Glucagon-Like Peptide-1 Infusion Must Be Maintained for 24 h/day to Obtain Acceptable Glycemia in Type 2 Diabetic Patients Who Are Poorly Controlled on Sulphonylurea Treatment

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OBJECTIVE — To assess the efficacy and safety of glucagon-like peptide-1 (GLP-1) on the plasma glucose level when given as a continuous infusion for either 16 or 24 h per day to type 2 diabetic patients who were poorly controlled on sulfonylurea treatment.

RESEARCH DESIGN AND METHODS — This single-center, randomized, parallel, double-blind, placebo-controlled trial was conducted in 40 hospitalized patients who were randomized to receive infusions of either placebo or GLP-1 4 or 8 ng · kg^{-1} · min^{-1} for either 16 or 24 h per day for 7 days. At predetermined intervals, 24-h profiles of glucose, glucagon, and insulin were measured. Adverse events and clinical chemistry and hematology were recorded.

RESULTS — For all active treatment groups, the change in average glucose (area under the curve [AUC] for day 7 minus AUC for day 0 divided by 24 h) was statistically significantly different from placebo ($P \leq 0.001$). The GLP-1 8 ng · kg^{-1} · min^{-1} dose given for 24 h was more efficacious than any of the other doses ($P \leq 0.05$). Nocturnal and fasting plasma glucose levels at day 7 were greater in the 16-h groups compared with the 24-h groups ($P \leq 0.05$). Insulin AUC did not show any treatment effect for any of the treatment groups when change was assessed from day 0 to day 7. However, for the 16-h groups, the pattern of the insulin profiles changed; the insulin profiles were considerably higher during the initial 3–4 h after restart of the GLP-1 infusion on day 7, although there was a tendency for insulin levels to decrease during the afternoon and evening. Glucagon AUC decreased significantly for all active treatment groups compared with placebo. GLP-1 was generally well tolerated.

CONCLUSIONS — This study demonstrated that GLP-1 should be given continuously to obtain the most optimal glycemic control. Because of the short plasma half-life of native GLP-1, long-acting derivatives should be developed to make GLP-1 treatment clinically relevant.

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Glucagon-like peptide-1 (GLP-1)-amide (7-36) and GLP-1 (7-37) are proteins comprised of 30 and 31 amino acids, respectively, secreted from L-cells in the intestinal mucosa into the circulation after intake of mixed meals. GLP-1 is an insulinotropic and glucagonostatic hormone. Together with gastric inhibitory polypeptide, it contributes to postprandial insulin response (1,2). In short-term studies, it has been demonstrated that intravenous infusion of GLP-1 at doses of ≥4 ng · kg^{-1} · min^{-1} stimulates insulin secretion, decreases glucagon secretion, and normalizes plasma glucose in hyperglycemic type 2 diabetic patients. Likewise, gastric emptying is delayed, which leads to a slower absorption of nutrients. This should further contribute to a postprandial reduction in plasma glucose.

We have previously studied the effect of GLP-1 administered continuously for 7 days in type 2 diabetic patients (3). Doses of GLP-1 4 and 8 ng · kg^{-1} · min^{-1} were shown to be safe and very efficacious. The effect of GLP-1 on glucose metabolism was slightly better during the initial 10–12 h of infusion than during the rest of the 7-day treatment period. Therefore, we decided to investigate whether an infusion of GLP-1 for 16 h per day (from 8:00 A.M. to 12:00 A.M.) for 7 days would be superior to a continuous infusion of the same dosage for 24 h per day for 7 days. The former study investigated patients that responded relatively well to sulfonylurea (SU) treatment. In order to study whether GLP-1 would also be efficacious in diabetic patients with a more deteriorated metabolic control, we decided to investigate diabetic patients who were considered to be SU failures.

