Long-Lasting Antidiabetic Effect of a Dipeptidyl Peptidase IV–Resistant Analog of Glucagon-Like Peptide-1

Rémy Burcelin, Wanda Dolci, and Bernard Thorens

Glucagon-like peptide-1(7-37) (GLP-1) is the most potent insulinotropic hormone characterized thus far. Because its activity is preserved in non–insulin-dependent diabetes mellitus (NIDDM) patients, it is considered a potential new drug for the treatment of this disease. One limitation in its therapeutic use is a short half-life in vivo (5 minutes), due in part to a fast degradation by the endoprotease dipeptidylpeptidase IV (DPPIV). Recently, it was reported that GLP-1 became resistant to DPPIV when the alanine residue at position 8 was replaced by a glycine (GLP-1-Gly8). We report here that this change slightly decreased the affinity of the peptide for its receptor (IC_{50}, 0.41 ± 0.14 and 1.39 ± 0.61 nmol/L for GLP-1 and GLP-1-Gly8, respectively) but did not change the efficiency to stimulate accumulation of intracellular cyclic adenosine monophosphate (cAMP) (EC_{50}, 0.25 ± 0.05 and 0.36 ± 0.06 nmol/L for GLP-1 and GLP-1-Gly8, respectively). Second, we demonstrate for the first time that this mutant has an improved insulinotropic activity compared with the wild-type peptide when tested in vivo in an animal model of diabetes. A single injection of 0.1 nmol GLP-1-Gly8 in diabetic mice fed a high-fat diet can correct fasting hyperglycemia and glucose intolerance for several hours, whereas the activity of 1 nmol GLP-1 vanishes a few minutes after injection. These actions were correlated with increased insulin and decreased glucagon levels. Interestingly, normoglycemia was maintained over a period that was longer than the predicted peptide half-life, suggesting a yet undescribed long-term effect of GLP-1-Gly8. GLP-1-Gly8 thus has a markedly improved therapeutic potential compared with GLP-1, since it can be used at much lower doses and with a more flexible schedule of administration.

THE LOSS OF GLUCOSE-INDUCED insulin secretion by the pancreatic β cell is a characteristic of non–insulin-dependent diabetes mellitus (NIDDM).[1] In the treatment of NIDDM when diet and exercise are no longer sufficient to correct the diabetic syndrome, postprandial insulin secretion is stimulated by administration of insulin secretagogues such as sulfonylureas.[2] These substances stimulate insulin secretion by directly inhibiting the activity of K^+-adenosine triphosphate channels, thereby depolarizing the β-cell plasma membrane and stimulating insulin secretion. However, the initial efficacy of sulfonylureas vanishes after a few years of treatment. This is thought to be due to β-cell exhaustion resulting, at least in part, from the action of sulfonylureas solely on the stimulation of insulin secretion without a concomitant positive effect on insulin biosynthesis. New clinically useful insulin secretagogues are therefore required.

The insulinotropic hormone glucagon-like peptide-1(7-37) (GLP-1) is the most potent stimulator of glucose-induced insulin secretion characterized thus far.[3-5] It is produced in the intestinal L cells as a proteolytic processing product of the preproglucagon molecule,[6] and is secreted in the blood following nutrient ingestion, particularly glucose and fatty acids. GLP-1 binds to a specific β-cell plasma membrane receptor of the G protein–coupled receptor family that is linked to the activation of adenylyl cyclase.[7-9] GLP-1 insulinotropic activity is measurable with peptide concentrations as low as 1 to 10 pmol/L and is detected only in the presence of normal or elevated glucose levels.[10-12] Besides its effect on insulin secretion, GLP-1 also stimulates transcription of the insulin gene and translation of the insulin mRNA.[13,14] Importantly, the insulinotropic effect of this peptide is preserved in patients with NIDDM, even those with secondary failure to sulfonylureas.[15-18] This combined with the glucose dependence of its action and its positive effects on insulin biosynthesis make GLP-1 an attractive potential new agent for the treatment of type 2 diabetes. However, a significant drawback in the therapeutic use of this peptide, is its short half-life in vivo, about 5 minutes.[19] This is due in part to a rapid inactivation by the circulating endopeptidase dipeptidylpeptidase IV (DPPIV),[20-22] which hydrolyzes peptides or protein after the second amino-terminal residue, provided that the sequence is X-Pro/Ala. DPPIV action on GLP-1 produces GLP-1(9-27), a relatively weak antagonist of the GLP-1 receptor.[23] Recent data show that GLP-1-Gly8 has a longer N-terminal half-life of 3.3 minutes versus 0.9 minutes for GLP-1, as determined by the use of specific antibodies.[24]

Here, we demonstrate that when tested in vivo in glucose-intolerant mice fed a high-fat diet, GLP-1-Gly8 shows a strongly improved efficacy to correct glucose intolerance compared with the wild-type peptide, as well as a marked improvement of basal glycemia. In addition, normoglycemia was maintained over a period that was longer than the predicted peptide half-life, suggesting a yet undescribed long-term effect of GLP-1-Gly8. Due to its improved stability and the probable absence of antagonist formation, the efficacy of the mutant peptide required smaller dosages and was longer-lasting.

