Blood Glucose Lowering and Glucagonostatic Effects of Glucagon-Like Peptide I in Insulin-Deprived Diabetic Dogs

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To establish potential effects of glucagon-like peptide I (GLP-I) on blood glucose control in insulin-deficient states, GLP-I [GLP-I(7-36) amide; 10 pmol · kg^{-1} · min^{-1}] was infused intravenously in six fasting, canine C-peptide–negative, chronically diabetic dogs for 8 h. Blood samples were saved for the analysis of hormones, metabolites, and turnover rates of glucose (6-^{3}H-glucose), alanine (U-^{14}C-alanine), and urea ($^{15}N_2$-urea) starting 22 h after the last subcutaneous dose of exogenous insulin. Circulating plasma GLP-I levels rose under infusion from 2.9 ± 0.8 to 41.4 ± 10.1 pmol/l. This was efficient to significantly reduce the preexisting diabetic hyperglucagonemia. Since in the utilized model functioning pancreatic β-cells are lacking, GLP-I had no insulinogenic effect. Compared with control experiments in the same animals receiving saline infusion, glycemia dropped from 20.8 ± 1.9 to 16.2 ± 1.0 mmol/l ($P < 0.05$). This was in parallel to the infusion of GLP-I and was most likely caused by a decrease of elevated glucose production since overall glucose turnover decreased with no alteration in glucose metabolic clearance. Alanine turnover was significantly reduced, obviously reflecting a decline in alanine production in relation to changed muscle glucose uptake under conditions of lower glycemia and overall glucose turnover. There was, however, neither an effect of GLP-I on alanine conversion into circulating glucose nor an effect on urea production rate, indicating unchanged gluconeogenesis from amino acid precursors. We conclude that the blood glucose–lowering effect of GLP-I in an animal model of insulinopenia was shown to be due to a reduction in hepatic glucose output, possibly secondary to reduction in glucagon concentrations leading to decreased glycogenolysis. Whether GLP-I might be therapeutically useful in clinical insulin-deficient diabetes needs to be verified. *Diabetes* 46:824–828, 1997

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GLP-I, glucagon-like peptide I; MPE, Mol percentage enrichment.

After meal ingestion, glucagon-like peptide I (GLP-I) is released into the portal circulation from the mucosa of the lower small bowel and, putatively, from the colon by various food constituents including glucose (1,2). GLP-I has been described as acting as a physiological postprandial secretagogue (3–5). In addition, it may reduce postmeal hyperglycemia due to some pharmacological effects such as delayed gastric emptying (6) and reduction of pancreatic glucagon concentrations (7). Accordingly, it has been reported that GLP-I is able to reduce the meal-related insulin requirement of type I diabetic patients (3,8). On the basis of these findings, GLP-I(7-36) amide was suggested as a tool for glycemic control in non-insulin-dependent diabetic patients (9,10).

It is, however, still an unsettled issue whether direct metabolic effects of GLP-I occur as such. In normal humans, an increase of minimal model–based glucose effectiveness has been shown after intravenous infusion of GLP-I (11). A recent study provided evidence that GLP-I released due to meals promotes glucose disappearance by various mechanisms augmenting insulin secretion, stimulating insulin-independent glucose uptake, and/or enhancing the suppression of hepatic glucose output (12). Also, stimulation of glycogen synthesis was observed in isolated rat liver (13) and skeletal muscle (14). Other investigators could not establish a direct effect of GLP-I on glucose metabolism in those tissues (15–17). Therefore, this study was designed to clarify whether GLP-I can exert metabolic effects on its own. This was done in an experimental insulin-dependent diabetes model under a situation of diabetes-related hyperglucagonemia but independent from the availability of exogenous insulin. Previous studies in insulin-dependent diabetic dogs maintained normoglycemic have shown that the turnover of alanine (18) and the catabolism of amino acids as indicated by urea production rate (19) may serve as sensitive indicators of effects of an altered insulin-to-glucagon ratio. Therefore, in addition to glucose flux rates, these variables were studied to evaluate potential metabolic effects of GLP-I. This is the first report on the efficiency of GLP-I in blood glucose control of insulin-deprived chronic diabetic dogs.