RESEARCH DESIGN AND METHODS

— This study was approved by the Institutional Review Board at the University of Miami School of Medicine. Written informed consent was obtained from all participants. The study consisted of 40 type 2 diabetic patients (aged 37–69 years) who were considered to be SU failures. SU failure was defined as a fasting plasma glucose (FPG) of ≥10 mmol/l while on the maximum recommended dose of SU for ≥1 month before enrollment. The patients' characteristics

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Abbreviations: AE, adverse events; ANOVA, analysis of variance; AUC, area under the curve; BG, blood glucose; DPP-IV, dipeptidyl peptidase IV; FPG, fasting plasma glucose; GLP-1, glucagon-like peptide-1; SU, sulfonylurea.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Patient characteristics at baseline*

Characteristics	Placebo group	GLP-1 4 ng · kg^{-1} · min^{-1} 16-h group	GLP-1 8 ng · kg^{-1} · min^{-1} 16-h group	GLP-1 4 ng · kg^{-1} · min^{-1} 24-h group	GLP-1 8 ng · kg^{-1} · min^{-1} 24-h group
n	8	8	8	8	8
Female/male (*n*)	1/7	2/6	5/3	3/5	4/4
Age range (years)	37–69	43–62	42–66	50–67	52–68
Weight range (kg)	61–99	67–110	68–108	62–99	75–117
Height range (cm)	163–183	157–180	147–180	148–178	155–183
HbA_{1c} (%)	9.8 ± 2.6	9.9 ± 1.9	9.5 ± 2.3	8.8 ± 2.2	10.1 ± 1.4
FPG (mmol/l)	12.7 ± 1.7	13.5 ± 2.5	14.2 ± 4.1	12.5 ± 3.7	15.9 ± 4.2

Data are means ± SD unless otherwise indicated.

are shown in Table 1. The patients had a history of type 2 diabetes for ≥1 year and had been treated with oral antidiabetic drugs for ≥6 months. After a 2-week SU washout period, the patients' FPG had to be ≤20 mmol/l to be randomized to treatment. All 40 patients (with all data available) were included in the analyses. Two patients were discontinued after 2 days (placebo) and 4 days (GLP-1 8 ng · kg^{-1} · min^{-1}) of treatment, respectively. In both instances, the patients were discontinued because of poor venous access.

Study design

The study was a single-center, randomized, placebo-controlled, double-blind, parallel trial. All eligible patients underwent a 2-week SU washout period as outpatients. Thereafter, they were admitted to the clinic for 10 days (from day −1 to day 8). During the initial 36 h, the patients were observed off medication. Thereafter, they were randomized to the placebo group or to one of four groups receiving GLP-1 for 7 days (from day 1 to day 7). Finally, they were observed off study medication for 24 h (day 8) before being discharged.

Study medication

A frozen solution (concentration 200 μg/ml) of sterile biosynthetic GLP-1 (Pfizer, Groton, CT) and a sterile frozen placebo (phosphate-buffered saline, pH = 7) were used. The study drug vial for the individual patient was removed from the freezer and allowed to equilibrate to room temperature. A 4-ml dose of human serum albumin (Albumin [Human] Albutein U.S.P., 25%, NDC 49669-5213-3; Kendall McGaw, Irvine, CA) was added to a 500-ml saline infusion bag (0.9% sodium chloride injection, U.S.P., NDC 0264-7800-10; Kendall McGaw). Thereafter, a quantity of GLP-1 or placebo was added. The needed quantity was calculated for each individual patient based on body weight, and the final preparation was administered to the patients at an infusion rate of 30 ml/h. The infusion bags were changed each day at 8:00 A.M. and 12:00 A.M. The patients who were randomized to receive study medication for 16 h per day received placebo during the night. An independent pharmacist who was not otherwise involved in the study performed the preparation of the study medication. To verify that the GLP-1 concentration was correct, a 2-ml sample from the infusion bag was collected, frozen, and later analyzed for GLP-1 content. Aseptic techniques were used at all steps of the preparation.

Diet regimen and schedule

During the entire study, the patients received a weight-maintaining diet in accordance with the American Diabetes Association's recommendations. A dietitian interviewed patients and planned their diet on an individual basis. During the inpatient period, the clinic prepared the meals and beverages. The patients were instructed to not consume anything other than the diet provided by the site. They were also instructed to consume all food and beverages given to them. Breakfast was served immediately after sampling for FPG, which was obtained at 7:30 A.M., and lunch and dinner were served immediately after glucose sampling at 1:00 P.M. and 6:00 P.M., respectively.