MATERIALS AND METHODS

Peptides, Cells, and Cell Culture

GLP-1, GLP-1-Gly8, and diprotin A were purchased from Bachem (Bubendorf, Switzerland) and purified to 97% by high-performance liquid chromatography (HPLC). Clone 5 cells are Chinese hamster lung fibroblasts stably expressing the rat GLP-1 receptor.[9] These cells were

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cultured and used for peptide binding experiments and cyclic adenosine monophosphate (cAMP) production as previously described.[9,25]

Stability of GLP-1 Analogs

Peptides were incubated in the presence of 20 μmol/L purified DPPIV in a total volume of 50 μL 25-mmol/L triethanolamine, pH 7.8, for 15 minutes at 37°C. The incubation reaction was then acidified, and the peptides were analyzed by HPLC using a Nucleosil phenyl reverse-phase column (125 × 3.0 mm; Macherey-Nagel, Oensingen, Switzerland) with isocratic elution conditions as previously described.[26] The sensitivity of the method allowed detection of 0.1% of the degradation product (GLP-1(9-37)). Alternatively, peptides were incubated at an initial concentration of 10 nmol/L in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) at 37°C for different periods and in the presence or absence of 0.2 mmol/L diprotin A. The remaining biological activity of the peptides was then assessed by measuring the production of intracellular cAMP in clone 5 cells induced by an aliquot of the incubation medium diluted 1:10. The activity of the sample was compared with a standard dose-response curve for cAMP production generated using the corresponding peptide diluted in the same incubation medium.

Animal Studies

Six-week-old male C57Bl/6JIco mice (18 to 20 g, IFFA Credo, L'Arbresle, France) were fed ad libitum with either normal chow pellets ([NC] energy content, 12% fat, 28% protein, and 60% carbohydrate) or a high-fat (HF) diet (energy content, 72% fat [corn oil and lard], 28% protein [cow milk casein], and <1% carbohydrate) containing added minerals, vitamins, and cellulose (ICN, Costa Mesa, CA). The animals were kept on a 9 PM to 9AM dark cycle. Blood was collected at 2 PM in the fed state. For fasting experiments, food was removed at 9AM

Intraperitoneal Glucose Tolerance Test

Food was removed 5 hours before initiation of an intraperitoneal glucose tolerance test ([IPGTT] 1 g glucose/kg body weight). Blood glucose levels were monitored using a glucometer (Bayer, Zurich, Switzerland) with 2.5 μL tail blood. The area under the curve (AUC) was calculated in millimolars per minute for the indicated periods. Insulin and glucagon were analyzed by radioimmunoassay (Linco, St Louis, MO) with blood collected from the orbital sinus of separate sets of animals.

Statistical Analysis

Results are expressed as the mean ± SE. Statistical analyses were performed by Student's *t* test for unpaired data and considered significantly different at a *P* level less than .05.

RESULTS

In Vitro Studies

GLP-1-Gly8 binding to the GLP-1 receptor was evaluated in displacement experiments. Clone 5 cells were incubated in the presence of tracer amounts of radioiodinated GLP-1 and increasing concentrations of wild-type or mutant GLP-1 peptides. Figure 1A shows that the IC_{50} values for displacement of

