A synthetic glucagon-like peptide-1 analog with improved plasma stability

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Abstract

Glucagon-like peptide-1 (GLP-1) is the most potent endogenous insulin-stimulating hormone. In the present study the plasma stability and biological activity of a GLP-1 analog, [Ser8]GLP-1(7–36)amide, in which the second N-terminal amino acid alanine was replaced by serine, was evaluated in vitro and in vivo. Incubation of GLP-1 with human or rat plasma resulted in degradation of native GLP-1(7–36)amide to GLP-1(9–36)amide, while [Ser8]GLP-1(7–36)amide was not significantly degraded by plasma enzymes. Using glucose-responsive HIT-T15 cells, [Ser8]GLP-1(7–36)amide showed strong insulino-tropic activity, which was inhibited by the specific GLP-1 receptor antagonist exendin-4(9–39)amide. Simultaneous i.v. injection of [Ser8]GLP-1(7–36)amide and glucose in rats induced a twofold higher increase in plasma insulin levels than unmodified GLP-1(7–36)amide with glucose and a fivefold higher increase than glucose alone. [Ser8]GLP-1(7–36)amide induced a 1.5-fold higher increase in plasma insulin than GLP-1(7–36)amide when given 1 h before i.v. application of glucose. The insulino-tropic effect of [Ser8]GLP-1(7–36)amide was suppressed by i.v. application of exendin-4(9–39)amide. The present data demonstrate that replacement of the second N-terminal amino acid alanine by serine improves the plasma stability of GLP-1(7–36)amide. The insulino-tropic action in vitro and in vivo was not impaired significantly by this modification.

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Introduction

Glucagon-like peptide-1 (GLP-1) is a neuroendocrine hormone of the distal gut with a strong insulino-tropic action. It originates from intestinal L-cells by post-translational processing of the preproglucagon molecule into GLP-1(7–37) and GLP-1(7–36)amide, which are the biologically active forms of GLP-1. It is secreted into GLP-1(7–37) and GLP-1(7–36)amide, which are the biologically active forms of GLP-1. The anti-diabetogenic effect of GLP-1 is limited by its short plasma half-life. Physiologically, GLP-1 is degraded by plasma dipeptidyl-peptidase IV (DPP IV) with a half-life of 12–30 min (Deacon et al. 1995, Kieffer et al. 1995). The N-terminal two amino acids (His-Ala-) are cleaved resulting in a loss of biological activity of the shortened GLP-1 molecule. Degradation of GLP-1 by DPP IV seems to require alanine or proline at the second N-terminal position. Peptides with the N-terminus His-Ser- are not significantly degraded by the peptidase (Mentlein et al. 1993). Therefore, substitution of the second N-terminal amino acid alanine by serine might improve the anti-diabetogenic effect of exogenously administered GLP-1 by prolongation of its plasma half-life. It has been shown that such a modification does not impair the interaction of GLP-1 with its receptor in vitro (Adelhorst et al. 1994). To evaluate whether the anti-diabetogenic effect of GLP-1 could be improved by this modification, we synthesized a GLP-1 analog with serine instead of alanine, [Ser8]GLP-1(7–36)amide, and investigated its plasma stability and biological activity in vitro and in vivo.
Materials and Methods

Peptides

GLP-1(7–36)amide (molecular mass 3297.5 Da) was obtained in analytical purity from Saxon Biochemicals GmbH (Hannover, Germany). Modified GLP-1(7–36)amide with the serine instead of alanine in position 8 ([Ser8]GLP-1(7–36)amide) and the GLP-1 receptor antagonist exendin-4(9–39)amide were synthesized using the Fmoc strategy on TentaGel S RAM resin (Rapp, Tübingen, Germany) with an ABI 433A peptide synthesizer (Applied Biosystems, Foster City, CA, USA).

After cleavage of the peptides from the resin, the crude products were purified by preparative reverse-phase HPLC (RP-HPLC) performed on a Waters Prep Nova-Pak HR C18 silica gel column (5 × 30 cm, 6 µm particle size, 6 nm pore size) with a mixture of aqueous 0.1% trifluoroacetic acid (TFA) and methyl cyanide. Subsequent analytical RP-HPLC was performed on a Vydac C18 silica gel column (0.46 × 25 cm, 5 µm particle size, 30 nm pore size) with solvents A (0.1% TFA in water) and B (80% methyl cyanide in 0.1% TFA in water) at a flow rate of 1 ml/min. The samples were eluted with 5% solvent B for 5 min and then with a linear gradient of 5–95% solvent B over 30 min. The correct molecular mass of the peptides was confirmed by plasma desorption mass spectrometry (PDMS) on a BioIon 20 time-of-flight mass spectrometer (BioIon, Uppsala, Sweden). PDMS calculation for [Ser8]GLP-1(7–36)amide was 3313.7 Da and for exendin-4(9–39)amide 3486.9 Da.