Experimental procedures

On day −1, the patients were admitted to the clinic at 8:00 P.M. and fasted overnight. On day 0, a screening FPG (glucose meter reading) was taken. If the value was between 10 and 20 mmol/l, the patient was permitted to continue in the study. Thereafter, the eligible patients were randomized. An intravenous catheter was inserted into one of the patient's forearms for blood sampling purposes only; it was kept patent with a heparin lock. At 7:30 A.M., the first of the blood samples for the 24-h glucose profile (24 points) and 13-point profiles for insulin and glucagon were obtained. On day 1, breakfast was served immediately after FPG, and at 8:00 A.M. an intravenous catheter was inserted into the forearm of the arm that was not being used for blood sampling and was attached to the infusion bag with the study medication via an infusion pump (AccuPro Pump; Kendall McGaw). Infusion continued for 7 entire days. Blood samples for the glucose profiles were obtained on days 1, 4, and 7 and, for the insulin and glucagon profiles, on day 7 (glucose profiles for days 1 and 4 are not displayed). On day 8, the GLP-1 infusion was stopped at 8:00 A.M. Blood glucose (BG) was analyzed at 20-min intervals until 9:00 A.M. The patients were discharged after lunch and resumed their diabetes treatment under the supervision of the clinic.

Laboratory procedures

Glucose. Blood was collected into tubes containing potassium oxalate and sodium fluoride, mixed immediately, and centrifuged at 2,000–2,300 rpm for 20 min. Plasma was transferred into a transport tube and refrigerated. A DuPont Dimension chemistry analyzer (Brookfield, CT) analyzed glucose using the glucose-hexokinase method.

Insulin. Blood was collected in a tube and allowed to clot for 20 min and was

Table 2—*24-h mean values and changes from baseline for glucose, insulin, and glucagons*

	Placebo group	GLP-1 4 ng · kg^{-1} · min^{-1} 16-h group	GLP-1 8 ng · kg^{-1} · min^{-1} 16-h group	GLP-1 4 ng · kg^{-1} · min^{-1} 24-h group	GLP-1 8 ng · kg^{-1} · min^{-1} 24-h group
Glucose (mmol/l)					
Baseline	15.3	15.2	14.8	17.2	18.3
Change (day 1)	−0.2	−3.2*	−4.4*	−4.7*	−6.6*†
Change (day 4)	+1.1	−3.4*	−3.7*	−4.6*	−10.9*†
Change (day 7)	+1.3	−3.9*	−4.7*	−5.2*	−7.7*†
Insulin (μU/ml)					
Baseline	37	54	49	36	30
Change	−3	+3	−5	−7	−7
Glucagon (pg/ml)					
Baseline	84	107	118	115	114
Change	+6	−7	−30‡	−24‡	−30‡

Compared with placebo, *$P < 0.001$; compared with other active treatments, †$P < 0.05$; compared with placebo, ‡$P < 0.05$.

then centrifuged at 2,000–2,300 rpm for 20 min. Serum was transferred to transport tubes and frozen immediately at −20°C. A radioimmunoassay method was used for the analyses of insulin.

Glucagon. Blood was collected into tubes containing EDTA and aprotinin, centrifuged at 2,000–2,300 rpm for 20 min, and then immediately frozen at −20°C. Glucagon was analyzed using a glucagon-chemiluminescent antibody-PDC immulite technique. All analyses were performed at Medlab, San Antonio, Texas.

Statistical analysis

The primary efficacy assessment was the 24-h plasma glucose profile measured on days 1, 4, and 7. The secondary efficacy assessments were the 24-h insulin and glucagon profiles measured on day 7. From the 24-h profiles, the average area under the curve (AUC) was calculated. The change in average glucose AUC from baseline (day 0) to days 1, 4, and 7 was subjected to an analysis of variance (ANOVA), with treatment as the fixed effect. Contrasts addressed the difference among the five treatment groups. A separate ANOVA was applied for days 1, 4, and 7. Similar methods were applied to the change in average insulin and glucagon AUCs from baseline to day 7. Furthermore, FPG and nocturnal plasma glucose (measured at 4:00 A.M.) on day 7 were analyzed as described above. For all analyses, $P = 0.05$.

RESULTS

Plasma glucose

The highest mean changes in plasma glucose occurred on day 7 for all groups. All of the active treatment groups had a statistically significantly larger mean decrease in the AUC from baseline to day 7 compared with placebo ($P \leq 0.001$). For AUC in the GLP-1 8 ng · kg^{-1} · min^{-1} 24-h group, the average decrease from baseline to day 7 was statistically significant compared with the other three active treatment groups ($P < 0.05$) (Table 2).