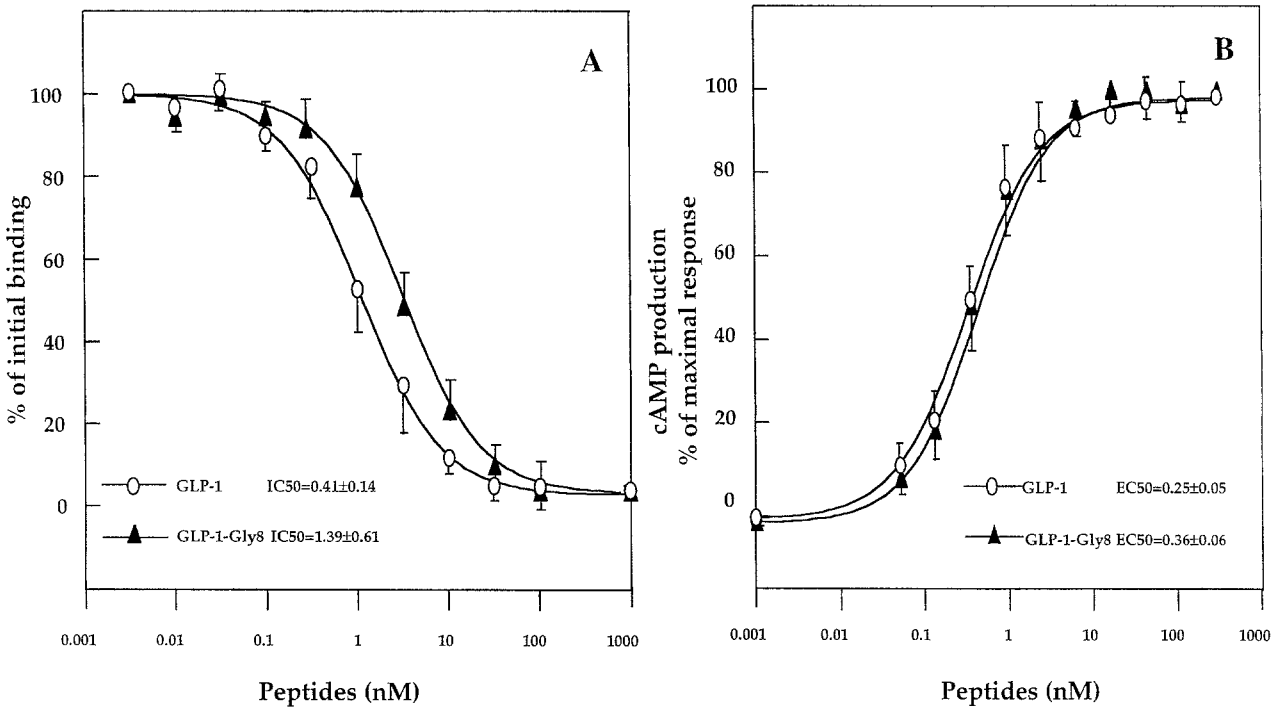

Fig 1. Binding and activation of adenylyl cyclase by GLP-1 and GLP-1-Gly8 occur with the same affinity and efficacy. (A) Displacement of ^{125}I-GLP-1 binding to its receptor by increasing concentrations of GLP-1 or GLP-1-Gly8. Chinese hamster lung fibroblast cells expressing GLP-1 receptor (clone 5 cells) were incubated at 4°C in the presence of radioiodinated GLP-1 (20 pmol/L) and in the presence of increasing concentrations of cold GLP-1 or GLP-1-Gly8. Curves are the mean ± SE of 4 different experiments. IC_{50s} (nmol/L, mean ± SE, n = 4) for displacement of radioiodinated GLP-1 by GLP-1 or GLP-1-Gly8 are indicated. Difference between the 2 IC_{50s} is not significant. (B) Dose-dependent accumulation of cAMP in clone 5 cells in response to increasing concentrations of GLP-1 or GLP-1-Gly8. cAMP production was measured after exposure of clone 5 cells to indicated concentrations of peptides. Curves are mean ± SE of 4 different experiments. EC_{50} (nmol/L mean ± SE) for both peptides is indicated.