Plasma stability of GLP-1(7–36)amide and [Ser8]GLP-1(7–36)amide in vitro

GLP-1(7–36)amide and [Ser8]GLP-1(7–36)amide (1 µg/µl peptide in phosphate buffer at pH 7.4) were incubated with human or rat plasma (final sample volume 500 µl; final peptide concentration 20 µM) for 0 or 15 min or 1, 2, 3, 4, 5, 6 or 9 h at 37 °C. The incubation was terminated by the addition of 20 µl aqueous 10% TFA. The samples were applied to C18 reverse-phase cartridges (size: 3CC/100MG; Bond-Elut; Varian, Harbor City, CA, USA) washed with 2 ml aqueous 0.1% TFA, and eluted with 1 ml 60% acetonitrile in aqueous 0.1% TFA. After lyophilization and dissolution of the samples in 100 µl 0.1 M phosphate buffer (pH 7.4), analytical RP-HPLC was performed on a Nucleosil C18 column (30 nm pore size, 7 µm particle size; Macherey-Nagel, Düren, Germany) with solvents A (0.1% TFA in water) and B (70% acetonitrile in 0.1% aqueous TFA) at a flow rate of 1.5 ml/min. The peptides were eluted with a linear gradient of solvent B from 40 to 70% for 45 min using Waters chromatography pumps equipped with a Waters automated gradient controller (Eschborn, Germany). Elution of the peptides was monitored by measuring UV absorption at 220 nm (LKB 2151 variable wavelength monitor; Pharmacia, Freiburg, Germany) and collected for characterization by mass spectrometry and RIA. Recovery at the end of the entire procedure including pre-extraction, lyophilization and RP-HPLC was approximately 65%.

RIA and electrospray ionization (ESI) mass spectrometry

GLP-1(7–36)amide, [Ser8]GLP-1(7–36)amide and their N-terminal truncated metabolites were detected by RIA using a polyclonal antibody (GA 1178; Affinity Research, Nottingham, UK), which recognizes the C-terminus of the peptides. Synthetic GLP-1(7–36)amide was allowed to react with 125I in the presence of Chloramine-T (Cooper 1981) and purified by RP-HPLC. Aliquots (40 µl) of the samples were incubated with 50 µl antibody solution and 50 µl 125I-labeled GLP-1(7–36)amide (3000 c.p.m.) for 5 days. Antibody-bound GLP-1 was separated from free GLP-1 by precipitation with a carbon/dextran suspension and centrifugation at 6000 g for 20 min.

The molecular mass of the peptides was determined by mass spectrometry using a Micromass AutoSpec-T tandem mass spectrometer with an electrospray interface. The samples were applied by direct loop injection or from the HPLC apparatus (ABI 140B pumps; splitter: LC-packings 1:20; column: 15 cm × 0.3 mm Vydac C18; 30 nm pore size, 5 µm particle size; detector: ABI 759 at 220 nm). Solvent A contained aqueous 0.05% TFA, and solvent B aqueous 80% acetonitrile in 0.02% TFA. The flow rate was 4 µl/min at a linear gradient of 1% acetonitrile/min.