RESEARCH DESIGN AND METHODS

Animals. Six insulin-dependent diabetic ASDI strain (Versuchstierzucht Groß Börnecke, Germany) dogs of either sex were used. Their mean age was 44 ± 3 months, the duration of diabetes was 27 ± 5 months, and the body weight was 24 ± 1 kg. For comparison, selected variables, including pancreatic glucagon values, were measured in six healthy control dogs of the same strain

(25 ± 2 months, 24 ± 2 kg). These nondiabetic dogs fasted the same length of time as the diabetic dogs did and were also infused with saline (see below). The goal and the design of the study was approved by the Animal Ethics Committee of the Country Mecklenburg-Vorpommern, Germany.

Diabetes was induced by means of subtotal pancreatectomy (~90% of estimated organ weight) in combination with intra-pancreaticoarterial infusion of 2 mg/kg streptozotocin (20). The animal model was proven canine C-peptide negative. The stool showed no signs of steatorrhea. Daily metabolic control was achieved by means of three subcutaneous injections of regular insulin (Actrapid HM, 40 IU/ml, Novo Nordisk Pharma, Mainz, Germany; average dose, 1.4 ± 0.4 IU · kg^{-1} · d^{-1}) and of two pelleted meals (Noblesse, Paragon Petcare, Nettetal-Leuth, Germany), adjusted to avoid ketonuria and to maintain stable body weight and glycemic excursions ranging between 4 and 12 mmol/l.

Protocol. Each diabetic animal underwent two experiments (control vs. GLP-I). On the day before the experiment, the last pre-experimental meal was given at 2:00 P.M., and the last subcutaneous insulin dose was applied at 4:00 P.M. White blood cell count was 7.9 ± 0.4 × 10^9 · l^{-1} and hematocrit was 42 ± 2%. On the experimental day, the animals came to the laboratory at 4:00 A.M. They were free-standing in a Pavlov harness and were acutely instrumented with cannulas in the iliac vein for all infusions, in the upper caval vein for blood sampling to determine specific activities and enrichments, and in the cephalic vein for online blood glucose monitoring. GLP-I(7-36) amide was infused over 8 h starting at 6:00 A.M., i.e., 14 h after the last subcutaneous insulin dose. Additionally, from 10:00 A.M. to 2:00 P.M., primed constant infusions of $^{15}N_2$-urea (99% enrichment; Cambridge Isotope Labs, MA), D-6-^{3}H-glucose (specific activity 32 mCi/mmol; Amersham, Bucks, U.K.), and L-U-^{14}C-alanine (specific activity, 154 mCi/mmol; Amersham) were applied. Prime and constant tracer doses were 270 μmol/kg and 0.450 μmol · kg^{-1} · min^{-1} $^{15}N_2$-urea, 5.0 μCi/kg and 41.7 nCi · kg^{-1} · min^{-1} tritiated glucose, and 1.25 μCi/kg and 12.5 nCi · kg^{-1} · min^{-1} ^{14}C-alanine, respectively. Blood glucose was determined at intervals of 10 min throughout, and tracer-based turnover rates were estimated at intervals of 15 min between 12:00 and 2:00 P.M., i.e., 20–22 h after the last insulin administration. Hormones and metabolites were determined before the beginning of GLP-I administration and after 360 and 480 min of GLP-I infusion, respectively.

In all GLP-I infusion and control experiments, the isotopic steady state of the applied tracer was attained after 135 min of tracer infusion. This is shown for the infusion experiments with GLP-I for tritium and ^{14}C-labeled glucose, ^{14}C-alanine, and ^{15}N-labeled urea (Fig. 1).