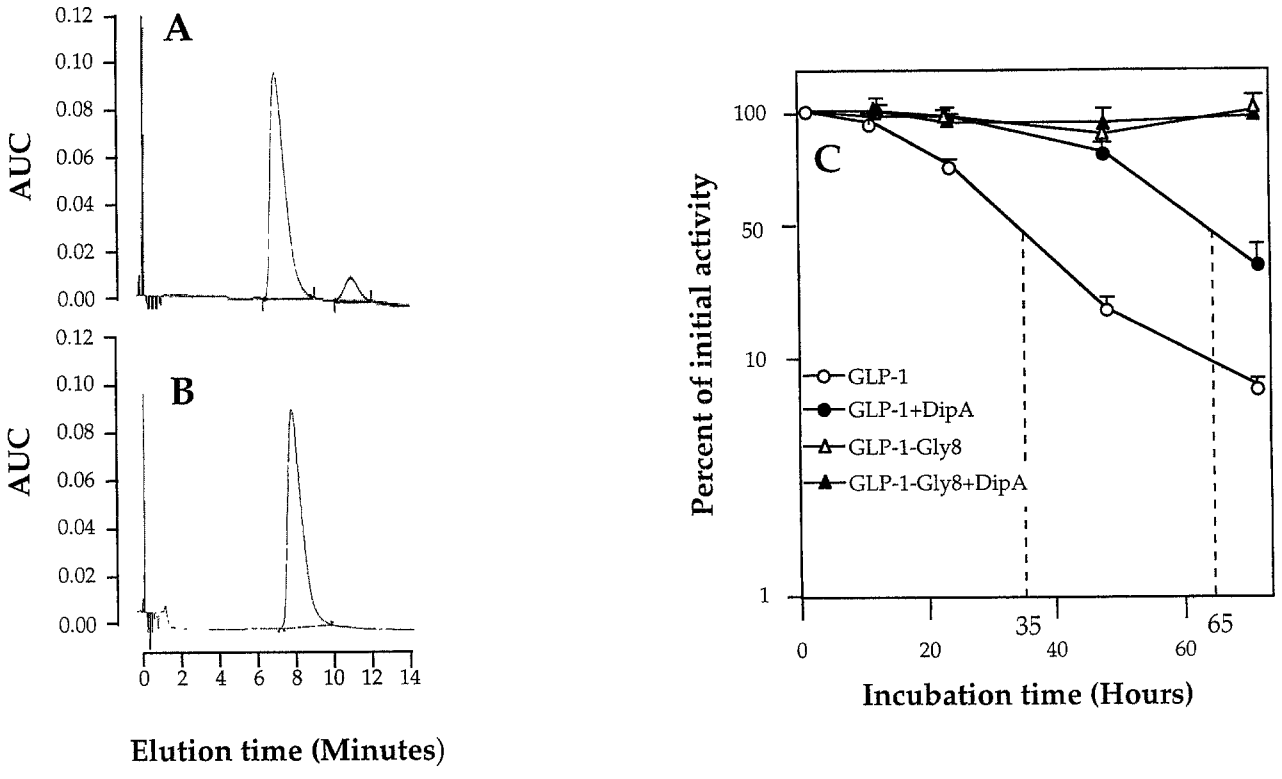

Fig 2. Increased stability of GLP-1-Gly8 to degradation by DPPIV. (A) GLP-1 or (B) GLP-1-Gly8 were incubated in the presence of purified DPPIV, and peptides separated by HPLC. Intact peptides are eluted at 7.5 minutes and GLP-1(9-37) at 10 minutes. 10% of GLP-1 is converted to the cleaved product and <0.1% of GLP-1-Gly8 is degraded. (C) GLP-1 or GLP-1-Gly8 was incubated for indicated periods in DMEM containing 10% FBS. Biological activity of peptides was then measured on stimulation of cAMP formation by clone 5 cells. Loss of GLP-1 activity can be prevented in part by diprotin A (Dip A). GLP-1-Gly8 was completely resistant to inactivation.

bound radioiodinated GLP-1 by GLP-1 or GLP-1-Gly8 were not statistically different (0.41 ± 0.14 *v* 1.39 ± 0.61 nmol/L, respectively). The dose-dependent induction of cAMP accumulation in clone 5 cells by both peptides also displayed the same EC_{50} (Fig 1B).

The resistance of GLP-1-Gly8 to degradation by DPPIV was first evaluated by incubating the peptide in the presence of the purified enzyme and analyzing the reaction products by HPLC. The intact peptides (GLP-1 and GLP-1-Gly8) eluted with a retention time of 7.5 minutes, whereas the cleaved products (GLP-1(9-37)) had a retention time of 10.5 minutes (Fig 2A). In conditions where 10% of GLP-1 was converted to GLP-1(9-39), less than 0.1% of GLP-1-Gly8 was recovered as cleaved peptide, indicating a marked resistance of the peptide to degradation by DPPIV (Fig 2B). The stability of GLP-1-Gly8 was further analyzed by incubation of the peptide in the presence of FBS that naturally contains DPPIV activity.[27,28] Importantly, this assay was designed to measure bioactivity of the remaining peptide by assessing the ability of the incubation medium to activate cAMP production by clone 5 cells. The half-life of GLP-1 was about 35 hours when incubated in the presence of FBS, and this half-life was extended to 65 hours when diprotin A, a specific DPPIV inhibitor,[29] was added at the beginning of the experiment (Fig 2C). In contrast, in the same conditions, GLP-1-Gly8 remained stable for more than 72 hours, even in the absence of diprotin A. Similar observations were made when the peptides were incubated in the presence of baby hamster kidney (BHK) cells cultured in the same medium (not shown).

In Vivo Studies

C57Bl/6J mice obtained at 6 weeks of age were fed NC or a HF, carbohydrate-free diet. Body weight was significantly higher in the HF group (n = 12) after 8 weeks of diet versus the control group (n = 12; 30.2 ± 0.6 *v* 28.1 ± 0.3 g, respectively) and remained about 30% higher up to 26 weeks (38.5 ± 0.8 *v* 30.3 ± 0.3 g, respectively). Fed blood glucose levels were lower in the HF versus NC group, since no carbohydrates were present in the HF diet (Table 1). Fasting induced a rapid decrease in blood glucose in NC mice, whereas glycemia remained stable in the HF group (5.7 ± 0.2 and 7.2 ± 0.1 mmol/L after 6-hour fast in NC and HF mice, respectively).