Cell culture experiments

The insulinotropic activities of GLP-1(7–36)amide and [Ser8]GLP-1(7–36)amide were evaluated by static incubation with hamster islet tumor (HIT) cells. HIT-T15 cells at passage 60 were obtained from American Type Culture Collection (Rockville, MD, USA). The cells were cultured using RPMI 1640 medium containing 100 IU/ml penicillin, 100 µg/ml streptomycin, 10% heat-inactivated horse serum and 5% heat-inactivated fetal bovine serum at 37 °C in 5% CO2/95% air. All experiments were performed at passage 63. One million cells were plated per well in six-well plates. On the third day the growth medium was removed, the cells were washed with Krebs-Ringer bicarbonate (KRb) medium (Hill & Boyd 1985), and preincubated with KRb. After 1 h, the medium was replaced by GLP-1(7–36)amide or [Ser8]GLP-1(7–36)amide at final concentrations of 0.1, 0.3, 1, 3, 10, 30 or 100 nM dissolved in KRb and supplemented with 4 mM glucose (n=6 for each incubation time point). Incubation was performed for 30 min at 37 °C. The same experiments were also performed in the presence of 10 and 100 nM of the GLP-1 receptor.
antagonist exendin-4(9–39)amide. In control experiments HIT cells were incubated with KRB alone or 4 mM glucose dissolved in KRB without the addition of GLP-1 or [Ser^8]GLP-1. After termination of the stimulation, the medium was removed for quantification of insulin in the supernatant by RIA (Fehmann et al. 1991) using a porcine standard (Sigma, München, Germany). The cells were scraped, and their protein content was quantified by a dye-binding assay (Coomassie Brilliant Blue G-250; Bio-Rad, Munich, Germany).

In vivo experiments

The in vivo effect of GLP-1(7–36)amide and [Ser^8]GLP-1(7–36)amide was evaluated in male Wistar rats (270–300 g). The animals were maintained under standardized conditions and were fed ad libitum, with free access to water. Before the experiments (12–14 h) the rats were deprived of food. After anesthesia with subcutaneous injection of sodium pentobarbital (50 mg/kg body weight; Nembutal) the animals were placed on a water-perfused table to maintain a constant body temperature of 37 °C. The left femoral vein and the left carotid artery were cannulated, and 500 IU heparin was injected i.v. Each experiment was performed with six animals. Blood samples were collected from the left carotid artery before and 2.5, 5, 10, 20, 30 and 60 min after injection of glucose. After centrifugation (24 000 g for 2 min), the plasma was frozen in liquid nitrogen and stored at –20 °C.

In a first series of experiments, glucose (1 g/kg body weight) was injected i.v. simultaneously with GLP-1(7–36)amide (1 µg/kg body weight) or [Ser^8]GLP-1(7–36)amide (1 µg/kg body weight). In a control group, glucose (1 g/kg) was injected i.v. without application of GLP-1(7–36)amide or [Ser^8]GLP-1(7–36)amide.

In a second series of experiments, GLP-1(7–36)amide (1 µg/kg) and [Ser^8]GLP-1(7–36)amide (1 µg/kg) were injected 60 min before i.v. application of glucose (1 g/kg). In a further six animals [Ser^8]GLP-1(7–36)amide (1 µg/kg) was injected simultaneously with the GLP-1 receptor antagonist exendin-4(9–39)amide (100 µg/kg) 60 min before i.v. application of glucose (1 g/kg).

Plasma insulin was quantified by RIA (Thorens & Waeber 1993) using a rat insulin standard (Novo, Mainz, Germany). Glucose was measured by the glucose oxidase method with a Beckmann glucose analyzer 2.

Statistical analysis

Data are shown as means ± s.e.m. and were analysed using GraphPad Prism 2.00 software for PC-DOS. In vitro data were analysed using two-tailed t-tests. In vivo data were analysed by calculation of the area under the curve. Statistical analysis was performed by ANOVA.

Results

In vitro stability of GLP-1(7–36)amide and [Ser^8]GLP-1(7–36)amide

Addition of GLP-1(7–36)amide or [Ser^8]GLP-1(7–36)amide to human or rat plasma and immediate RP-HPLC yielded one product eluted with a retention time of 25.6 min for the serine-modified GLP-1 or 25.8 min for the unmodified GLP-1. The peptides were identified by ESI mass spectrometry. The molecular masses were 3297.6 Da for GLP-1(7–36)amide and 3317.7 Da for [Ser^8]GLP-1(7–36)amide. Their respective theoretical masses were 3297.5 and 3313.7 Da. In addition, a plasma-derived product with a retention time of 34.8 min was identified by ESI mass spectrometry as albumin.

