Glucagon-Like Peptide-1 Has No Insulin-Like Effects in Insulin-Dependent Diabetic Dogs Maintained Normoglycemic and Normoinsulinemic

E.-J. Freyse, S. Knospe, T. Becher, O. El Hag, B. Göke, and U. Fischer

A pharmacological concentration of glucagon-like peptide-1 (GLP-1) in the insulin-deficient state clearly decreases the blood glucose level. Therefore, this study was designed to evaluate a putatively relevant effect of the gastrointestinal peptide as an adjuvant to insulin replacement therapy. GLP-1 (GLP-1(7-36) amide 10 pmol · kg^{-1} · min^{-1}) was infused intravenously over 8 hours in nine fasting, C-peptide–negative diabetic dogs. The animals were under normoglycemic control by glucose-controlled insulin infusion (GCII) during the night before and during GLP-1 administration. During the paired control tests, the animals received saline infusion instead of GLP-1. In addition to the insulin infusion rates required to maintain normoglycemia, hormones, metabolites, and the turnover rates for glucose (6-^{3}H-glucose), alanine (U-^{14}C-alanine), and urea ($^{15}N_2$-urea) were measured during the final 2 hours of GLP-1 administration. Circulating plasma GLP-1 levels increased from 3 ± 1 to 17 ± 7 pmol/L. There was no significant difference in the insulin infusion rate between the experimental and control groups (0.43 ± 0.05 *v* 0.40 ± 0.05 mU · kg^{-1} · h^{-1}, average over the entire interval). Glycemia was maintained at a practically identical level (4.9 ± 0.3 *v* 4.8 ± 0.4 mmol/L). Also, the concentration of plasma insulin—which was not hyperinsulinemic—and pancreatic glucagon remained unaltered. We found no appreciable effect of GLP-1 on glucose production and metabolic clearance, alanine turnover and the formation of glucose from alanine (1.8 ± 0.2 *v* 1.4 ± 0.2 μmol · kg^{-1} · min^{-1}), or the urea production rate as a measure of overall amino acid catabolism (4.1 ± 0.4 *v* 4.1 ± 0.4 μmol · kg^{-1} · min^{-1}). Thus, no conclusive adjuvant effect of GLP-1 was ascertained in insulin-treated diabetic dogs under normoglycemic control.

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) has been proposed as a tool for glycemic control in diabetes mellitus (DM) type 2,[1-4] mainly on the basis of its insulin-stimulating and glucagon-inhibiting potency. In DM type 1, contradictory findings were reported. On one hand, increased minimal model–based glucose effectiveness[5] and decreased meal-related insulin requirement in isoglycemic clamps[6] were shown at elevated GLP-1 concentrations. However, for insulin sensitivity, no significant effect of GLP-1 could be ascertained during a euglycemic-hyperinsulinemic clamp.[7] In insulin-dependent diabetic dogs, we were able to show that exogenous GLP-1 is effective to reduce endogenous glucose production and thereby fasting hyperglycemia in the insulin-deficient diabetic state.[8]

Therefore, a study was undertaken in dogs with experimental DM type 1 to search for potential additive therapeutic effects of GLP-1 under near-normoglycemic, normoinsulinemic conditions maintained by a glucose-controlled insulin infusion (GCII). When GLP-1 was used at dosages and over intervals that, according to the literature, are expected to be effective,[3,7,8] no influence was found either on the insulin dose required to maintain normoglycemia or on the glucagon concentration and tracer-determined metabolic rates of glucose, alanine, and urea. The findings reported here do not point to additive insulin-like effects of GLP-1 under basal insulin concentrations in DM type 1.

From the Diabetes Institute "Gerhardt Katsch," Karlsburg; and the Clinical Research Unit for Gastrointestinal Endocrinology, Philipps-University Marburg, Germany.

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Address reprint requests to E.-J. Freyse, MD, Institut für Diabetes "Gerhardt Katsch" Karlsburg e.V., Greifswalder Str. 11e, D-17495 Karlsburg, Germany.