Table 1. Glucose, Insulin, and Glucagon Levels in Fed and 24-Hour Fasted C57Bl/6J Mice After 16 to 18 Weeks of NC or HF Diet

	Fed		Fasted	
Parameter	NC	HF	NC	HF
Glucose (mmol/L)	8.2 ± 0.2*	7.1 ± 0.2	4.9 ± 0.2*	6.9 ± 0.4
Insulin (μU/mL)	76.3 ± 7.8*	43.8 ± 8.4	19.8 ± 4.9*	9.7 ± 1.4
Glucagon (pg/mL)	59.2 ± 2.0*	117.7 ± 7.4	35.5 ± 3.2	28.8 ± 5.5

*Significantly different *v* corresponding HF group, $P < .05$, $n \geq 5$.

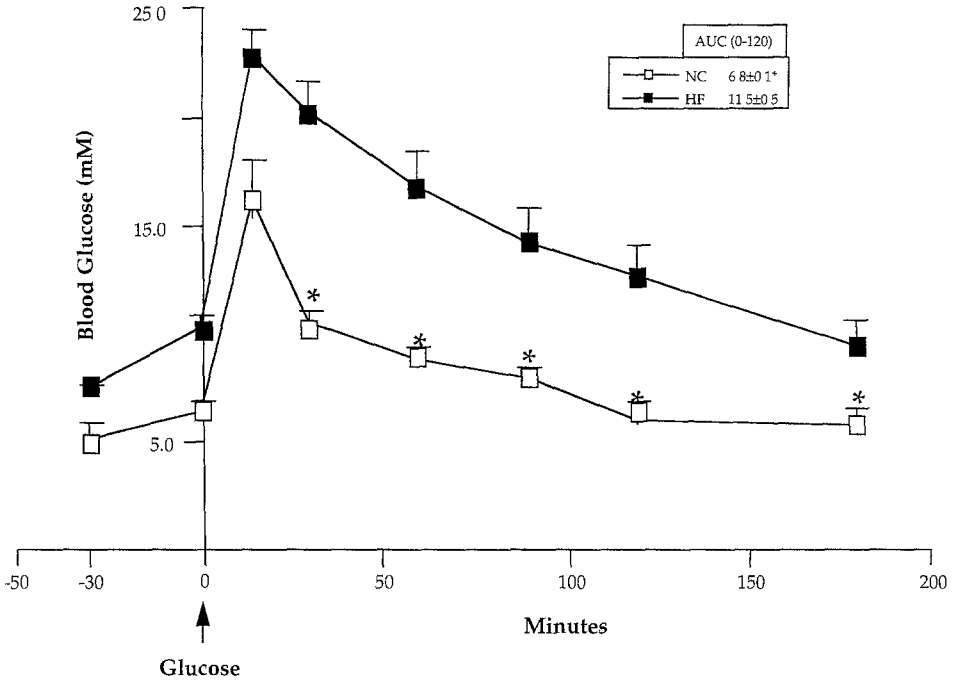

Fig 3. Glucose intolerance in C57Bl/6J mice fed a HF diet. IPGTT (1 g/kg) was performed after a 5-hour fast in NC or HF mice. (Inset) AUC(0-120). *Significantly different *v* corresponding HF-fed, saline-injected mice, $P < .05$; n ≥ 6 for each group.

Insulin levels in the fed state were lower in HF compared with NC mice. Twenty-four hours of fasting decreased insulin concentrations in both groups, and insulin levels were significantly lower in the HF group compared with the NC group. Glucagon levels in the fed state were higher in HF versus NC mice, but were not different after fasting (Table 1). These data therefore indicate the presence of a diabetic phenotype induced by HF feeding.

GLP-1 and GLP-1-Gly8 were first evaluated for the ability to correct the glycemic excursions in HF mice during the IPGTT performed after a 5-hour period of fasting. Figure 3 shows the IPGTT curves obtained with NC and HF mice. HF mice show higher glycemia at the time of glucose injection and a much higher and prolonged increase in blood glucose. This is reflected by the 1.7-fold higher AUC for the first 2 hours (AUC(0-120)) for HF compared with NC mice. Table 2 presents blood glucose and plasma insulin and glucagon levels 30 minutes after initiation of the IPGTT. Glycemia was twofold higher and plasma insulin was 50% lower in HF versus control mice, and glucagon levels were unchanged (Table 2). There were thus the impaired insulin to glucose and insulin to glucagon ratios characteristic of type 2 diabetes. When injected at 30 minutes, 1 nmol GLP-1 led to a correction of the AUC calculated between 60 and 120 minutes. However, this correction was not as good as that obtained with 1 nmol GLP-1-Gly8, but was similar to the value obtained with 0.1 nmol GLP-1-Gly8 (Fig 4). Furthermore, with the mutant but not with the wild-type peptide, the correction of glycemia was associated with an increase in circulating insulin and a decrease in plasma glucagon measured at 60 minutes after initiation of the IPGTT (Table 2).