The 24-h glucose profiles on day 7 are shown in Fig. 1. Baseline profiles are compared with day 7 profiles in the left column of Fig. 2. When GLP-1 was administered in the 16-h groups, FPG and nocturnal plasma glucose (4:00 A.M.) were statistically significantly higher compared with the 24-h groups on day 7 ($P < 0.05$), with the exception of FPG in the GLP-1 8 ng · kg^{-1} · min^{-1} 24-h group compared with the GLP-1 4 ng · kg^{-1} · min^{-1} 16-h groups ($P < 0.1$). Thus, during the night, the glucose values increased between 11:00 P.M. and 4:00 A.M. in the 16-h groups, whereas the plasma glucose values further decreased during the night, reaching a nadir at 4:00 A.M., in the two 24-h groups.

Serum insulin

Analyses of the change from baseline to day 7 in the mean plasma insulin values (AUC) indicated that there was no statistically significant treatment effect on plasma insulin (Table 2). The plasma insulin profiles on day 0 and day 7 are displayed for all groups in the center column of Fig. 2. However, for the two 16-h groups, the pattern of the profiles changed from day 0 to day 7. In both groups, the insulin concentrations were considerably higher during the initial 3–4 h after the restart of GLP-1 infusion on day 7, whereas there was a tendency for insulin levels to decrease during the afternoon and evening. In the 24-h groups, there were no changes in the pattern of insulin profiles.

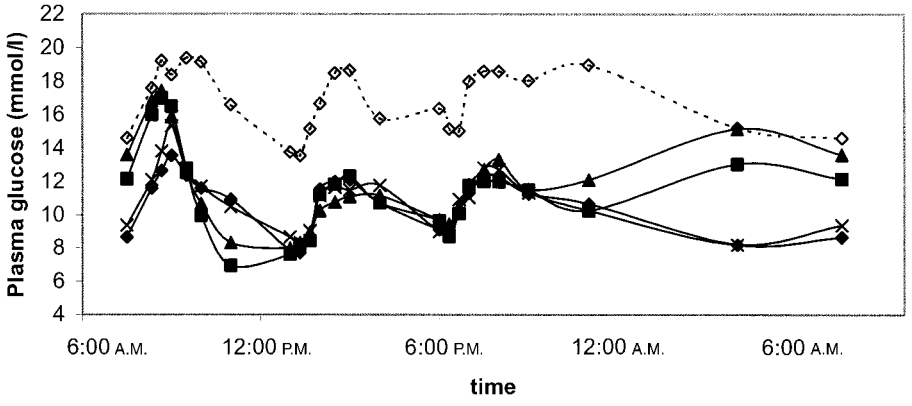

Figure 1—*24-h plasma glucose profiles for the five groups on day 7. - -◇- -, placebo group; —■—, GLP-1 4 ng · kg^{-1} · min^{-1} 16-h group; —▲—, GLP-1 8 ng · kg^{-1} · min^{-1} 16-h group; —◆—, GLP-1 4 ng · kg^{-1} · min^{-1} 24-h group; —×—, GLP-1 8 ng · kg^{-1} · min^{-1} 24-h group.*