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MATERIALS AND METHODS

Animals

Nine insulin-dependent diabetic ASDI-strain (Versuchstierzucht Groß, Börnecke, Germany) dogs of either sex were used (age, 34 ± 5 months). The duration of diabetes was 12 ± 3 months, and the body weight was 24 ± 1 kg. The aim and design of the study were approved by the Animal Ethics Committee of the Country Mecklenburg-Vorpommern, Germany.

Diabetes was induced by means of subtotal pancreatectomy (approximately 90% of estimated organ weight) in combination with intrapancreaticoarterial infusion of 2 mg/kg streptozotocin.[9] Once diabetes was established, usually after 3 weeks or less, the animals were canine C-peptide–negative, and steatorrhea did not occur. Daily metabolic control was maintained by means of three subcutaneous injections of regular insulin (Actrapid HM 40 IU/ml; Novo Nordisk Pharma, Mainz, Germany; mean dose, 1.6 ± 0.2 IU · kg^{-1} · d^{-1}) and two pelleted meals (138 g carbohydrate, 79 g protein, and 10 g fat per meal, Noblesse; Paragon Petcare, Nettetal-Leuth, Germany) adjusted to avoid ketonuria and to maintain stable body weight, with glycemic excursions between 4 and 12 mmol/L.

Protocol

Each diabetic animal underwent two experiments in random order 2 weeks apart (GLP-1 *v* control). On the day before the experiment, the last meal was provided at 2 PM and the last subcutaneous insulin dose at 4 PM. The white blood cell count was 9.5 ± 0.8 × 10^9 · L^{-1} and hematocrit 42 ± 1%.

At 7 PM the evening before the experiment, the animals were taken to the laboratory and kept freestanding in a Pavlov harness. Cannulas were inserted into one cephalic vein for glucose monitoring and into one saphenous vein for insulin administration. Normoglycemia was restored and maintained during the night by GCII on the basis of plasma glucose determinations at intervals of 10 to 20 minutes and subsequent adjustment of the insulin dose infused by a high-precision syringe pump (Perfusor E; Braun, Melsungen, Germany). At 5 AM the following morning, the animals were allowed a 15-minute walk outside the laboratory to void. Then, a central venous catheter was inserted for additional blood sampling. GLP-1 (7-36) amide (Saxon Biochemicals, Hannover, Germany; 10 pmol · kg^{-1} · min^{-1} in a bovine serum albumin–primed solution containing an appropriate concentration to allow for the infusion rate of 6.25 mL/h) or saline (6.25 mL/h) were infused over 8

hours by a Perfusor secura FT pump connected to the established infusion line.

In addition, primed constant tracer infusions were applied from 10 AM until the end of the experiment. $^{15}N_2$-urea (99% enrichment; Cambridge Isotope Laboratories, Cambridge, MA), D-6-^{3}H-glucose (specific activity, 32 mCi/mmol; Amersham, Bucks, UK), and 1-U-^{14}C-alanine (specific activity, 154 mCi/mmol; Amersham) were used at doses previously described.[8]

During GLP-1 infusion, plasma glucose was determined at intervals of 10 minutes throughout, specific radioactivity and isotopic enrichment were estimated between 12 noon and 2 PM at intervals of 15 minutes, and hormones and metabolites were analyzed at the beginning and after 360 and 480 minutes of GLP-1 infusion. All infusates were prepared and applied as described previously.[8]

Analyses and Calculations

Plasma concentrations of GLP-1 were estimated after Sep Pak KC 18 extraction of plasma (Waters, Milford, MA) by a competitive radioimmunoassay using ^{125}I-labeled GLP-1 (7-36) amide (specific activity, 74 Tbq/mmol[10,11]) and the specific antibody GA 1178 (Affinity Research, Nottingham, UK). The antibody was 100% reactive both with GLP-1 (1-36) amide and with truncated GLP-1 (7-36) amide. There was no cross-reactivity with gastric inhibitory peptide, pancreatic glucagon, glicentin, oxyntomodulin, or GLP-2.[8,10,12] Assay sensitivity was 2 fmol/tube, and the intraassay and interassay coefficients of variation were 3.4% and 5.2%, respectively.