GLP-1 or GLP-1-Gly8 were then injected into the peritoneal cavity of HF mice at several time points before initiation of an IPGTT. When preinjected at −30 minutes, 1 nmol GLP-1 did not normalize the glucose tolerance (Fig 5A), whereas 1 nmol GLP-1-Gly8 almost completely normalized it. This was quantified by calculating the AUC(0-120) of the IPGTT (Fig 5A and B). Figure 5B also shows that the glucose AUC(0-120) was corrected when 1 nmol GLP-1-Gly8 was injected intraperitoneally up to 4 hours, but not 24 hours, before the start of the

Table 2. Plasma Hormones and Glucose Levels in NC- and HF-Fed Mice During an IPGTT

	30 Minutes After IPGTT		HF Mice 60 Minutes After IPGTT		
Parameter	NC	HF	Saline	GLP-1	GLP-1-Gly8
Glucose (mmol/L)	10.4 ± 0.7*	20.2 ± 1.5	13.5 ± 0.2	11.1 ± 0.7*	7.2 ± 1.7†
Insulin (μU/mL)	55.2 ± 6.1*	24.6 ± 4.9	8.0 ± 0.9	6.8 ± 1.0	25.7 ± 1.2†
Glucagon (pg/mL)	59.1 ± 2.0	53.2 ± 4.0	53.0 ± 3.7	51.1 ± 11.1	38.2 ± 2.9†
Insulin/glucose ratio	5.5	1.2	0.51	0.75	2.44†
Insulin/glucagon ratio	0.93	0.49	0.15	0.12	0.67†

NOTE. An IPGTT (1 g/kg body weight) was performed with 5-hour fasted NC or HF mice. Thirty minutes after initiation of the test, HF mice received an intraperitoneal injection of saline or saline containing 1 nmol GLP-1 or GLP-1-Gly8. Glucose, insulin, and glucagon levels were measured at 60 minutes.

*Significantly different *v* corresponding HF group, $P < .05$, n ≥ 5.

†Significantly different *v* corresponding HF saline group, $P < .05$, n ≥ 5.

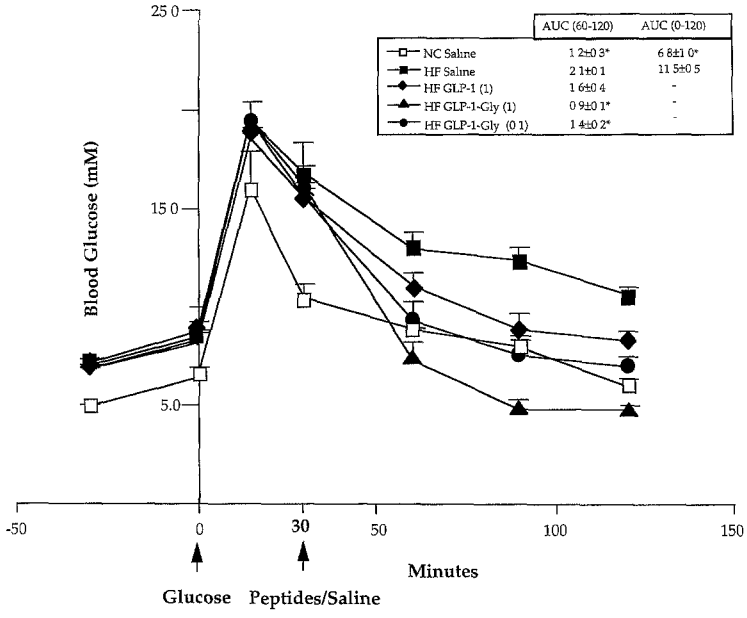

Fig 4. Improvement of glucose tolerance by GLP-1 or GLP-1-Gly8 injection. IPGTT was performed after a 5-hour fast in NC or HF mice. Saline, 1 nmol GLP-1, or 1 or 0.1 nmol GLP-1-Gly8 were injected intraperitoneally 30 minutes after the glucose challenge. Both GLP-1 and GLP-1-Gly8 corrected the glycemic levels as measured by the AUC (60-120 minutes). However, 0.1 nmol GLP-1-Gly8 was as efficient as 1 nmol GLP-1 (n ≥ 5 for each group). For HF and NC saline groups, mean of all experiments is presented (n ≥ 10).