The animals were randomly subjected at intervals of 2 weeks either to GLP-I infusion or to control test, applying 6.25 ml/h of 0.154 mol/l NaCl. GLP-I(7-36) amide (Saxon Biochemicals, Hannover, Germany) was applied at a dose of 10 pmol · kg^{-1} · min^{-1}. It was dissolved in a stock solution of 1 mg/6 ml saline containing 60 mg bovine serum albumin (Albumin Fraction V, Boehringer Mannheim, Mannheim, Germany). Body weight–dependent aliquots of this solution were freshly diluted with saline to allow the application of 50 ml GLP-I infusate within the indicated interval of 8 h. All infusions were applied by means of high-precision syringe pumps (Perfusor Secura FT, B. Braun, Melsungen, Germany).

Analyses. Plasma concentration of glucose was determined by means of a glucose analyzer (Beckman Analyzer, Fullerton, CA). For the calculation of specific radioactivities, lactate, alanine, and glucose were measured in chromatographed plasma extracts by means of standard enzymatic-spectrophotometric procedures. Neutralized, chromatographed alanine or glucose-containing fractions were also used for the measurement of the respective ^{14}C radioactivities using a Model 2650 Packard Tricarb Liquid Scintillation Spectrometer (Packard, Vienna, Austria), as detailed previously (21). Plasma β-hydroxybutyrate was measured by means of a standard enzymatic-spectrometric procedure as detailed previously (18). The activity of tritiated glucose was measured after deproteination and lyophilization of plasma and redissolution of the extracts as described before (22). $^{15}N_2$-urea was analyzed by means of the SSQ 710 mass spectrometer (Finnigan MAT, San Jose, CA) employing deproteinized plasma samples after cation exchange chromatography (Dowex 50 WX8), drying under nitrogen gassing, and derivation with BSTFA (*N,O*-bis-trimethylsilyltrifluor-acetamide, Merck, Darmstadt, Germany). Using established formulas (23), the Mol percentage enrichments (MPE) were calculated from the ion intensities of the singly charged fragments m/e with the molecular masses 189, 190, and 191. They represent the unlabeled (^{14}N/^{14}N) moiety, and the single- (^{15}N/^{14}N; M+1) and double-^{15}N-labeled (^{15}N/^{15}N; M+2) portions of derivatized urea, respectively.

Radioimmunoassays of plasma insulin and pancreatic glucagon have been described before (24). Plasma concentrations of GLP-I were estimated after Sep Pak KC 18 extraction (Waters, Milford, MA) by means of a competitive radioimmunoassay (2) using ^{125}iodine-labeled GLP-I(7-36) amide (specific activity 74 Tbq/mmol) (2,25) and the specific antibody GA 1178 (Affinity Research, Nottingham, U.K.). The antibody was 100% reactive with both GLP-I (1-36) amide and truncated GLP-I(7-36) amide. There was no cross-reactivity with GIP, pancreatic glucagon, glicentin, oxyntomodulin, or GLP-II (2,26). Sensitivity of the assay was 2 fmol/tube, and the intra- and inter-assay coefficients of variation were 3.4 and 5.2%, respectively.

Calculations. The mean labeling data in metabolic steady state (Fig. 1) obtained between 390 and 480 min were used. Steele's equation (27) was used to calculate *1*) the flux rates of glucose and alanine from their specific activity data (21), and *2*) the production rate of urea from its MPE data considering the amount of urea infused (23).

As detailed before (24), the percentage of glucose formed from alanine was assessed from the specific ^{14}C activities of plasma glucose and alanine considering the metabolic exchange of carbon among the different gluconeogenetic pathways (28), and this figure was used to calculate rates of glucose production from alanine considering the simultaneously measured glucose flux rates. All tracer kinetic calculations were performed for the six 15-min intervals between 12:30 and 2:00 P.M. according to the protocol. Under steady-state conditions of specific activity and MPE (Fig. 1), the average flux rates of the respective data sets were presented as means ± SE. Paired Student's *t* test was used to assess statistical significance at the $P < 0.05$ level; for comparison of data between nondiabetic and diabetic dogs, the nonpaired *t* test was applied. The SPSS/PC+ program V3.1 (SPSS, Chicago, IL) was used for statistical evaluation.