All other analytical methods used have been detailed previously as follows: plasma glucose and urea with Beckman Instruments (Fullerton, CA) analyzers, glucose and alanine concentration and specific radioactivity from chromatographed plasma extracts,[13,14] and ^{15}N-isotope enrichment by means of SSQ 710 mass spectrometer (Finnigan MAT, San Jose, CA).[8]

Applying established formulas, glucose and alanine flux rates were calculated from their specific activity,[15] $^{15}N_2$-urea mole percent enrichment (MPE) was calculated from the ion intensity of the singly charged fragments *m/e* 189, 190, and 191, and on this basis, urea production rates were calculated considering the amount of urea infused.[16]

The percent glucose formed from alanine was assessed from the specific ^{14}C activity of plasma glucose and alanine, considering the metabolic exchange of carbon among the different gluconeogenetic pathways.[17] These data were used to calculate the rate of glucose production from alanine using the simultaneously measured glucose flux rate.

The mean ± SEM are presented for tracer kinetic estimates during the final six 15-minute intervals according to protocol when steady-state levels of specific activity and MPE were established, as shown previously.[8] Student's paired *t* test was used to assess statistical significance at a *P* level less than .05. The SPSS/PC+ program version 3.1 (SPSS, Chicago, IL) was used for statistical evaluation.

RESULTS

At the beginning of GCII, all animals were slightly hyperglycemic, but normoglycemia was generally restored by 2 AM on the experimental day (Fig 1). There was no significant difference in glycemia during the further course of the two experiments or between GLP-1 and control experiments (Fig 1 and Table 1). Also, no appreciable difference could be ascertained in analyzing the insulin amount used during the two investigations (Fig 2). Plasma GLP-1 increased due to infusion of this hormone from 3 ± 1 at time 0 to steady-state levels of 17 ± 7 pmol/L (360 minutes) and 15 ± 7 pmol/L (480 minutes, $P < .05$ *v* 0 minutes), respectively. However, in the control experiments, GLP-1 concentrations remained unaltered: 3 ± 1, 2 ± 1, and 2 ± 1 pmol/L at the respective times. Also, plasma concentrations of insulin and pancreatic glucagon did not show any difference either during GLP-1 administration or between the test and control groups (Fig 2).