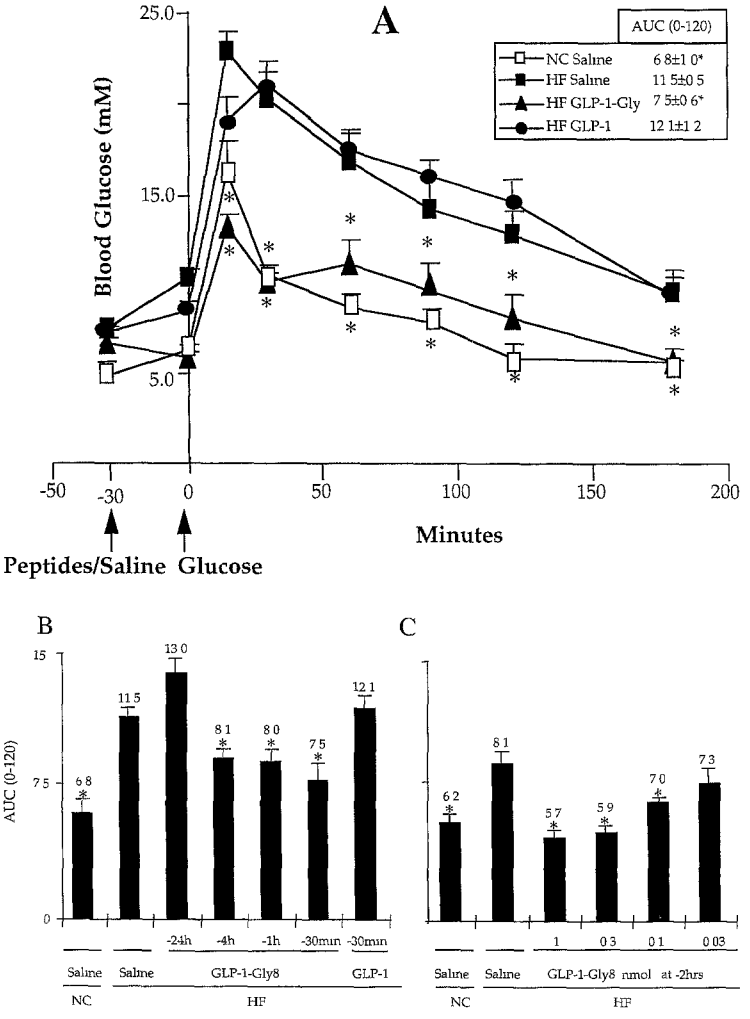

Fig 5. Effect of GLP-1 and GLP-1-Gly8 injection before intraperitoneal glucose challenge. (A) IPGTT was performed as in Fig 3 on NC or HF mice; 1 nmol GLP-1 or GLP-1-Gly8 was injected intraperitoneally 30 minutes before the glucose challenge. Whereas GLP-1-Gly8 almost completely corrected glucose tolerance, GLP-1 did not improve glycemic excursion. (Inset) AUC(0-120). *Significantly different *v* corresponding HF-fed, saline-injected mice, $P < .05$; n ≥ 5 for each group. (B) AUC(0-120) calculated from IPGTT performed as described in (A) for mice that received saline or 1 nmol of peptides intraperitoneally 24, 4, 1 hour, or 30 minutes before the glucose challenge. Injection of 1 nmol GLP-1-Gly8 up to 4 hours before initiation of IPGTT led to complete correction of glucose intolerance, whereas GLP-1 injection 30 minutes before was without effect (n ≥ 5 for each group). (C) AUC(0-120) calculated from the IPGTT performed as described in (A) for mice that received 2 hours before initiation of the IPGTT either saline solution or the indicated amount (nmol) of GLP-1-Gly8. *Significantly different *v* corresponding HF-fed, saline-injected mice, $P < .05$; n ≥ 5 for each group.

Table 3. Effect of GLP-1 on Fasting Blood Glucose Levels

Group	Treatment	Time of Injection	Fasting Blood Glucose (mmol/L)
NC	None	—	5.7 ± 0.2*
HF	None	—	7.2 ± 0.1
	GLP-1 1 nmol	−10 min	6.0 ± 0.1*
		−30 min	7.8 ± 0.5
	GLP-1-Gly8 1 nmol	−10 min	5.3 ± 0.1*
		−30 min	5.9 ± 0.4*
		−4 h	6.1 ± 0.3*
		−24 h	7.4 ± 0.3
NC	Saline	−2 h	5.4 ± 0.2†
HF	Saline	−2 h	6.9 ± 0.8
	GLP-1-Gly8	−2 h	
	1.0 nmol		5.5 ± 0.3†
	0.3 nmol		5.7 ± 0.2†
	.01 nmol		5.5 ± 0.3†
	.003 nmol		6.6 ± 0.3

NOTE. C57Bl/6J mice fed a NC or HF diet were fasted for 5 hours before blood glucose measurement. The mice previously received 1 nmol GLP-1 or GLP-1-Gly8 as an intraperitoneal injection at the indicated times before blood sampling, or the indicated amount of GLP-1-Gly8 2 hours before measurement of glycemia.