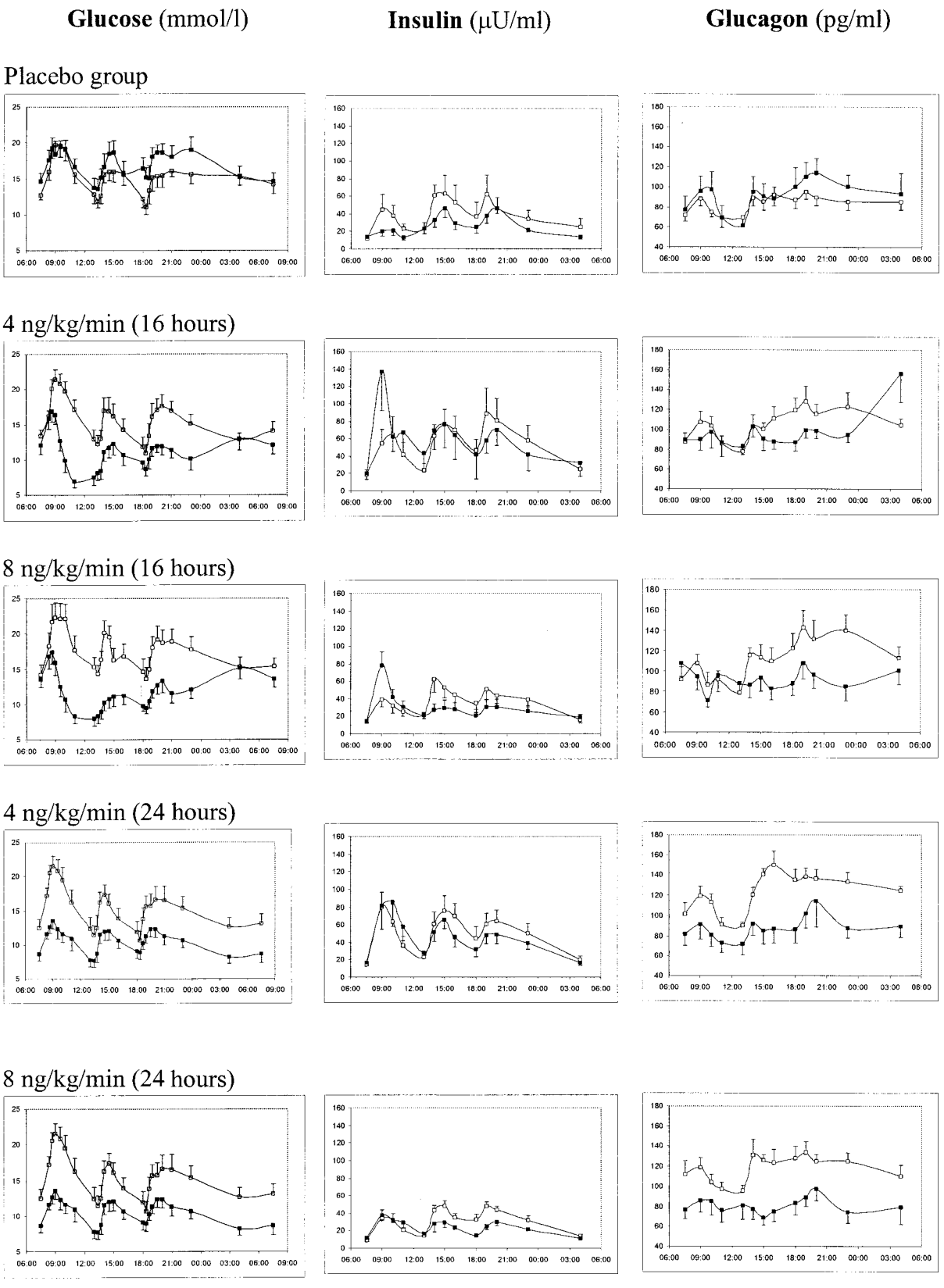

Figure 2—*The left column shows the 24-h plasma glucose profiles on day 0 (□) and on day 7 (■) for the placebo group and the four active treatment regimens. The curves in the center column represent the corresponding insulin profiles and the curves in the right column represent the glucagon profiles. The bars are SEM.*

Table 3—*Overall AE with a rate of occurrence in two or more patients*

	Placebo group	GLP-1 4 ng · kg^{-1} · min^{-1} 16-h group	GLP-1 8 ng · kg^{-1} · min^{-1} 16-h group	GLP-1 4 ng · kg^{-1} · min^{-1} 24-h group	GLP-1 8 ng · kg^{-1} · min^{-1} 24-h group
Patients with at least one event	6	5	7	4	7
Total number of unique events	14	14	21	7	29
Cellulitis	2	0	0	1	2
Fever	1	1	0	0	2
Chills	0	1	0	0	1
Headache	3	2	2	2	3
Injection site reaction	2	0	0	0	2
Abdominal pain	2	2	2	0	1
Back pain	0	0	1	0	3
Diarrhea	0	0	1	0	1
Dyspepsia	0	0	3	2	2
Nausea	1	2	4	0	4
Vomiting	1	2	3	1	3
Dizziness	0	1	3	0	1
Tremor	0	1	0	0	1

Data are *n*.

Plasma glucagon

Analyses of the change from baseline to day 7 showed that, except for the GLP-1 4 ng · kg^{-1} · min^{-1} 16-h group, there was a statistically significantly larger mean decrease ($P < 0.05$) in the glucagon AUC in the active treatment groups compared with the placebo group. All four active treatment groups showed a mean decrease in glucagon, whereas patients in the placebo group indicated a mean increase (Table 2). In the two 16-h groups, the day 7 profiles were almost overlapping with the day 0 profiles until the afternoon, when day 7 profiles decreased until the active infusion was replaced with placebo at midnight (Fig. 2, right column).