The overall rates of endogenous glucose production and

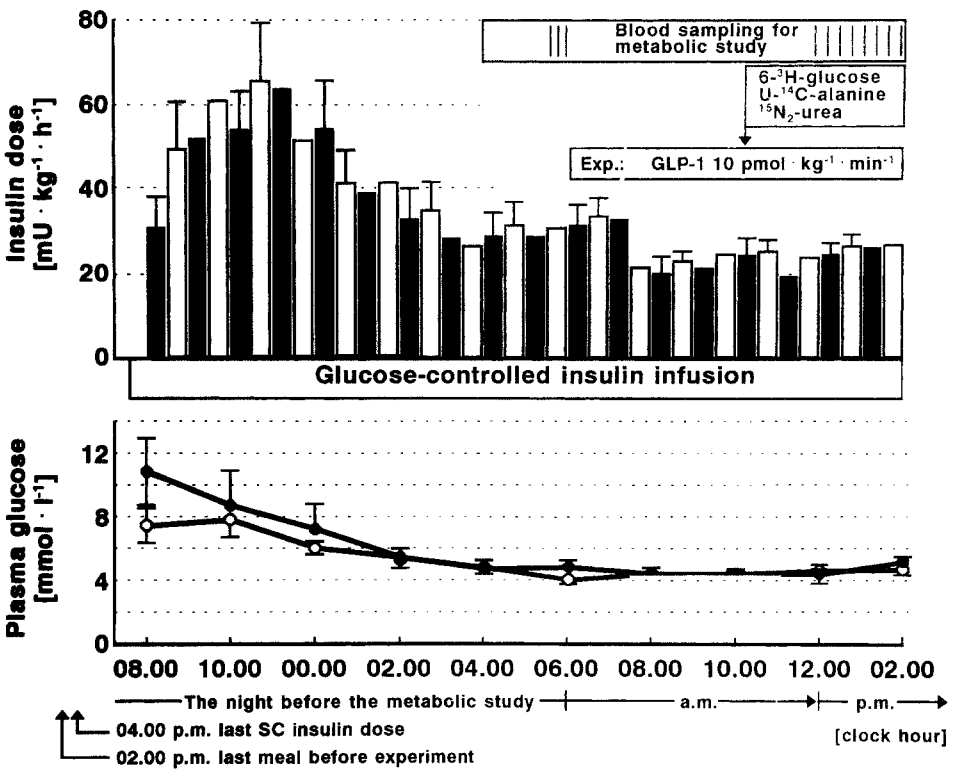

Fig 1. Glycemia and insulin dose pattern in glucose-controlled insulin-infused diabetic dogs, paired metabolic studies, with infusion of GLP-1(7-36)amide (■, ●) or saline (□, ○). Time schedules for blood sampling and primed continuous tracer infusion are shown. Data are means ± SEM.

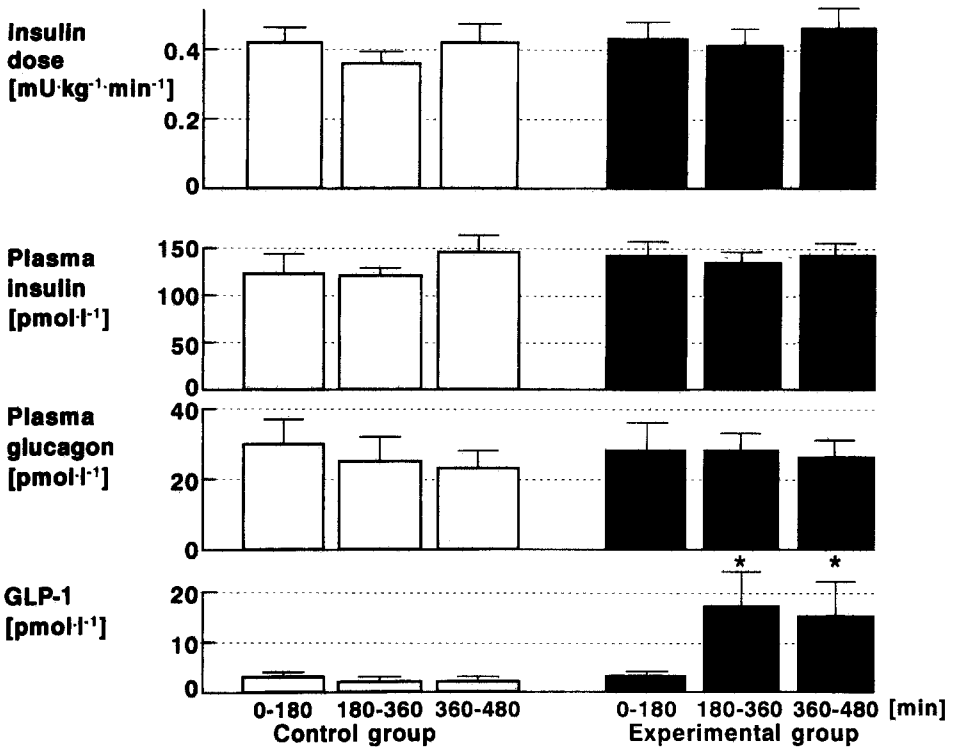

Fig 2. Insulin infusion rate at defined time intervals during 8-hour infusion of GLP-1 (test group) or saline (control group) and steady-state concentration of plasma insulin, glucagon, and GLP-1. *$P < .05$ *v* 0 minutes.

peripheral glucose utilization (metabolic clearance rates) were practically identical in GLP-1 and control tests. Furthermore, no significant difference was found in alanine metabolism (plasma concentration, flux rate, or gluconeogenesis from alanine). This corresponds well to the lack of a significant difference in the plasma concentration and production rate of urea (Table 1).