*Significantly different *v* corresponding noninjected HF group, $P < .05$, $n \geq 5$.

†Significantly different *v* corresponding HF saline-injected group, $P < .05$, $n \geq 5$.

IPGTT. Figure 5C shows that when administered intraperitoneally 2 hours before initiation of the IPGTT, complete correction of glucose intolerance could be achieved with 0.3 nmol of GLP-1-Gly8, and a significant effect was still observed with 0.1 nmol.

We then evaluated the ability of GLP-1-Gly8 to control glycemia in 5-hour fasted mice. A single injection of 1 nmol GLP-1-Gly8 maintained normoglycemia for at least 4 hours, whereas the effect of 1 nmol GLP-1 had already vanished after 30 minutes. Alternatively, when GLP-1-Gly8 was injected 2 hours before blood was sampled, normoglycemia was maintained with as little as 0.1 nmol (Table 3).

DISCUSSION

The present study shows that substitution of the alanine normally present at the second position of GLP-1 by a glycine improves the resistance of the peptide to the action of DPPIV. This was demonstrated by showing the resistance of the peptide to hydrolysis by the purified enzyme, and by the preserved biological activity of the mutant peptide incubated up to 72 hours in the presence of FBS. Importantly, this Ala to Gly substitution did not change the binding affinity or the capacity of the peptide to activate the production of cAMP.

The effectiveness of the DPPIV-resistant form of the peptide to control glycemic levels was demonstrated by several experiments. First, when injected at 30 minutes after initiation of an IPGTT, GLP-1-Gly8 showed an approximately 10-fold more potent therapeutic effect, since 0.1 nmol of the mutant peptide produced the same glycemic correction as 1 nmol GLP-1. This effect was mediated by the insulinotropic effect of the peptide, since insulin levels increased and glucagon levels decreased 30 minutes after injection of the mutant peptide. Second, preinjection of 1 nmol GLP-1-Gly8 up to 4 hours before initiation of the IPGTT completely corrected the glucose intolerance, whereas the same amount of GLP-1 had no effect even when injected 30 minutes before the IPGTT. And third, when injected 2 hours before the IPGTT, 0.3 nmol completely corrected the glucose intolerance and 0.1 nmol still had a significant effect.

This improved physiological action of GLP-1-Gly8 was unexpected from previously published data, which reported that the half-life in pig serum of the GLP-1-Gly8 mutant was increased about sixfold and the half-life in vivo was increased about threefold compared with GLP-1.[24] The basis for the improved biological efficiency of this peptide is not known. However, it may be due to the fact that cleavage of GLP-1 by DPPIV leads to the formation of GLP-1(9-37). This peptide is a low-affinity antagonist of the GLP-1 receptor and can be found in vivo at a 10-fold excess over GLP-1.[23] Because of its resistance to DPPIV, GLP-1-Gly8 is unlikely to produce significant concentrations of this antagonist. This may therefore explain, in part, the persistence of the in vivo efficacy of the mutated peptide. In addition, a single injection of GLP-1-Gly8 decreased glucagon levels much more efficiently than GLP-1 and for a prolonged period. The improved insulin to glucagon ratio may have significant effects on hepatic glucose production, thereby further reinforcing the antidiabetic effect of GLP-1-Gly8.

Several studies have been performed to evaluate the therapeutic potential of GLP-1 in the control of diabetic hyperglycemia in human patients.[15,16,18] In initial clinical assays, GLP-1 was given intravenously either as a bolus at the beginning of a meal or as a continuous infusion over the time of observation, and a possible prolonged correction of glycemia after cessation of peptide administration was not evaluated. However, recently, Rachman et al[30] infused GLP-1 overnight to diabetic patients at a rate that led to correction of basal hyperglycemia. Following discontinuation of GLP-1 infusion, a breakfast was given to the patients and the glycemia immediately returned to the diabetic level, indicating no prolonged effect of GLP-1. In an effort to develop GLP-1 as a therapeutically useful molecule, this peptide was administered either as a subcutaneous injection[31-33] or as a buccal tablet.[34] Although good correction of the glycemic excursion could be obtained, the therapeutic efficacy of the peptide was also short-lived.

In conclusion, we have demonstrated that GLP-1-Gly8, due to its resistance to inactivation by DPPIV and probable absence of antagonist production, increases insulin secretion and normalizes glucagon and glucose levels for a longer time than the natural peptide, and hence can better improve glycemic control in HF diabetic mice. GLP-1-Gly8 therefore represents a significantly improved form of this incretin hormone, since lower doses can be used for the correction of diabetic hyperglycemia and with a more flexible schedule of administration.

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