Incubation with human plasma resulted in a decrease in the GLP-1(7–36)amide peak, while a second peak with a retention time of 27.0 min appeared. The second peak represented the N-terminally truncated GLP-1 metabolite GLP-1(9–36)amide with a molecular mass of 3092.5 Da compared with a theoretical value of 3089.4 Da. After 15 min incubation with human plasma, about one-third of GLP-1 was recovered as the N-terminally truncated peptide, while after 60 min incubation about two-thirds of the initial GLP-1 was degraded to GLP-1(9–39)amide (Fig. 1). Incubation of [Ser^8]GLP-1(7–36)amide with human plasma did not result in any significant degradation of the peptide (Fig. 2). After 4 h incubation, GLP-1(7–36)amide was almost completely metabolized, whereas most of [Ser^8]GLP-1(7–36)amide was still intact. However, small products of [Ser^8]GLP-1(7–36)amide were eluted from the RP-HPLC column later than [Ser^8]GLP-1(7–36)amide after prolonged incubation. Even after 9 h incubation [Ser^8]GLP-1(7–36)amide could still be detected.

Incubation of GLP-1(7–36)amide or [Ser^8]GLP-1(7–36)amide with rat plasma resulted in truncation of GLP-1(7–36)amide to GLP-1(9–36)amide whereas [Ser^8]GLP-1(7–36)amide was not significantly degraded. However, after 60 min incubation with rat plasma, a higher proportion of GLP-1(7–36)amide was degraded compared with the corresponding experiments with human plasma (Figs 1 and 2).

Stimulation of insulin secretion by GLP-1(7–36)amide and [Ser^8]GLP-1(7–36)amide from HIT cells

Basal insulin secretion from HIT-T15 cells in the absence of glucose, GLP-1(7–36)amide or [Ser^8]GLP-1(7–36)amide was 0.74 ± 0.04 pg/30 min per µg protein. In the presence of 4 mM glucose, insulin secretion increased to 1.06 ± 0.16 pg/30 min per µg protein (P=0.08 vs incubation with KRB alone). Both peptides, GLP-1(7–36)amide and [Ser^8]GLP-1(7–36)amide, dose-dependently enhanced glucose-induced insulin release (Fig. 3).
The highest insulin release was measured after stimulation of the cells with 10 nM GLP-1(7–36)amide (2.63 ± 0.11 pg/30 min per µg protein) or 10 nM [Ser³]GLP-1(7–36)amide (2.54 ± 0.10 pg/30 min per µg protein). The maximal insulin secretion induced by the two peptides did not differ significantly (P=0.55).

Figure 1 RP-HPLC profile of 10 nmol GLP-1(7–36)amide after 0, 15, 60 min and 4 h incubation with human plasma and after 60 min incubation with rat plasma in vitro. GLP-1(7–36)amide and GLP-1(9–39)amide were identified by mass spectrometry. UV detection was at 220 nm.
Addition of the specific GLP-1 receptor antagonist exendin-4(9–39)amide significantly inhibited the insulinotropic effect of GLP-1(7–36)amide and [Ser8]GLP-1(7–36)amide. However, the inhibition was stronger after stimulation with GLP-1(7–36)amide than with [Ser8]GLP-1(7–36)amide. In the presence of 10 nM exendin-4(9–39)amide the stimulatory effect of the serine-modified GLP-1 was significant at a concentration of 10 nM (P=0.002), whereas a 100 nM concentration of the unmodified GLP-1 was necessary to increase insulin

Figure 2  RP-HPLC profile of 10 nmol [Ser8]GLP-1(7–36)amide after 0, 15, 60 min and 4 h incubation with human plasma and after 60 min incubation with rat plasma in vitro. UV detection was at 220 nm.
secretion significantly ($P=0.0004$). Addition of 100 nM exendin-4(9–39)amide completely inhibited the insulino-tropic activity of GLP-1(7–36)amide. Under the same conditions, 100 nM [Ser$^8$]GLP-1(7–36)amide was still able to induce significant insulin secretion ($P=0.003$).

Plasma glucose and insulin levels after simultaneous injection of GLP-1(7–36)amide and [Ser$^8$]GLP-1(7–36)amide in rats

The basal glucose levels of rats receiving glucose or glucose and one of the two peptides ranged from 107 to 114 mg/100 ml and did not differ significantly in the three groups ($P>0.05$). After bolus injection of glucose, the mean plasma glucose level increased to $477\pm31$ mg/100 ml within 2.5 min in rats receiving GLP-1(7–36)amide and to $478\pm17$ mg/100 ml in rats receiving [Ser$^8$]GLP-1(7–36)amide. In the control animals, the glucose level increased to $519\pm20$ mg/100 ml (Fig. 4). During the following 60 min, the glucose level declined to $99\pm5$ mg/100 ml in those animals that had received [Ser$^8$]GLP-1(7–36)amide. In rats in which the unmodified
peptide had been injected and in the control animals, mean plasma glucose concentrations did not decline to basal levels during the 60 min. The area under the curve (AUC) was $13,590 \pm 2,137 \text{ mg/ml per min}$ in the control experiments vs $12,830 \pm 2,372 \text{ mg/ml per min}$ in the experiments using GLP-1 and $10,970 \pm 1,068$ using [Ser$^8$]GLP-1 ($P<0.0001$).