Table 1. Glucose, Alanine, and Urea Metabolism in Insulin-Dependent Diabetic Dogs Maintained Normoglycemic by GCII

Parameter	Control Group (saline)	Experimental Group (GLP-1)
Glucose		
Plasma level (mmol/L)	4.8 ± 0.4	5.2 ± 0.5
Endogenous Ra ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	20.0 ± 1.0	18.3 ± 4.0
MCR ($mL \cdot kg^{-1} \cdot min^{-1}$)	3.9 ± 0.4	3.4 ± 0.5
Alanine		
Plasma level (mmol/L)	0.38 ± 0.08	0.34 ± 0.06
Flux rate ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	10.1 ± 1.9	9.8 ± 2.6
Glucose production from alanine ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	1.4 ± 0.2	1.8 ± 0.2
Urea		
Plasma level (mmol/L)	1.7 ± 0.3	1.3 ± 0.2
Production rate ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	4.1 ± 0.1	4.1 ± 0.4

NOTE. Data are the mean ± SEM of individual averages during 6 15-minute intervals between 390 and 480 minutes of GLP-1 (7-36) amide infusion, which was 20 and 22 hours after the last subcutaneous dose of regular insulin. No significant difference for all variables.

Abbreviations: Ra, rate of appearance; MCR, metabolic clearance rate.

DISCUSSION

GLP-1 has been proposed as a potentially therapeutic alternative in the treatment of DM type 2.[18] This proposal was deduced from both the insulinogenic and glucagon-lowering effects of GLP-1 as predominantly shown under in vitro conditions,[2] in healthy man,[1] in insulin-dependent diabetic man with significant C-peptide response,[19] or in non–insulin-dependent diabetic man.[3] Also, an insulin-like action of GLP-1 has been suggested by some investigators[5,6,20,21] but could not be verified by others.[7,22-24]

In a previous study, we were able to demonstrate a blood glucose-lowering effect of GLP-1 in hyperglycemic insulin-deficient hyperglucagonemic diabetic dogs. This effect was caused by a reduction in hepatic glucose production but not by stimulation of peripheral glucose utilization, and it was paralleled by a weak reduction in plasma pancreatic glucagon levels. However, in the experimental design chosen, the circulating "pancreatic" glucagon was mostly of gastric origin.[25] Also, because of a lack of sufficiently functioning β cells,[8,9] no appreciable insulinogenic effect of GLP-1 was taken into consideration.

The latter two conditions also apply to the present experiments. We used an established insulin-dependent diabetic animal model maintaining both normoglycemia upon adequate insulinization and near-normoglucagonemia. We looked for potential insulin-like effects of GLP-1 in controlling glycemia. The dose of GLP-1 administered and its consecutive plasma concentrations measured accordingly were in the low-pharmacological range, and can basically be expected to be effective. Also, under in vivo conditions, the 8-hour duration of GLP-1

infusion has reportedly been efficient to influence blood glucose control.[3,8]

It has been assumed that a reduction in plasma glucagon may be responsible for the blood glucose-lowering effect of GLP-1 in human type I diabetic patients exhibiting some residual β-cell activity.[19] However, in this study, no such decline could be ascertained in the measured near-normal plasma glucagon levels. This observation is not necessarily specific to the dog model used, taking into consideration that both pancreatic[9] and gastric[25] α cells appear to respond sensitively to administration of insulin[13,14,17] or GLP-1 in the absence of insulin.[8]

Considering these circumstances, the lack of influence of GLP-1 on the insulin dose required to maintain normoglycemia or the rate of glucose production and utilization, of the alanine flux and its contribution to gluconeogenesis, or of amino acid catabolism (ie, urea production) unequivocally shows that there is no therapeutically relevant insulin-like effect of GLP-1 in DM type I. It is therefore concluded that the claimed potential adjuvant role of GLP-1 in diabetes therapy may only become apparent in the presence of insulin-deficient hyperglucagonemia and/or functioning β cells.

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