RESULTS

In the diabetic animals, hyperglycemia was 19.7 ± 1.4 mmol/l glucose when the study was initiated in the morning. At this time, β-hydroxybutyrate levels were already distinctly elevated compared with nondiabetic controls (0.18 ± 0.06 vs. 0.03 ± 0.01 mmol/l; $P < 0.05$). Also, plasma lactate was higher (0.43 ± 0.11 vs. 0.28 ± 0.05 mmol/l; $P < 0.05$) and alanine levels were diminished (0.15 ± 0.02 vs. 0.25 ± 0.05 mmol/l; $P < 0.05$) compared with nondiabetic dogs under similar conditions. In the diabetic dogs, the average plasma insulin concentrations were 24 ± 9 pmol/l, which was not significantly different from the lower detection limit of the assay (<40 pmol/l). The nondiabetic control animals, however, showed distinctly higher insulin levels (78 ± 12 pmol/l, $P < 0.05$). These levels showed no significant changes during the entire observation (Fig. 2). Basal plasma concentrations of GLP-I were not different between diabetic and nondiabetic animals (2.4 ± 0.8 vs. 1.4 ± 0.4 pmol/l, NS). During GLP-I infusion, steady-state levels reached ranged around 40 pmol/l. Plasma pancreatic glucagon concentrations were significantly elevated in the diabetic animals (70 ± 8 pmol/l) compared with nondiabetic controls (28 ± 2 pmol/l; $P < 0.05$). During GLP-I treatment, these values significantly declined at 360 and 480 min, respectively (42 ± 8 and 44 ± 8 pmol/l; $P < 0.05$), as shown in Fig. 2. There was a constant decrease of glycemia in the diabetic dogs during GLP-I infusion, which reached significance 4 h after the onset and appeared to attain a steady-state level from hour 6 of hormone administration (Fig. 2). The values of glucose concentrations at 480 min were 16.4 ± 1.1 and 19.6 ± 1.2 mmol/l during the GLP-I and saline infusions, respectively. The decrease in glycemia was related to a GLP-I–mediated reduction in steady-state glucose flux rates that essentially represents the overall endogenous glucose production (Table 1). There was no complete restoration to normal values as in healthy dogs, which, under identical conditions, were 5.2 ± 0.2 mmol/l for the blood glucose concentration and 13.1 ± 0.4 μmol · kg^{-1} · min^{-1} for the endogenous glucose production rate. The metabolic clearance rate of glucose, which in nondiabetic dogs was 2.5 ± 0.1 ml · kg^{-1} · min^{-1}, was also significantly reduced in the diabetic animals (see Table 1). This was not influenced by administration of GLP-I.

