a delay and only a small overall reduction in glycaemic increments.

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