Intravenous application of [Ser$^8$]GLP-1(7–36)amide simultaneously with glucose induced significantly higher plasma insulin levels than injection of GLP-1(7–36)amide and glucose or glucose alone with a maximum of insulin secretion after 5 min (Fig. 4). The corresponding AUC values were $392 \pm 53 \text{ ng/ml per min}$ using the serine-modified GLP-1, $284 \pm 59 \text{ ng/ml per min}$ for native GLP-1, and $241 \pm 93 \text{ ng/ml per min}$ in the control experiments ($P<0.0001$).

PLasma glucose and insulin levels after i.v. injection of GLP-1(7–36)amide or [Ser$^8$]GLP-1(7–36)amide 60 min before i.v. administration of glucose in rats

Intravenous administration of glucose 60 min after i.v. application of GLP-1(7–36)amide or [Ser$^8$]GLP-1(7–36)amide increased the mean plasma glucose concentration within 2.5 min to $462 \pm 32 \text{ mg/100 ml}$ and $433 \pm 22 \text{ mg/100 ml}$ respectively (Fig. 5). After 60 min, almost basal glucose concentrations were observed in [Ser$^8$]GLP-1(7–36)amide-treated rats, whereas the glucose levels were still elevated in those animals that had received the unmodified peptide. The AUC was $11,670 \pm 632 \text{ mg/ml per min}$ in the group with the modified GLP-1 vs $14,330 \pm 1,320 \text{ mg/ml per min}$ after injection of native GLP-1, and $13,900 \pm 585 \text{ mg/ml per min}$ after [Ser$^8$]GLP-1(7–36)amide plus the GLP-1 receptor antagonist exendin-4(9–39)amide ($P<0.0001$).

Discussion

GLP-1 is degraded in human and rat plasma in vitro and in vivo by the action of DPP IV (Mentlein et al. 1993, Deacon et al. 1995). The N-terminal two amino acids are cleaved by this enzyme resulting in the biological inactivation of GLP-1 (Buckley & Lundquist 1992). In accordance with previous reports, we could determine a time-dependent degradation of GLP-1(7–36)amide to GLP-1(9–36)amide as the major metabolite. DPP IV is found in plasma and is also localized on the surface of capillary endothelial cells, at kidney brush-border membranes, on the surface of hepatocytes, on the surface of some T-lymphocyte subpopulations, and on thymocytes (Loijda 1979, Mentlein et al. 1984, Nausch & Heymann 1985, McCaughan et al. 1990). DPP IV is responsible for degradation and inactivation of several biologically active peptides such as substance P (Ahmad et al. 1992), growth-hormone-releasing factor (Frohmann et al. 1989, Boulanger et al. 1992), gastric inhibitory peptide/glucagon-like peptide-dependent insulinotropic peptide (Mentlein et al. 1993) and peptide histidine methionine (PHM) (Mentlein et al. 1993).
All these peptides share considerable sequence similarity at their N-termini since they start with either His-Ala- or Tyr-Ala-. It has been shown that DPP IV acts as a highly specific aminopeptidase, which cleaves the N-terminal two amino acids if the second amino acid is alanine or proline. With this concept in mind we synthesized a GLP-1 analog with a serine instead of alanine at the second N-terminal position. This analog showed a significantly prolonged plasma half-life when it was incubated with human or rat plasma. It can be concluded that this modification protects the GLP-1 molecule from the degrading activity of DPP IV. The enzyme possesses two substrate-binding and -cleaving sites (P₁ and P₂). In the P₂ position a bulky N-terminal amino acid is preferred, whereas in the P₁ position almost no amino acid other than proline or alanine is accepted. P₁ seems to be a hydrophobic substrate-recognition site since substrates with synthetic hydrophobic oxa- or thia- derivates of the proline ring or hydrophobic alkyl derivatives induce high DPP IV activity (Rahfeld et al. 1991). In contrast, serine seems to be too hydrophilic to be a good substrate for DPP IV. The mean plasma half-life of GLP-1(7–36)amide was reported to be about 20 min (Buckley & Lundquist 1992), in agreement with the present observations. Replacement of the second N-terminal amino acid alanine with serine increased the plasma stability of GLP-1 significantly so that, even after 9 h incubation with human or rat plasma, no significant degradation could be observed. However, small amounts of metabolites could be detected after prolonged incubation of [Ser⁸]GLP-1(7–36)amide with plasma. These metabolites could not be identified by ESI mass spectrometry because of insufficient amounts. Since their retention times during RP-HPLC differed from those of GLP-1(9–36)amide, it can be speculated that they may be generated by unspecific degradation after initial truncation by DPP IV (Deacon et al. 1998).