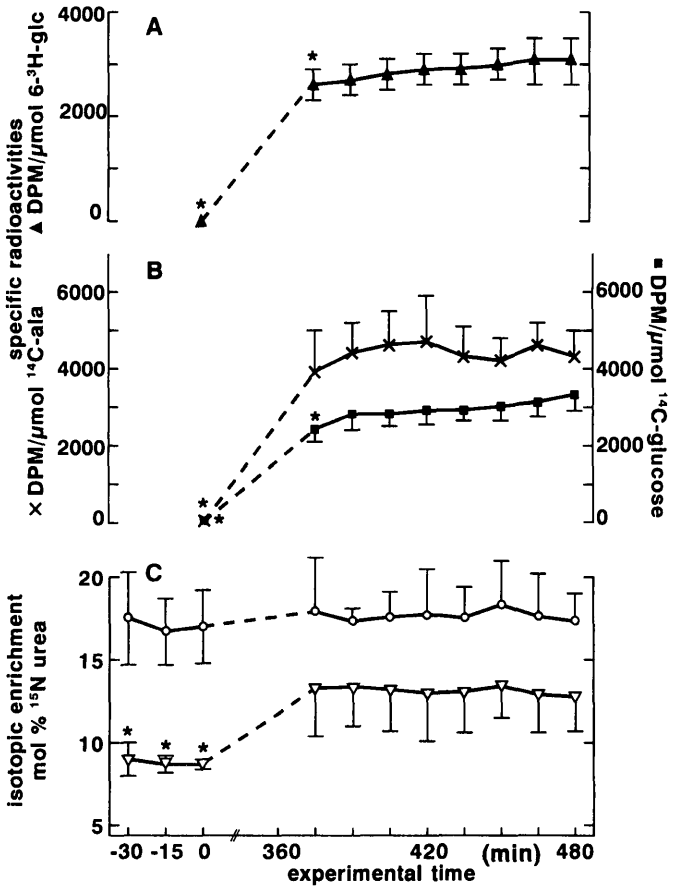

FIG. 1. Plasma enrichment of double (M+2, △) and single (M+1, ○) ^{15}N-labeled urea in the lower panel; specific radioactivities of ^{14}C-alanine (×) and ^{14}C-glucose (■) in the middle panel; and specific radioactivities of 6-^{3}H-glucose (▲) in the upper panel before infusion of the respective tracer and during the final isotopic steady state in six insulin-deprived diabetic dogs infused with GLP-I for 8 h (0 to 480 min) and with tracers for 4 h (240 to 480 min) attaining steady state at experimental time 375 min (controls not shown). Data are means ± SE. *P < 0.05 vs. final level.

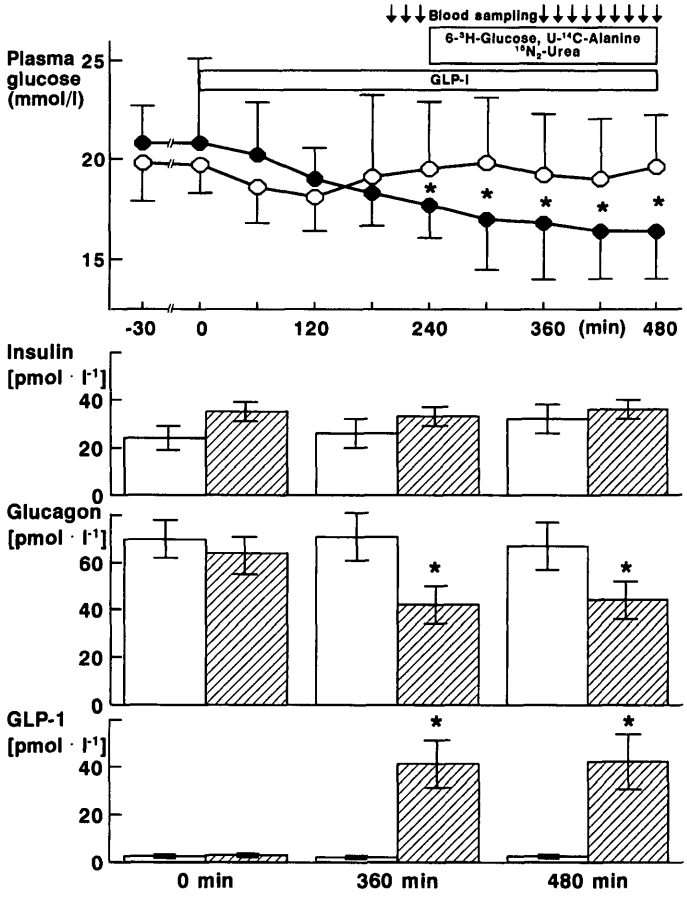

FIG. 2. Plasma glucose pattern in diabetic dogs between 13 and 22 h after the last subcutaneous dose of regular insulin: paired experiments, either with infusion of GPL-1(7-36) amide (●, ▨) or saline (○, □). Also presented are the average plasma concentrations of insulin, pancreatic glucagon, and GLP-I at the indicated experimental time. n = 6. Data are means ± SE. *P < 0.05 between GLP and control tests.

During GLP-I treatment, there was a significant reduction of the alanine turnover (Table 1). This did not apply to the plasma concentrations of alanine, which remained in the same range (0.15 ± 0.02 vs. 0.15 ± 0.03 μmol/l; NS). Furthermore, there was no significant difference in the plasma lactate levels between GLP-I treated and untreated diabetic animals (0.33 ± 0.05 vs. 0.43 ± 0.11 μmol/l; NS). In the diabetic dogs, the rate at which glucose was produced from alanine was always significantly elevated compared with nondiabetic animals (0.4 ± 0.1 μmol · kg^{-1} · min^{-1}). This, again, was not modified by GLP-I treatment. Accordingly, no influence of GLP-I administration could be found for the urea production rates (Table 1).