Safety

The adverse events (AEs), which occurred at a rate of two or more, are displayed in Table 3. Patients in the two high-dose groups (GLP-1 8 ng · kg^{-1} · min^{-1} for 16 and 24 h) reported the highest number of AEs (21 and 29 events, respectively), whereas patients in the two low-dose groups (GLP-1 4 ng · kg^{-1} · min^{-1} for 16 and 24 h) reported 14 and 7 events, respectively, which was similar to the AEs reported by the placebo group (14 events). The most frequently reported AEs were headache, nausea, vomiting, and dyspepsia. With the exception of a slightly higher incidence of nausea and vomiting, no clinically significant dose or length-of-infusion trends were observed in the incidence of any of the individual AEs reported. The majority of AEs were mild to moderate in intensity. Three patients experienced AEs that were considered to be severe (headache in a placebo patient, vomiting in a patient in the GLP-1 4 ng · kg^{-1} · min^{-1} 16-h group, and diarrhea in a patient in the GLP-1 8 ng · kg^{-1} · min^{-1} 16-h group. In all cases, the severe AEs resolved while the patients were receiving a study drug. No clinically significant changes from baseline were observed in laboratory parameters, vital signs, physical examination, or electrocardiogram.

CONCLUSIONS — It has previously been demonstrated that it is possible to normalize or improve the glycemic control in type 2 diabetic patients by both intravenous and subcutaneous infusion of GLP-1 at doses of ~4 ng · kg^{-1} · min^{-1} or higher (4–11). However, these studies have ranged from 4 to 6 h in duration for either fasting patients or patients receiving a single meal. In a recent study (6), it was shown that a continuous 48-h subcutaneous infusion of GLP-1 at a rate of ~4–8 ng · kg^{-1} · min^{-1} lowered fasting and postprandial glucose values in type 2 diabetic patients. Our study showed that a continuous infusion of GLP-1 instantaneously resulted in a marked and sustained improvement in the glycemic control of type 2 diabetic patients who were considered to be SU failures. When GLP-1 infusion was discontinued, BG levels increased and FPG returned to baseline before breakfast the following day. Likewise, most of the decrease in plasma glucose occurred in the actively treated groups from day 0 to day 1 and was maintained throughout the study.

The plasma insulin AUCs were unchanged from baseline to day 7. However, in the two 16-h groups, insulin levels increased considerably in the first few hours after the restart of GLP-1 infusion, followed by a decreased secretion later in the day. This corresponds well with the high FPG and the postbreakfast glucose values in these groups. Thus, GLP-1 may not increase the total daily secretion of insulin, but it may facilitate a more appropriate secretion pattern. The increased morning insulin levels were not seen in the two 24-h groups, presumably because of the much lower glucose values. If we had measured the insulin levels when infusion was initiated on day 1, we would probably have observed the same insulin secretion pattern seen in the 16-h groups. In previous studies (4,10,11), it has been shown that insulin levels return to baseline when glucose levels are normalized or at least lowered significantly. This is in agreement with the glucose-dependent properties of GLP-1 (1,5,8,9,11,12).

It is interesting that the glucagon-lowering effect of GLP-1 was not observed in our study until several hours after restart of the active infusion in the 16-h groups, as seen in several acute studies in which glucagon was lowered almost in-

stantly. However, during longer-term treatment, glucagon lowering contributes to an improved glycemic control, an effect that was also seen in this study. Other studies have shown that glucagon suppression occurs almost instantly (4,5). In the 24-h groups, glucagon secretion was suppressed throughout the day.

In conclusion, intravenous infusion of GLP-1 led to a major sustained improvement in the glycemic control of type 2 diabetic patients who were considered to be SU failures. Future longer-term studies will reveal if it is possible to bring patients into normoglycemia. To maintain nocturnal control, GLP-1 should be continuously present. Thus, continuous treatment appears to be superior to interrupted regimens.

For reasons of practicality, intravenous infusion is not relevant in long-term treatment. Therefore, sustained release formulations, dipeptidyl peptidase IV (DPP-IV)–resistant analoges, or oral mimetic compounds for either subcutaneous injection or oral administration would be appropriate should GLP-1 ever become a relevant treatment for type 2 diabetes. Simple DPP-IV–resistant analoges have been shown to have minimally extended half-lives because they are rapidly cleared by renal excretion (13). Thus, they would have to be administered three to four times daily. Another option would be to develop long-acting GLP-1 analoges with sufficiently long terminal half-lives to secure glycemic control for 24 h.

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