In a previous study it was shown that the interaction of GLP-1 with its receptor is not impaired by the serine modification (Adelhorst et al. 1994). In agreement with this observation, we found preserved insulinotropic activity of the modified GLP-1 in HIT cells. HIT cells possess most of the differentiated functions of normal β-cells including the ability to synthesize and secrete insulin after stimulation with glucose (Hill & Boyd 1985, Ashcroft et al. 1986) or GLP-1 (Hill & Boyd 1985). Both peptides, GLP-1(7-36)amide and [Ser⁸]GLP-1(7–36)amide, were able to stimulate dose-dependently the glucose-induced insulin secretion of these cells. Maximal insulin secretion was observed at a concentration of 10 nM, decreasing at higher concentrations. This observation is in agreement with previous studies on primary islets (Göke et al. 1993b).

To evaluate the insulinotropic activity of [Ser⁸]GLP-1(7–36)amide in vivo we used a rat model. It has been shown that GLP-1 is the strongest peptidergic stimulator of insulin secretion in rats and humans (Thorens & Waeber 1993). The modified GLP-1 was able to lower glucose levels significantly and to induce significant elevation of insulin secretion in rats. It is remarkable that in vivo [Ser⁸]GLP-1(7–36)amide induced significantly higher insulin levels than native GLP-1(7–36)amide, whereas this effect was not observed in the cell culture system. This difference can be explained by the absence of significant degrading activity in HIT cells. It remains the aim of further studies to investigate this phenomenon in primary islet cells. However, the effect of the GLP-1 receptor antagonist exendin-4(9–39)amide on insulin secretion from HIT cells after stimulation with GLP-1(7–36)amide or [Ser⁸]GLP-1(7–36)amide was more pronounced for GLP-1 than for [Ser⁸]GLP-1. Furthermore, insulin secretion in vivo was even stimulated when [Ser⁸]GLP-1(7–36)amide was injected 1 h before application of glucose. Under the same conditions, unmodified GLP-1 (7–36)amide induced a significantly lower rate of secretion of insulin. This observation can be explained by the prolonged plasma half-life of the modified peptide. The elevation of the plasma insulin concentration after injection of GLP-1(7–36)amide may result from the direct stimulatory effect of the injected glucose and by the fact that GLP-1 was injected in supraphysiological doses as compared with previous studies (Thorens & Waeber 1993). When [Ser⁸]GLP-1(7–36)amide was injected simultaneously with exendin-4(9–39)amide, a significantly lower rate of insulin secretion was observed. Exendin-4(9–39)amide is a specific GLP-1 receptor antagonist, which competes with GLP-1(7–36)amide for the same binding site on the GLP-1 (Göke et al. 1993b, Kolligs et al. 1995). Since exendin-4(9–39)amide was able to inhibit the activity of [Ser⁸]GLP-1(7–36)amide, it can be assumed that the insulinotropic action of the serine-modified GLP-1 was mediated by interaction with the GLP-1 receptor.

The results of the present experiments demonstrate that replacement of the second N-terminal amino acid alanine with serine significantly increases the plasma stability of GLP-1(7–36)amide against DPP IV without impairing its insulinotropic activity. This may indicate that this modification could improve the potential of GLP-1 in the treatment of type-II diabetes. In further studies the potency of the serine-modified analog will be evaluated after administration by a route, such as subcutaneous injection, that would be more relevant to potential clinical application.

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