DISCUSSION

The experimental diabetic dog model investigated appears to be appropriate for the study of GLP-I effects under the conditions of diabetic hypoinsulinemia and hyperglucagonemia. Basal GLP-I levels observed in this study correspond to those measured in other species (29,30), and the levels attained during GLP-I infusion appear to be in the upper range of normal increments after meals (29) or after oral carbohydrate loads (2). Indeed, the metabolic effects observed may be considered physiologically relevant. Because of the loss of pancreatic tissue and of β-cells in particular (20), no appreciable insulinogenic effect can be expected by GLP-I in the experimental model chosen. There is, however, a moderate diabetic hyperglucagonemia that may also be maintained by the gastric A cell population that secretes pancreatic glucagon (31). It has previously been shown that in this chronic IDDM dog model, the diabetic hyperglucagonemia is, in fact, related to the degree of blood glucose control (32). The glucagonostatic effect of GLP-I has been previously reported in vitro (33,34) and in vivo in healthy volunteers (35), in NIDDM (9), and in insulin-treated IDDM (8). This is also shown in this study in dogs with hyperglucagonemia due to an insulin-deficient state. Also, it turned out that in parallel to the glucagon-lowering effect of GLP-I, both the elevated blood glucose concentration and the elevated glucose production rate are lowered, but not normalized, at nonavailability of insulin. Our data argue against two earlier discussed pathways putatively contributing to GLP-I–induced insulin-independent blood glucose lowering in IDDM:

- There is obviously no insulin-like effect of GLP-I as such on extrahepatic glucose utilization, as shown by

TABLE 1
Flux rates of glucose, alanine, and urea in insulin-deprived diabetic dogs under the infusion of GLP-I

	Saline	GLP-I
Glucose turnover ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	36.5 ± 3.3	25.9 ± 1.2*
Glucose metabolic clearance rate ($ml \cdot kg^{-1} \cdot min^{-1}$)	2.0 ± 0.2	1.7 ± 0.2
Alanine turnover ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	7.0 ± 1.4	3.9 ± 0.9*
Glucose production from alanine ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	1.5 ± 0.1	1.3 ± 0.2
Urea production ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	7.2 ± 0.7	7.1 ± 0.8

Data are means ± SE of individual averages during six 15-min intervals between 390 and 480 min of GLP-I(7-36) amide infusion, which was 20 and 22 h after the last subcutaneous dose of regular insulin. *$P < 0.05$.

the lack of any influence on the metabolic clearance rate of glucose.

- Gluconeogenesis from alanine, i.e., from amino acids in general, which is distinctly altered due to insulin withdrawal (36), is not additionally influenced by the administration of GLP-I.

This corresponds well to the lack of effects of GLP-I on urea production, which is considered to reflect the elimination of nitrogen moieties from overall amino acid catabolism (37). It seems to apply to gluconeogenesis in general, since there was no appreciable alteration by GLP-I of plasma lactate concentration. Thus, it may be speculated that GLP-I exerts its glucose-lowering effect in insulin-deficient diabetes via its glucagonostatic potency, reducing hepatic glucose output due to reduced glycogen breakdown. Earlier studies in dogs have shown that hepatic glucose output elevated due to fasting may be reduced by means of lowering glucagon levels (38,39), which is, in fact, accompanied by increased hepatic glycogen stores (40). Comparable effects of GLP-I on liver glycogen remain to be elucidated.

Taken together, relatively low doses of exogenous GLP-I(7-36) amide are efficient in reducing the endogenous glucose production and, thereby, fasting blood glucose concentration even in the insulin-deficient diabetic state. Whether it is useful in the metabolic management of IDDM in the presence of insulin replacement and greatly reduced hepatic glucose output and plasma glucagon values needs to be verified.

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